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BEFORE THE CONTROLLER OF PATENTS, KOLKATA

IN THE MATTER OF THE PATENTS ACT, 1970

And

IN THE MATTER OF THE INDIAN PATENT APPLICATION No. 3658/KOLNP/2009
DATED October 20, 2009 TITLED “NUCLEOSIDE PHOSPHORAMIDATE PRODRUGS”

IN THE NAME OF GILEAD PHARMASSET LLC

..... the Applicant

And

IN THE MATTER OF A REPRESENTATION BY WAY OF AN OPPOSITION UNDER
SECTION 25(1), PATENTS ACT, 1970 AND RULE 55, PATENTS RULES, 2003

BY **VECTOR BIOSCIENCES PVT. LTD.**, HAVING ITS ADDRESS AT F-7, IDA,
GANDHINAGAR, HYDERABAD; TELANGANA STATE – 500037, INDIA.

..... the Opponent

REPRESENTATION BY WAY OF OPPOSITION U/S 25(1)

1. A pre-grant opposition under Section 25(1) of the Patents Act, 1970, is being submitted by the Opponent against Indian Patent Application No. 3658/KOLNP/2009 (hereinafter referred to as the “Present Application”) in the name of Gilead Pharmasset LLC (hereinafter referred to as the “Applicant”).

OPPONENT’S BUSINESS AND ACTIVITIES

2. The Opponent is a company incorporated under the laws of India and involved in the development of various Active Pharmaceutical Ingredients / Drug Intermediates for commercialization in different markets. Being a research organization the Opponent has access to the latest technologies relating to manufacture of the pharmaceutical products and drug intermediates.
3. The Opponent submits that a patent confers on the patentee a monopoly over any invention. This monopoly allows the patentee to exclude interested parties such as the Opponent itself, from manufacturing or developing inventions, in this case medicines, around the patent for a period of twenty years. The Opponent is therefore concerned about the impact of product patent on availability of safe and effective treatment for various diseases including Hepatitis C.

I. PROSECUTION HISTORY OF THE PRESENT APPLICATION

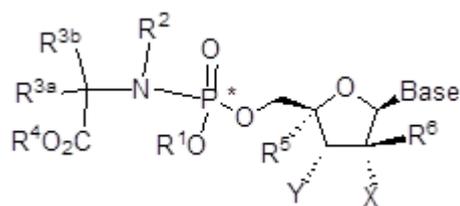
4. The Present Application - IN 3658/KOLNP/2009 titled “Nucleoside Phosphoramidate Prodrugs” was originally filed in the name of Pharmasset Inc. on October 20, 2009. The Applicant’s name was later changed to Gilead Pharmasset, LLC. The Present Application was published in India on March 19, 2010.
5. The Present Application is the national phase application of PCT/US2008/058183 dated March 26, 2008 and was published on October 9, 2008 bearing the publication number WO 2008/121634 (hereinafter referred as “WO ’634”). WO ’634 claims priority from US Provisional Application No. US 60/909,315 dated March 30, 2007, US Application No.

60/982,309 dated October 24, 2007 and US Patent Application 12/053,015 dated March 21, 2008.

6. The Applicant filed a request for examination on March 21, 2011. Thereafter, the Applicant amended the claims with claims totalling 14. It is pertinent to point out here that the duplicate Form-1 filed by the Applicant on April 1, 2014, incorrectly indicates US Application No. 60/666,230 as one of the priority applications instead of the US Application No. 12/053,015. The First Examination Report (FER) for the Present Application was issued on January 29, 2015. On November 4, 2015 the Applicant filed Form- 13 to amend the claims. The Applicant filed a response on November 27, 2015, and also made amendments to the claim, thereby bringing down the total number of claims to 5.

II. ANALYSIS OF THE PRESENT APPLICATION

7. The Present Application relates to Nucleoside Phosphoramidate prodrugs, which is claimed by the Applicant to be useful for treatment of viral diseases such as Hepatitis C. The Applicant claims that the mechanism with which the Phosphoramidate Prodrugs treat viral disease is by inhibiting the RNA polymerase that the hepatitis C virus uses to replicate its RNA.
8. The Applicant at internal page 3 of the complete specification claims that inhibition of HCV NS5B polymerase prevents formation of the double - stranded HCV RNA and therefore constitutes an attractive approach to the development of HCV-specific antiviral therapies. The Applicant further states that in order to function as a chain terminator, the nucleoside analog must be taken up by the cell and converted *in vivo* to a triphosphate to compete for the polymerase nucleotide binding site. According to the Applicant, the formation of the monophosphate by a nucleoside kinase is generally viewed as the rate limiting step of the three phosphorylation events (Refer to page 3 of the Present Application).
9. The complete specification of the Present Application discloses a compound of markush formula covering millions of compounds and its stereoisomers. The markush structure as disclosed on page 8 of the Present Application is given below :



Formula I

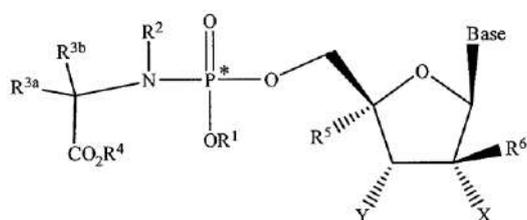
Wherein R^1 , R^2 , R^{3a} , R^{3b} , R^4 , R^5 , R^6 , X and Y represent a series of groups / substitutions on these groups. The complete specification also states that the compounds of the Formula I are racemic because of the chirality at phosphorous (see internal pages 18 – 19 of the Present Application).

III. CLAIMS OF THE PRESENT APPLICATION

Claims at the PCT stage

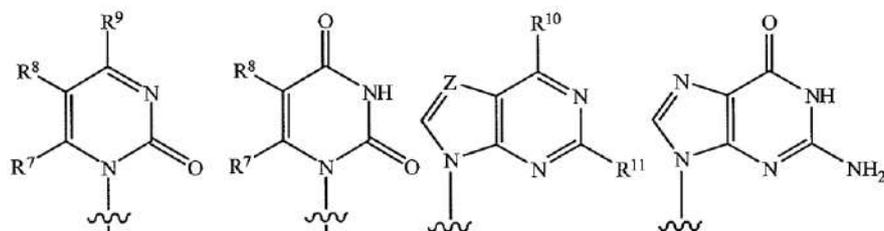
10. The Present Application had 12 claims at the PCT stage, discussed as under:

i) Claim 1 relates to a compound of Formula I



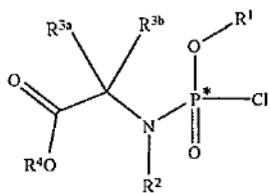
I

and its stereoisomer, salt, hydrate, solvate, or crystalline form thereof, where the base is a naturally occurring modified purine/pyrimidine represented by following structures:

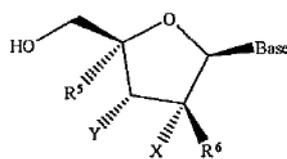


ii) Claim 2 relates to a compound, its stereoisomer, salt, hydrate, solvate or crystalline form thereof selected from a list of compounds, identified by their IUPAC names.

- iii) Claim 3 relates to a composition for treatment and/or prophylaxis of any viral agents disclosed therein, comprising a pharmaceutically acceptable medium selected from among an excipient carrier, diluent and compound of claim 1.
- iv) Claim 4 relates to a composition for treatment and/or prophylaxis of any viral agents disclosed therein, comprising a pharmaceutically acceptable medium selected from among an excipient, carrier, diluent and equivalent medium and a compound of claim 2.
- v) Claim 5 relates to a use of the compound of claim 1 in the manufacture of a medicament for the treatment of any condition the result of an infection by Hepatitis C virus, West Nile virus, yellow fever virus, dengue virus, rhinovirus, polio virus, Hepatitis A virus, bovine viral diarrhea virus or Japanese encephalitis virus.
- vi) Claim 6 relates to a use of a compound of claim 2 in the manufacture of a medicament for the treatment of any condition the result of an infection by Hepatitis C virus, West Nile virus, yellow fever virus, dengue virus, rhinovirus, polio virus, Hepatitis A virus, bovine viral diarrhea virus or Japanese encephalitis virus.
- vii) Claim 7 relates to a method of treatment which comprises administering a therapeutically effective amount of a compound of claim 1.
- viii) Claim 8 relates to a method of treatment which comprises administering a therapeutically effective amount of a compound of claim 2.
- ix) Claim 9 relates to a process for preparing compound of claim 1, the said process comprising reacting a substituted phosphorochloridate compound 4 with a nucleoside analog 5 reproduced below

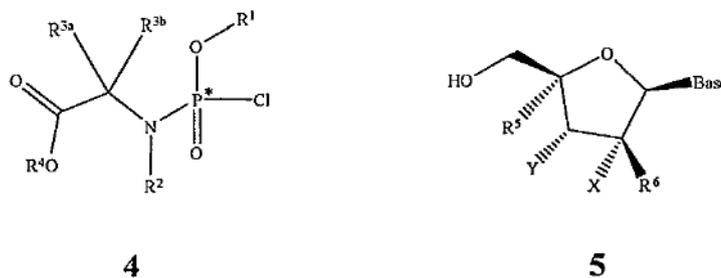


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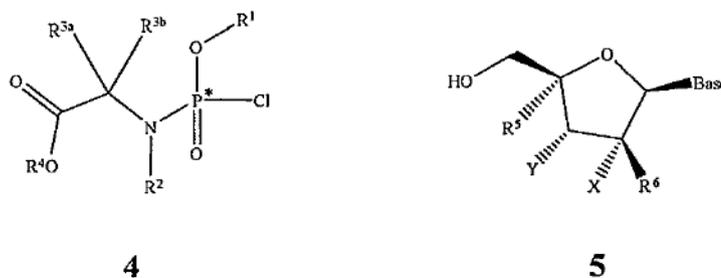


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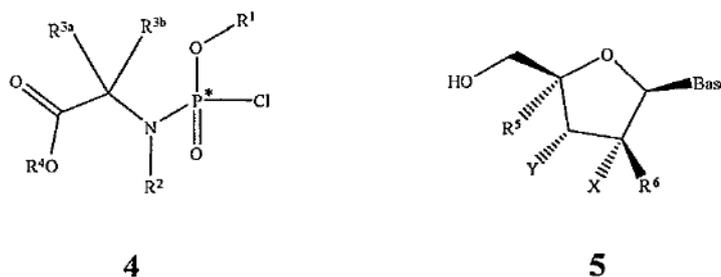
- x) Claim 10 relates to a process for preparing compound of claim 2, wherein the said process comprising reacting a substituted phosphochloridate compound 4 with a nucleoside analog 5 reproduced below-



- xi) Claim 11 relates to a product, its stereoisomer, salt, hydrate, solvate or crystalline form thereof, prepared by a process comprising reacting a substituted phosphochloridate compound 4 with nucleoside of analog 5, as reproduced under



- xii) Claim 12 relates to a product comprising a compound of claim 2, its stereoisomer, salt, hydrate, solvate or crystalline form thereof, prepared by a process comprising reacting a substituted phosphochloridate compound 4 with nucleoside of analog 5, as reproduced under



CLAIMS AS FILED AT THE INDIAN PATENT OFFICE

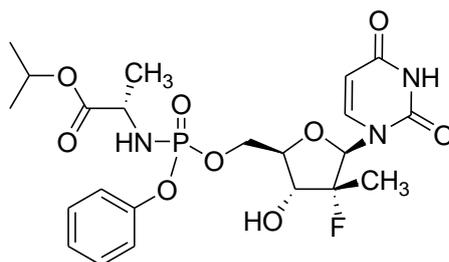
11. As against the 12 claims filed at the PCT stage, the Present Application was filed at the Indian Patent Office with only 10 claims (claims 7 and 8 as claimed in the PCT Application were deleted).

Amendment of claims

12. On December 26, 2011, the Applicant amended the claims for the first time. After the amendment, the total number of claims was 14. Further on November 27, 2015, the Applicant again amended the claims of the Present Application. After this amendment, the total number of claims was reduced to 5. These are the existing claims in the Present Application as set out below.

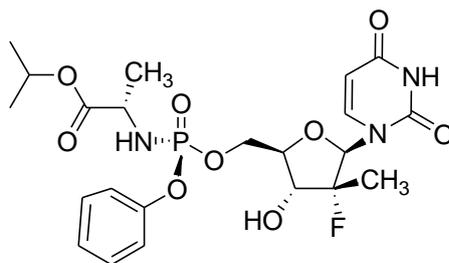
13. Claim 1 is independent claim and claims 2, 3, 4, 5 are dependent claims. These 5 claims are summarised below:

i) Claim 1 relates to (S)-2-{[(2R, 3R, 4R, 5R)-5-(2, 4-Dioxo-3, 4-dihydro-2H-pyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-ylmethoxy] phenoxy-phosphorylamino}-propionic acid isopropyl ester having the following structure



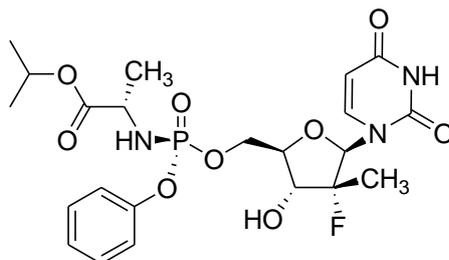
or a stereoisomer thereof.

ii) Claim 2 relates to the compound as claimed in claim 1 wherein the stereoisomer is (S)-isopropyl-2-(((S)-((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate having the following structure:



It is submitted that claim 2 claims a particular stereoisomer with the stereoisomer so identified at Phosphorus atom.

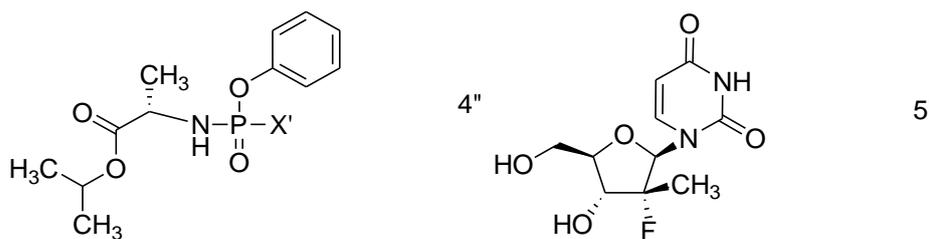
iii) Claim 3 relates to the compound as claimed in claim 1 wherein the stereoisomer is (S)-isopropyl-2-(((R)-((2R,3R,4R,5R)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate having the following structure :



It is submitted that claim 2 claims a particular stereoisomer with the stereoisomer so identified at Phosphorus atom.

iv) Claim 4 relates to a composition comprising the compound as claimed in any of the claims 1 to 3 and a pharmaceutically acceptable medium.

v) Claim 5 relates to a process for preparing the compound or a stereoisomer thereof as claimed in claim 1, said process comprising reacting a compound of 4'' (reproduced below) with a nucleoside analog 5' (reproduced below)



wherein X' is a leaving group.

IV. INCORRECT CLAIM OF PRIORITY DATE

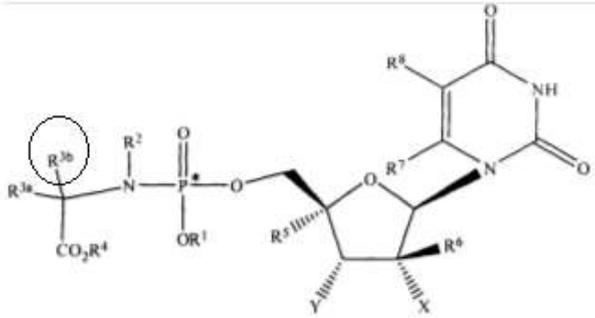
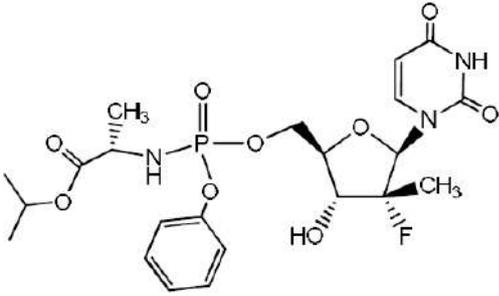
14. The Present Application was filed in India on October 20, 2009. The Present application claims the benefit of three priority dates namely,

- i) March 30, 2007 from US Provisional Application No. 60/909, 315 (hereinafter referred to as “US ’315”)
- ii) October 24, 2007 from US Provisional Application No. 60/982, 309 (hereinafter referred to as “US ’309”)
- iii) March 21, 2008 from US Patent Application No. 12/053, 015 (hereinafter referred to as “US ’015”)

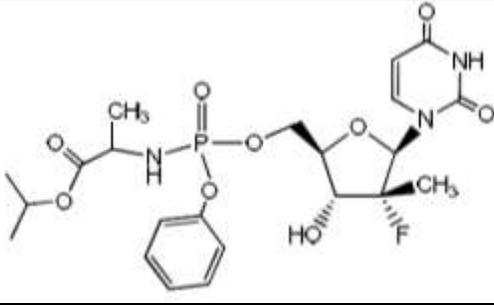
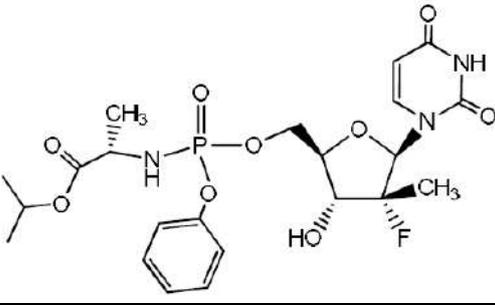
It is submitted that the Applicant has not specified as to which priority date corresponds to which claim. It is not open to the Applicant to claim 3 priority dates without specifying which claim(s) relates to which priority date.

Claims 1-5 cannot claim priority from 1st Priority date

15. It is submitted that the date claimed from US ’315 is not validly claimed for the entire set of claims in the Present Application. Claims 1-5 are not entitled to claim priority from US Provisional Application No ’315 i.e. 30.03.2007. The general structure and the specific structure of the compound in claim 1 is reproduced below for reference

General structure disclosed on page 8 of the US ’315 (on the basis of which 1 st Priority date is claimed)	Compound claimed in claim 1 of the Present Application
	

16. The support for the compound of claim 1 in the 1st priority date is to be allegedly found in compound IX-25-2, as provided on page 195 of complete specification of US ’315, on the basis of which the first priority date is claimed. It is submitted that the compound IX-25-2 in the US ’315 is not identical to compound of claim 1 of the Present Application and hence no priority can be claimed on the basis of US ’315.

Structure corresponding to compound IX-25-2 disclosed on page 195 of US '315	Compound claimed in claim 1 of the Present Application
	

17. It is submitted that the compound IX-25-2 at page 195 of the complete specification does not specify the stereochemistry of the asymmetric carbon linked to group R^{3b} (methyl group i.e. -CH₃). The compound of closest to claim 1 of the Present Application in fact follows from the selection from 3 lists of substituents or configuration. These lists have been identified as under-

- i) Selecting one structure from the lists II to XXXII (from page 97-661 of the complete specification);
- ii) Selecting one configuration for the asymmetric carbon from the list consisting of R and S;
- iii) Selecting one set from the list of 8 possible sets of substituents in each table

18. It is submitted that the compound of claim 1 cannot be derived directly and unambiguously either from the general formula or from compound IX-25-2 given in US '315. Therefore, it is not open to the Applicant to claim priority of US '315.

19. The Opponent would further like to rely on the Opposition proceeding against the corresponding patent number EP 2 203 462 granted to the Applicant in European Union. The Opponent submits that while granting the Auxiliary request 1 (a copy of which is annexed hereto as **Exhibit A**) in the opposition proceeding the said patent, the Opposition Division of the European Patent Office (order dated 31.10.2016 exhibited as **Exhibit B**) noted that the Auxiliary request 1 (that corresponds to claim 1 of the Present Application) was not entitled to priority from US '315. Therefore, claim 1 of the Present Application cannot claim priority from US '315.

20. It is submitted that the complete specification of US '315 also does not disclose the diastereomers of the compound in claim 1 of the Present Application. Therefore, the compounds as claimed in claims 2 and 3 have not been disclosed in US '315. The Opponent therefore submits that claims 2 and 3 cannot claim priority from US '315.
21. It is submitted that claim 4 relates to a composition comprising the compound of claims 1-3 and a pharmaceutically acceptable medium. As the compound in claim 1 cannot derive its priority from US '315, a composition formed from the compound thereof cannot claim priority from US '315.
22. Further, that claim 5 also cannot claim priority from US '315. The process for preparing the compound of claim 1, as claimed in claim 5 has not been described in US '315. It is therefore submitted that claims 1-5 of the Present Application cannot claim priority from US '315.
23. At the most, the Applicant may claim priority from US '309 (from which second priority date is claimed) or US '015 (from which the third priority date is claimed). However without identifying the corresponding claims to the priority applications, it is not allowed for the Applicant to claim priority from multiple priority dates.

V. SUMMARY OF GROUNDS CONSIDERED FOR OPPOSITION

24. The Opponent brings this opposition under the following grounds, amongst others, each of which are without prejudice to one another:
- i. Claims 1-5 the Present Application lack inventive step, and therefore fail under Sections 2(1)(j) and 2(1)(ja) of the Patents Act. Therefore, the Opponent brings this opposition under **Section 25(1)(e)**-that the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step, having regard to the matter published before the priority date in India or elsewhere in any document.
 - ii. Claims 1-3 of the Present Application do not satisfy the test of Section 3(d) of the Patents Act as the subject matter does not exhibit enhanced therapeutic efficacy.

Therefore, the Opponent brings this opposition under **Section 25(1) (f)** -that the subject of any claim of the complete specification is not an invention within the meaning of this Act.

- iii. The complete specification does not sufficiently and clearly describe the invention or the method in claims 1-5, by which it is to be performed. Therefore, the Opponent brings this opposition under **Section 25(1) (g)** -that that complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed.
- iv. The Opponent brings this opposition **under Section 25(1) (h)** of the Act-viz. that the Patent Applicant has failed to disclose the Controller information required by Section 8 or has furnished information which in any material particular was false to his knowledge.

VI. DETAILED GROUNDS

- i. **CLAIMS 1 TO 3 ARE OBVIOUS, DO NOT INVOLVE A TECHNICAL ADVANCE AND LACK INVENTIVE STEP AS DEFINED UNDER SECTION 2(1)(ja) AND THEREFORE HAVE TO BE REJECTED UNDER SECTION 25(1)(e) OF THE PATENTS ACT**

25. Section 2(1) (j) defines an “invention” as “a new product or process involving an inventive step and capable of industrial application.” Therefore, an alleged invention, in order to qualify for a patent, must satisfy the criteria of inventive step. Section 2(1)(ja) of the Patents Act defines an inventive step as “a feature of an invention that involves technical advance as compared to the existing knowledge ... and that makes the invention not obvious to a person skilled in the art”.

26. Sub-sections (j) and (ja) of Section 2(1) of the Patents Act thus require a Patent Applicant to show that the feature of the alleged invention involves a technical advance and that it is not obvious to a person skilled in the art. These requirements are laid down to ensure that patents, which result in a monopoly, are granted only to genuine inventions.

27. Section 25(1)(e) of the Patents Act provides a ground for opposition if the alleged invention is obvious and does not involve an inventive step having regard to matter published, as described in section 25(1)(b) of the Patents Act. The published matter to be considered under this provision includes matter published in India or elsewhere in any document before the priority date of the alleged invention. The Opponent submits that claims 1-3 of the Present Application lack an inventive step and therefore should be rejected.

28. At the time of the alleged invention, as will be explained below, the following were well known to persons skilled in the art:

- a. 2'-deoxy-2'-fluorocytidine was known as a potent anti-HCV agent and its *in vivo* uridine derivative was found to be inactive despite known to be possessing anti-HCV potency;
- b. ProTide approach/kinase bypass to activate an inactive nucleoside by phosphate prodrug formation was well known;
- c. L-alanine was well known as a preferred amino acid in ProTide approach;
- d. The stereoisomers arising from chirality at Phosphate in a Phosphoramidate were well known

A table representing the teaching of different prior art documents used in the following sections is produced below for ease of reference

Teaching →	2'-deoxy-2'-fluorocytidine is a potent anti-HCV agent and its in vivo uridine derivative is inactive	2'-deoxy-2'-fluorouridine-triphosphate has better half-life than 2'-deoxy-2'-fluorocytidine	Problem of non-permeability of 2'-deoxy-2'-fluorouridine through cell membrane may be overcome by making a monophosphate prodrug	ProTide approach can be used to activate an inactive nucleoside	L-alanine is a preferred amino acid in ProTide approach	Chirality at Phosphate in a Phosphoramidate gives two stereoisomers
Prior art ↓						
Clark <i>et al</i> (Exhibit C)	✓					
WO 2005/003147 (Exhibit D)	✓				✓	
Ma <i>et al</i> (Exhibit E)	✓	✓	✓			
McGuigan <i>et al</i> (Exhibit F)				✓		
Cahard <i>et al</i> (Exhibit G)				✓	✓	✓
Lee <i>et al</i> (Exhibit H)					✓	✓
Perrone <i>et al</i>					✓	

(Exhibit I)						
Sofia <i>et al</i> (Exhibit J)					✓	

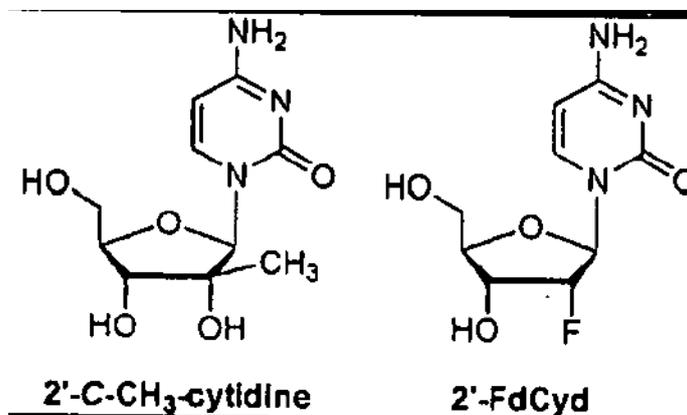
The ground of obviousness of the invention in the Present Application is discussed in detail below:

A. 2'-DEOXY-2'-FLUOROCYTIDINE WAS KNOWN AS POTENT ANTI-HCV AGENT AND ITS *IN VIVO* URIDINE DERIVATIVE WAS FOUND TO BE INACTIVE DESPITE KNOWN TO BE POSSESSING ANTI-HCV POTENCY.

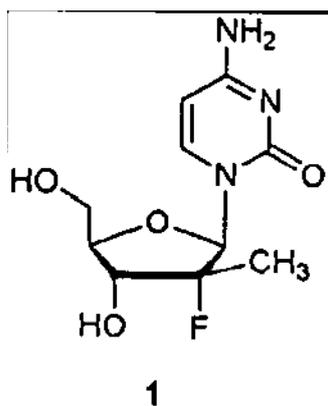
Clark et al (Published on web: 26.07.2005)

29. The Opponent relies on **Clark et al** titled "Design Synthesis, and Antiviral Activity of 2'-Deoxy-2'-fluoro-2'-C-methylcytidine, a Potent Inhibitor of Hepatitis C Virus Replication", J. Med. Chem. 2005, 48, pp. 5504-5508 (hereinafter referred as "Clark et al" and Exhibited as **Exhibit C**). This document was published in 2005, which is prior to the date of 1st Priority Application, and hence can be considered as a prior art. Clark et al notes that 2'-deoxy-2'-fluoro-2'-C-methylcytidine and 2'-C-methylcytidine were assayed in a sub genomic HCV replicon assay system and found to be potent and selective inhibitors of HCV replication (see abstract).

30. Clark et al points out that "several 2'-modified nucleoside analogues with potent inhibitory activity against the HCV NS5B polymerase have been identified. Among the most potent compounds in this class are 2'-deoxy-2'-fluorocytidine (2'-FdCyd) and 2'-C-methyl cytidine nucleosides." (see internal page 5504, RHS column, para 1, lines 2-6). The structure of these identified potent compounds are reproduced below for easy reference (see Figure 1 on internal page 5504)



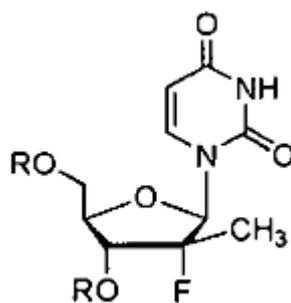
31. Clark *et al* further notes that “despite potent HCV inhibition of 2'-FdCyd, its therapeutic potential as an antiviral agent is diminished due to lack of selectivity between the cells and the target agents” (see internal page 5504, RHS column, para 1, lines 7-10). Hence, the authors of Clark *et al* worked towards synthesis and analysis of biological activity of a novel nucleoside analogue - 2'-fluoro-2'-C-methylcytidine (**compound 1**). The structure of **compound 1** is reproduced below for reference (see Figure 1, internal page 5504)



32. The novel nucleoside analogue, viz. compound 1, was tested for anti-HCV activity in both a cell-based quantitative real-time RT-PCR assay and surrogate bovine viral diarrhea virus (BVDV) assays. On comparison of compound 1 with 2'-deoxy-2'-fluorocytidine (2'-FdCyd) and 2'-C-methyl cytidine, **compound 1** demonstrated similar potency as 2'-FdCyd in the HCV replicon assay. Further, “dynamic profiling of the cell growth in this replicon assay revealed no cytostasis for compound 1 at the HCV replicon EC₉₀ value.” (see internal page 5506, RHS column, para 1, lines 3-5). However, much like 2'-FdCyd, but unlike 2'-C-methyl cytidine, **compound 1 was inactive** in BVD assays. (see internal page 5506, RHS column, para 1). The table comparing the anti-HCV activity and cellular toxicity of compound 1 with 2'-FdCyd, but unlike 2'-C-methyl cytidine is reproduced below for reference (see internal page 5506):

compound	cpBVDV ^a (MDBK cells)		HCV replicon ^b	
	EC ₉₀ (μM) ^b	CC ₅₀ (μM)	EC ₉₀ (μM)	CC ₅₀ ^c (μM)
1	>100	>100	5.40 ± 2.6	>100
9	>100	>100	>100	>100
2-C-MeCyd	2.30 ± 0.1	>100	19.0 ± 5.7	>100
2-FdCyd	>100	>100	6.50 ± 1.6	>100

33. Clark *et al* also states that “the degradation of enzymes cytidine deaminase (CDA) and deoxycytidine monophosphate deaminase (dCMP-DA) are responsible for *in vivo* metabolic conversion of cytidine or cytidine monophosphate to uridine. **To facilitate future *in vitro* studies of compound 1, 2`-deoxy-2`-fluoro-2`-C-methyluridine (9) was prepared**”.(see internal page 5506, LHS column, para 2, lines 4-11). The structure of compound 2`-deoxy-2`-fluoro-2`-C-methyluridine (**compound 9**) is being reproduced below for reference-



Compound 9

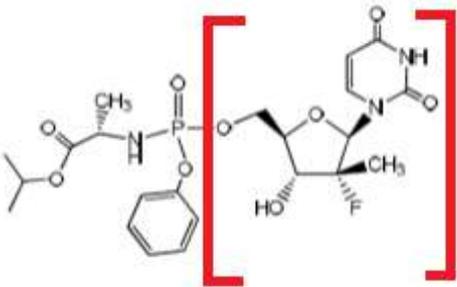
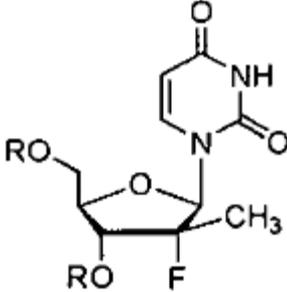
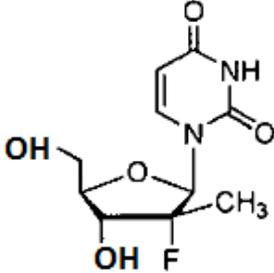
34. Clark *et al* also tested compound 9 for anti-HCV activity in both a cell-based quantitative real-time RT-PCR assay and surrogate bovine viral diarrhea virus (BVDV) assays. However, compound 9 demonstrated neither any activity nor cytotoxicity in any assay. (see internal page 5506, RHS column, para 1, lines 9-10). Compound 9 is disclosed to have an EC₉₀ of greater than 100 μM and a low cytotoxicity CC₅₀ (refer Table 2, page 5506, RHS column).

35. The Opponent submits Clark *et al* teaches that *in vivo* metabolism was converting the cytidine derivate - compound 1, into uridine. In light of this, Clark *et al* was motivated to make a uridine derivative of **compound 1** in order to test whether such a compound might show better activity. Hence, if compound 1 is envisaged for *in vivo* studies, the skilled person would also consider its *in vivo* metabolite i.e. compound 9 for the development of an anti-viral agent. This applies all the more because **compound 9** (and its *in vivo* precursor-compound 1) are described in Clark *et al* to be not cytotoxic, which is an obvious prerequisite for its use as medicament.

36. Therefore, in view of the foregoing, it is submitted that the person skilled in the art, based on teachings of Clark *et al* would identify compound 9 to be the starting compound of interest and would try to look into alternative approaches to make the compound 'active'. In

other words, the technical problem to be solved would now be as to how to activate the *in vivo* derivative of a compound 1 which is known to possess high anti-HCV potency.

37. In fact, it may be seen that compound 9 of Clark *et al* and compound of claim 1 of the Present Application have identical nucleoside. A tabular comparison of compound of claim 1 of the Present Application and compound 9 disclosed in Clark *et al* is produced below-

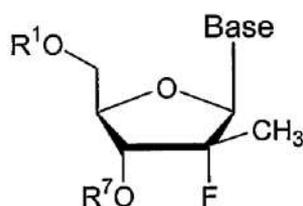
Compound in claim 1 of the Present Application	Compound 9 disclosed on internal page 5506 of Clark <i>et al</i>
 <p style="text-align: center;">Nucleoside</p>	 <p style="text-align: center;"> $b \left\{ \begin{array}{l} 8: R = Bz \\ 9: R = H \end{array} \right.$ </p> <p style="text-align: center;">Compound 9, where R is H is represented below</p> 

WO 2005/003147 (Published: April 21, 2004)

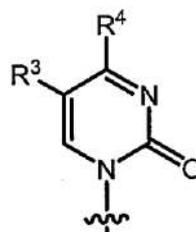
38. The Opponent relies on **WO 2005/003147** (hereinafter referred to as “WO ’147” and annexed hereto as **Exhibit D**), titled “Modified fluorinated nucleoside analogues” dated

April 21, 2004 and bearing a priority date of May 30, 2003. As the priority date of WO '147 is earlier than the priority date of the Present Application, it is a prior art document.

39. WO '147 relates to compounds for treating Flaviviridae virus, in particular for HCV (see "field of invention" on page 1 of the complete specification). The embodiment given at page 39, lines 3-9 of WO '147, discloses in the twelfth embodiment a compound, including its pharmaceutically acceptable salt **or its prodrug** of the embodiment. The twelfth embodiment is reproduced below:



Wherein the base is a structure as reproduced below:



Where R¹, R³ and R⁷ are H and R⁴ is NH₂ or OH.

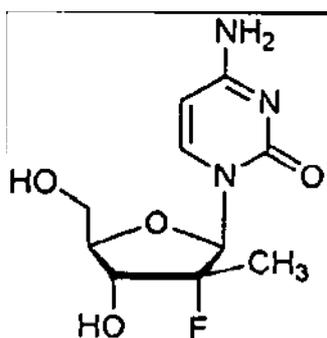
40. The twelfth embodiment in WO '147 also clearly includes a "prodrug". WO '147 indicates that, "*The invention also contemplates other embodiments, wherein the **prodrug of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L)** includes biologically cleavable moieties at the 3' and/or 5' positions. Preferred moieties are natural or **synthetic D or L amino acid esters**, including D or L- valyl, though preferably **L-amino acid esters**, such as L-valyl, and alkyl esters including acetyl. Therefore, this invention specifically includes 3'-L or D-amino acid ester and 3',5'-L or D-diaminoacid ester of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L)nucleosides, preferably L-amino acid, with any desired purine or pyrimidine base, wherein the parent drug optionally has an EC₅₀ of less than 15 micromolar, and even more preferably less than 10 micromolar...*". (see WO '147 at internal page 60, placitum 11-20)

41. Therefore, WO '147 provides **prodrug** of the identified nucleoside as a solution to the problem of non-availability of an active form of 2'-deoxy-2'-fluoro-2'-C-methyluridine, as recognised in Clark *et al.* The alleged solution provided by the Present Application is the phosphoramidate prodrug of β -D-2'-deoxy-2'-fluoro-2'-C-methyluridine wherein the phosphoramidate moiety is the phenyl phosphoramidate of L-alanine isopropyl ester.

Ma et al (Published: August 13, 2007)

42. The Opponent further relies on the publication by Ma *et al.*, titled “*Characterization of the Metabolic Activation of Hepatitis C Virus Nucleoside Inhibitor β -D-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) and Identification of a Novel Active 5'-Triphosphate Species*”, The Journal of Biological Chemistry, Vol. 282, No. 41, pp. 29812–29820, (hereinafter referred as “Ma *et al.*” and exhibited as **Exhibit E**) published on October 12, 2007. This document can be considered as a valid prior art considering the Applicant has wrongly claimed the priority date of March 30, 2007 and the effective date of priority should be October 24, 2007, as discussed in section titled ‘incorrect claim of priority’.

43. Ma *et al.* has described the incubation of 2'-Deoxy-2'-fluoro-2'-C-methylcytidine (cited as **PSI-6130** in Ma *et al.* and identical to **compound 1** from Clark *et al.*) with human hepatocytes which results into formation of the phosphate of its uridine analogue, **RO2433-TP**. The structure of **PSI-6130** is produced below for reference:



1

44. Ma *et al.* then “*determined whether the PSI-6130-derived uridine analog RO2433 could inhibit HCV replication targeting NS5B polymerase. Huh7 cells containing a subgenomic genotype 1b Con1 strain HCV replicon were incubated with RO2433 or PSI-6130 for 72 h,*

and dose-dependent inhibition of luciferase reporter activity was determined. RO2433 did not inhibit the HCV replication in the HCV subgenomic replicon system at concentrations up to 100 μM , whereas PSI-6130 inhibited HCV replication with a mean IC_{50} of 0.6 μM under the same assay conditions. **The lack of potency in the replicon could be related to inefficient compound phosphorylation.** To address whether the triphosphate of RO2433 directly inhibits the HCV RNA polymerase, the RNA synthesis activity of the native membrane-associated HCV replication complexes isolated from the same replicon cells was tested in the presence of RO2433-TP. **RO2433-TP inhibited the RNA synthesis activity of HCV replicase with a mean IC_{50} of 1.19 μM , whereas PSI-6130-TP inhibited HCV replicase with a mean IC_{50} of 0.34 μM .** RO2433-TP also inhibited the RNA synthesis activity of the recombinant HCV Con1 NS5B on a heteropolymeric RNA template derived from the 3-end of the negative strand of the HCV genome with an IC_{50} of 0.52 μM and K_i of 0.141 μM , as compared with an IC_{50} of 0.13 μM and K_i of 0.023 μM for PSI-6130-TP under the same assay conditions. **These results established that both RO2433-TP and PSI-6130-TP are intrinsically potent inhibitors of RNA synthesis by HCV polymerase.**” (see internal page 29815, RHS column, para 1, lines 4-31)

45. Ma *et al* also determined the half-life of PSI-6130 TP and RO 2433-TP. Here half-life is defined as the time needed for the triphosphates to be reduced to 50% that of the highest level of triphosphates after extra cellular parent compound removal (see internal page 29818, RHS column, table 4). The table is reproduced for reference below-

TABLE 4

Intracellular half-life of PSI-6130-TP and RO2433-TP

The half-life ($t_{1/2}$) values were calculated by nonlinear fitting of intracellular triphosphate concentrations to a single phase exponential decay equation. $t_{1/2}$ was defined as the time needed for the triphosphates to be reduced to 50% that of the highest level of the triphosphates after extracellular parent compound removal. Data shown are the mean \pm S.D. of values of four independent experiments using hepatocytes from four donors.

$t_{1/2}$, h (mean \pm S.D. ($n = 4$))	
PSI-6130-TP	RO2433-TP
4.7 \pm 0.6	38 \pm 16

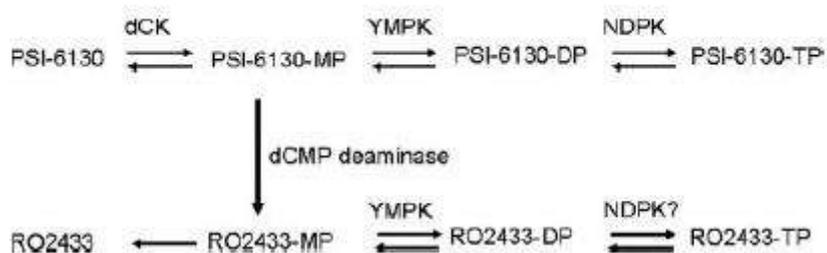
46. Therefore, Ma *et al* concluded that **“The longer intracellular half-life of RO2433-TP may have pharmacologic relevance for maintaining more constant concentrations of the**

antiviral triphosphate over the dosing period in clinical studies” (see internal page 29819, RHS column, para 3, lines 27-31)

Problem of non-permeability of RO2433 through cell membrane may be overcome by making a monophosphate prodrug

47. Ma *et al* further notes that “*Despite the intrinsic potency of RO2433-TP against HCV polymerase, RO2433 was not active in the HCV replicon system at concentrations up to 100 μM. RO2433 was either not phosphorylated in the replicon cells or could not penetrate the cell membrane. However, RO2433, when formed intracellularly from radiolabeled PSI-6130, dissociated rapidly across the cell membrane with a half-life faster than 30 min. Therefore, RO2433 is most likely not efficiently phosphorylated to form RO2433-MP. Similarly, the uridine analog of the HCV replication inhibitor R1479 (4-azidocytidine) was inactive in the replicon system. However, when delivered as a monophosphate prodrug, 4-azidouridine could be converted into a potent inhibitor of HCV replication, demonstrating that a block of monophosphate formation resulted in lack of antiviral activity of 4-azidouridine. Assuming a likely block of RO2433 phosphorylation to its monophosphate, RO2433-MP in human hepatocytes was most likely formed through the deamination of PSI-6130-MP by the cellular dCMP deaminase and subsequently further phosphorylated to RO2433-DP and -TP by uridine/ cytidine monophosphate kinase and possibly nucleoside* Despite the intrinsic potency of RO2433-TP against HCV polymerase, RO2433 was not active in the HCV replicon system at concentrations up to 100 μM. **RO2433 was either not phosphorylated in the replicon cells or could not penetrate the cell membrane.** However, RO2433, when formed intracellularly from radiolabeled PSI-6130, dissociated rapidly across the cell membrane with a half-life faster than 30 min. Therefore, RO2433 is most likely not efficiently phosphorylated to form RO2433-MP. Similarly, the uridine analog of the HCV replication inhibitor R1479 (4-azidocytidine) was inactive in the replicon system. However, when delivered as a monophosphate prodrug, 4-azidouridine could be converted into a potent inhibitor of HCV replication, demonstrating that a block of monophosphate formation resulted in lack of antiviral activity of 4-azidouridine. **Assuming a likely block of RO2433 phosphorylation to its monophosphate, RO2433-MP in human hepatocytes was most likely formed through the deamination of PSI-6130-MP by the cellular dCMP deaminase and subsequently further phosphorylated to RO2433-DP and -TP by**

uridine/cytidine monophosphate kinase and possibly nucleoside diphosphate kinase. The proposed metabolic pathway for PSI-6130 is illustrated in Fig. 7. Using a primer-directed nucleotide incorporation assay mediated by HCV NS5B, we demonstrated that the incorporation of both PSI-6130-MP and RO2433-MP resulted in the complete blockage of the next nucleotide incorporation similar to that of the obligatory chain terminator 3-dCMP and 3-dUMP (Fig. 3, B and C). Therefore, the 2-C-methyl-2-Fluoro motif resulted in functional chain terminators on the respective uridine and cytidine analogs. It has been proposed that the chain termination activity of 2-C-methyl nucleotide analogs is related to a steric clash of the 2-methyl group with the ribose of the next incoming nucleotide substrate based on modelling of the NS5B initiation complex from bacteriophage 6 RNA-dependent RNA polymerase and NS5B crystal structures. Similar steric hindrance could occur with PSI- 6130-TP and RO2433-TP after incorporation due to the presence of the 2-C-methyl group” (see internal page 29819 LHS column, para 3, lines 39-59 and RHS column, para 1 and 2, lines 1-18). The proposed metabolic pathway of PSI-6130 is reproduced below for reference.



48. The Opponent submits that although the uridine derivative (**compound 9** of Clark *et al*) which had been synthesised because it was believed to be the *in vivo* metabolite of the cytidine analogue of **compound 1** was inactive, Ma *et al* motivates a person skilled in the art to identify the phosphorylated forms of both cytidine and uridine as having anti-viral activity and therefore possessing the realistic potential as anti-viral agents.

b. PROTIDE APPROACH/KINASE BYPASS TO ACTIVATE AN INACTIVE NUCLEOSIDE BY PHOSPHATE PRODRUG FORMATION WAS KNOWN

McGuigan *et al* (Published: 1994)

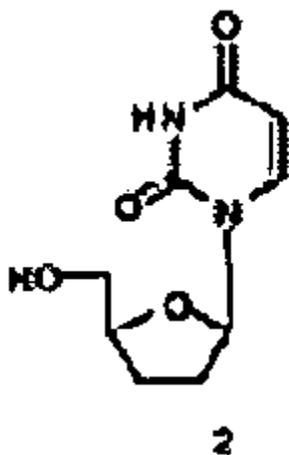
49. The Opponent relies on a document titled “Certain Phosphoramidate derivatives of dideoxy uridine (ddU) are active against HIV and successfully by-pass thymidine kinase” authored

by McGuigan *et al*, FEBS Letters 351 (1994) 11-14 (hereinafter referred to as McGuigan *et al* and exhibited as **Exhibit F**). McGuigan *et al* discovered that “*certain phosphate trimer derivatives of the inactive nucleoside analogue, dideoxy uridine (ddU) are inhibitors of HIV replication at μ M levels...certain phosphoramidate derivatives retain their activity in thymidine kinase deficient cells...The increased structural freedom in drug design that this allows may simulate the discovery of improved therapeutic agents.*” (see abstract)

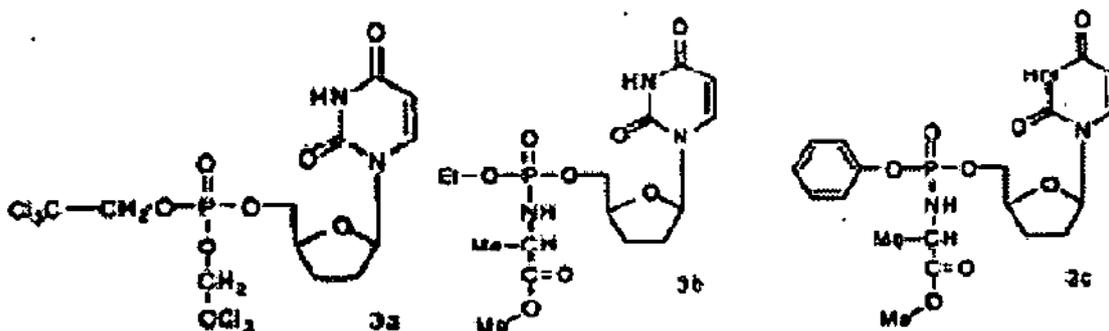
50. McGuigan *et al* further notes that “*We and others have pursued a masked phosphate approach in an attempt to improve on the therapeutic potential of the parent nucleoside analogues. In this approach, inactive phosphate derivatives of the nucleoside analogues are designed to penetrate the cell membrane and liberate the bio-active nucleotides intracellularly. Masking of the phosphate group is necessary on account of the extremely poor membrane penetration by the polar (charged) free nucleotide. One mechanism by which masked phosphates may lead to enhanced selectivity of action arises from what we have termed ‘kinase bypass’*” (see internal page 11, LHS column, para 2, lines 8-19). It adds that, “*We have recently reported on the success of this “kinase by-pass” strategy with several highly modified 3’-substituted nucleosides.* (see internal page 11, RHS column, lines 11-13).

It also states that, “*We now report the success of this approach with the simple nucleoside analogue dideoxy uridine (ddU,2). This is essentially inactive against HIV, but judicious phosphorylation leads to introduction of significant, selective anti-viral effect.*” (see internal page 11, RHS column, lines 14-18)

51. McGuigan *et al* worked on a parent nucleoside identified as compound **(2)**. The structure of compound **(2)** is reproduced below for reference (see internal page 13, LHS column):



52. McGuigan *et al* tested the above compound and its corresponding masked phosphates for their ability to inhibit replication of HIV-1 in C8166 cells, and in the thymidine kinase deficient [JM] cells. The masked phosphates of compound 2, particularly 3a, 3b and 3c are reproduced below- (see internal page 13, LHS column)



53. McGuigan *et al* notes that “...we have found that aryloxy phosphoramidates are especially potent phosphate blocking groups for AZT, and appear to release the free nucleotides within cells, on the basis of data in thymidine kinase-deficient cells...” (see internal page 13, RHS column, para 2, lines 2-6). Further, McGuigan states that, “ the parent nucleoside (2) is active only at the highest concentration tested... The bis (trichloroethyl) phosphate (3a) is approximately 5-10 times more active in each assay. On the other hand, the simple phosphoramidate (3b) is devoid of antiviral activity in this assay...However, the aryloxy phosphoramidate (3c) is a potent agent, being approximately 50-times more active than the parent nucleoside analogue” (see internal page 13, RHS column, para 2, lines 2-6 and line 12-25).The table depicting the activity of the parent compound and the masked phosphates is given under for reference (see Table 2. Internal page 14, LHS column)

Table 2
Anti-HIV1 activity of nucleoside and nucleotide analogues

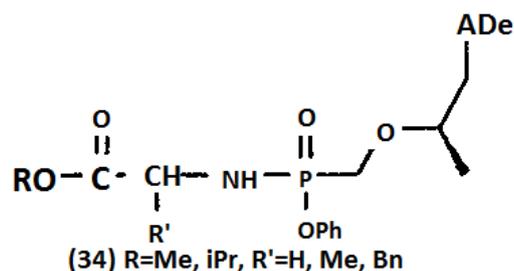
Compound	C8166		JM	
	ED ₅₀	CC ₅₀	ED ₅₀	CC ₅₀
2	200	> 1,000	1,000	> 1,000
3a	40	500	80	400
3b	> 1,000	> 1,000	400	400
3c	4	400	20	500

54. Therefore, as is evident from the table above, compound 3c showed the most activity. Further, McGuigan *et al* teaches that “*the antiviral activity of certain phosphate derivatives of the inactive nucleoside ddU. We note that aryloxy phosphoramidates are particularly efficacious, and attribute the introduction of activity to kinase by-pass.*” (see internal page 14, LHS column, para 2, lines 5-9).

Cahard *et al* (Published: 2004)

55. The Opponent further relies on a document titled “Aryloxy Phosphoramidate Triester as Pro-Tides” by Cahard *et al*, Mini-Reviews in Medicinal Chemistry, 2004, 4, pp. 371-381 (hereinafter referred as “Cahard *et al*” and exhibited as **Exhibit G**). The document was published in 2004, which is prior to the date of earliest priority of the Present Application. Therefore, Cahard *et al* qualifies as a prior art document. Cahard *et al* discusses the development of aryloxy phosphoramidate triesters as an effective protide motif for the intracellular delivery of charged bioactive anti-viral nucleoside monophosphates (see abstract).

56. Cahard *et al* notes that the authors “*sought to find a universal phosphate delivery motif that could be applied to a range of nucleosides*” (see internal page 374, LHS column, para 1, lines 2-6). It further states that “*one of the most remarkable demonstrations of effectiveness of the aryloxy phosphoramidate approach came from our application of the technology to the dideoxydihydro purine d4A [34]*”. (see internal page 374, RHS column, para 2, Lines 16-20). The structure of compound 34 is being reproduced below for reference (see internal page 375, LHS bottom)



It may be noted that compound 34 has a (2S)-isopropyl 2-(((phenoxy) phosphoryl) amino) propanoate moiety.

57. Cahard *et al* also pointed out that the Gilead group who has been active in the commercialisation of ANPs (Acyclic nucleoside phosphonates) have reported that aryloxy phosphoramidates (compound 34) of PMPA (tenofovir) are highly active anti-retrovirals. Cahard *et al* reached the same conclusion for PMPA and the closely related PMEA (adefovir) (see internal page 375, RHS column, para 3, lines 33-36). The Opponent submits that at least as early as 2004, a person skilled in the art would be aware that aryloxy phosphoramidates of an anti-viral (such as PMPA) show high activity.

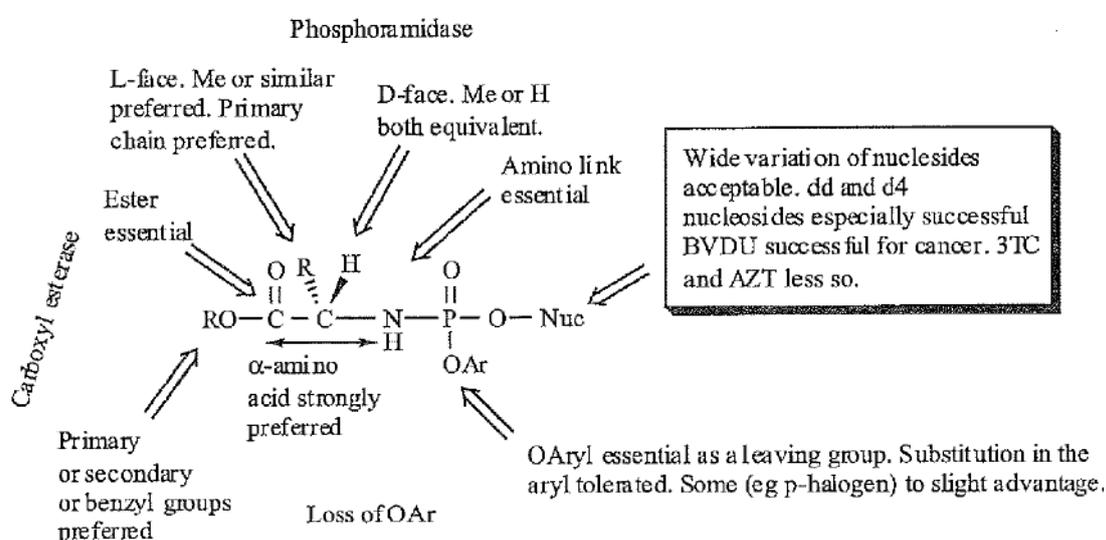
58. In the section “Conclusions” (see paragraph bridging pg. 379-380) of Cahard *et al* points out that the “*aryloxy phosphoramidate approach has emerged, along with SATE, CycloSAL, aryl phosphate diester and others as viable method for intracellular delivery of free monophosphates of a range of nucleoside analogues...the approach works well for d4T(stavudine) and a range of dd and d4 nucleosides. D4A (2'-3'-didehydro-2', 3'-dideoxyadenosine) is a particularly dramatic example with >1,000 fold boosts in potency on phosphoramidate formation...carbocyclic L-d4A has shown potency enhancements of almost 10,000 on phosphoramidate formation. We believe that this has reached the levels that we originally described over 10 years ago as 'kinase bypass', wherein an inactive nucleoside is activated by phosphate pro-drug formation. This suggests the prudence of pro-tide synthesis on a range of nucleoside analogues, and particularly not just those selected as active in initial screens; the inactivity of other structures may simply correspond to poor initial phosphorylation, which may now be by-passed with pro-tides*” (see internal page 380, LHS column, para 1, lines 2-18).

59. Therefore, Opponent submits that a person having ordinary skill in the art would consider aryloxy phosphoramidate of Cahard *et al* to be suitable for the provision of a phosphoramidate prodrug of compound 9 of Clark *et al*.

c. L-ALANINE WAS KNOWN AS A PREFERRED AMINO ACID IN PROTIDE APPROACH

Cahard *et al* (Published: 2004)

60. The Opponent relies on Cahard *et al* (**Exhibit G**), in particular, Figure 4 on pg. 380 which illustrates the preferred moieties of such a “viable” aryloxy phosphoramidate. The figure has been reproduced below:



It may be seen from the above figure that:

- the O Aryl substitution at the phosphorus atom is “*essential*”. The Opponent submits here that the claimed compounds of the Present Application have a OPh at this site);
- the α -amino acid is “*strongly preferred*”. According to pg. 380, LHS column at para 2 “alanine remains a good choice of amino acid”. It is submitted that the claimed compounds of the Present Application have an L-alanine moiety;
- the esterification of the amino acid moiety is “*essential*”. Primary alkyl, secondary alkyl or benzyl groups are “*preferred*”. It is submitted that one may look at the compounds of claims 1-3 of the Present Application have an iso-propyl ester moiety.

61. Hence, Fig. 4 and the corresponding general statements provide a strong motivation to screen various aryloxy phosphoramidate nucleoside prodrugs by varying the moieties in the

phosphoramidate part as taught by Cahard *et al*, bearing in mind that not only active parent nucleoside analogues but also those parent nucleoside analogues described in the art as inactive should be considered in the screening.

62. Further, Cahard *et al* states that “alanine has risen as the aminoacid of choice” with alkyl phosphoramidates. (see page 372, LHS, para 1, lines 1-3 and page 376 RHS, para 2, lines 17-19).

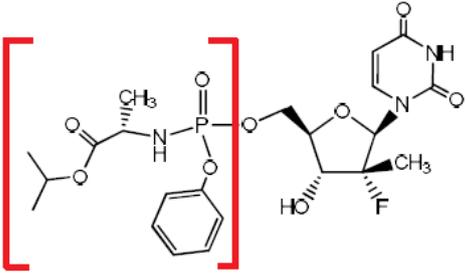
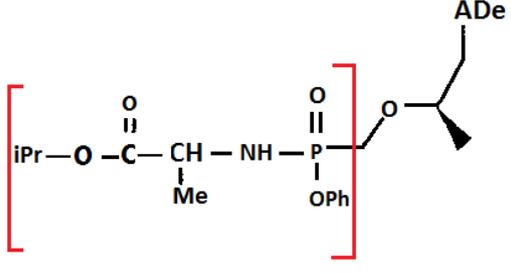
63. Cahard *et al* teaches, “Indeed, recent work from our labs in collaboration with GLaxoSmithKilne on the carbolylic L-d4A has shown potency enhancements of almost 10,000 on phosphoramidate formation. This suggests the prudency of pro-tide synthesis on a range of nucleoside analogues, and particularly not just those selected as active in initial screens; the inactivity of other structures may simply correspond to poor initial phosphorylation, which may now be by-passed with pro-tides.” (see internal page 380, LHS column, lines 4-20).

64. It is further concluded from Cahard *et al* that, “Alanine remains a good choice of amino acid, although the achiral α,α -dimethylglycine is a good alternative.” (see internal page 380, LHS column, lines 24-25). Cahard *et al* also teaches the, “ the issue of phosphate stereochemistry is worth considering” (see internal page 380, LHS column, lines 34-35)

65. The Opponent submits that, since Cahard *et al* clearly teaches that the protide approach is applicable to both nucleosides and acyclic nucleoside phosphonates, a person having ordinary skill in the art would be directly led to the phosphoramidate moiety of the compound of claim 1 of the Present Application. Therefore, a person having ordinary skill in the art (PHOSITA), starting with compound 9 of Clark *et al* would have considered applying the teachings of Cahard *et al* with (2S)-isopropyl 2-(((phenoxy)phosphoryl)amino) propanoate) of compound 34, and would have arrived at compounds of claim 1 of the Present Application with no need of inventive skill.

66. The prodrug moiety of the compound of claim 1 of the Present Application is compared with compound 34 (where R=iPr and R'=Me) disclosed in Cahard *et al*, in the table below.

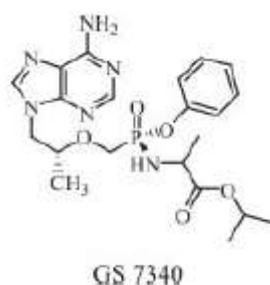
Compound of claim 1 of Present	Compound 34 disclosed in Cahard <i>et al</i>
---------------------------------------	---

Application	at page 375 (when R=iPr and R'=Me)
 <p data-bbox="263 548 470 582">Prodrug moiety</p>	

Lee et al (Published: 2005)

67. It is submitted that the Applicant at internal page 7, *placitum* 7-18 of the complete specification, identifies first phosphorylation bypass as a problem to effective biological activity of a nucleoside. In the same paragraph, the Applicant states that “*nucleoside phosphoramidate prodrugs have been shown to be precursors of the active nucleoside triphosphate and to inhibit viral replication when administered to viral infected whole cells*”. In this regard, the Applicant relies on paper authored by Lee, W.A., *et al.*, titled “*Selective Intracellular Activation of a Novel Prodrug of the Human Immunodeficiency Virus Reverse Transcriptase Inhibitor Tenofovir Leads to Preferential Distribution and Accumulation in Lymphatic Tissue*”, *Antimicrobial Agents and Chemotherapy*, 2005, 49, pp. 1898-1906 (hereinafter “**Lee et al**” and exhibited as **Exhibit H**).

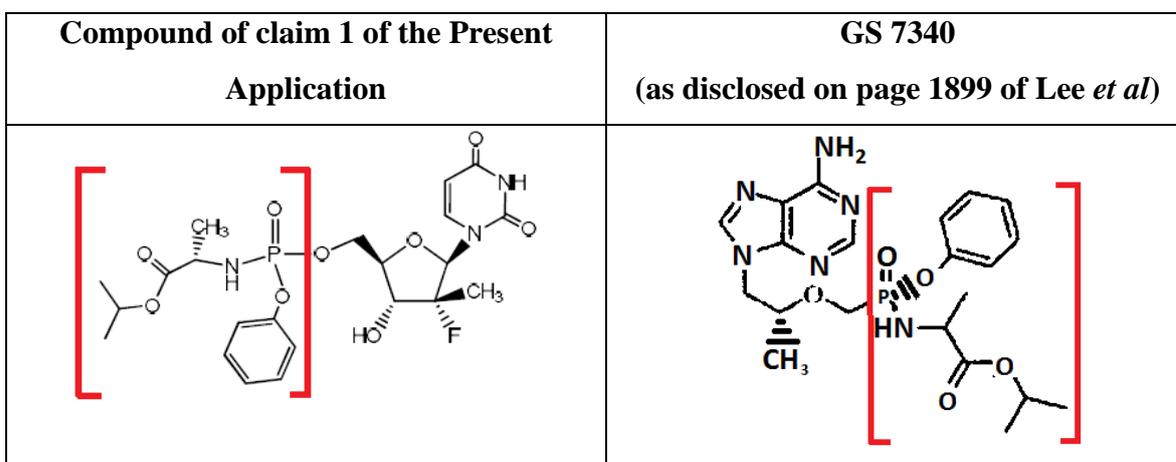
68. Lee *et al* discloses “GS 7340”- an isopropylalaninyl monoamidate phenyl monoester prodrug of an anti-HIV agent tenofovir. It states that this prodrug demonstrates extreme potent *in-vitro* activity (see internal page 1899, LHS, para 1, *lines* 1-3). The structure of GS 7340 is reproduced below for reference:



69. Lee *et al* teaches that isopropylalaninyl monoamidate phenyl monoester moiety may be used to overcome the problem of first phosphorylation bypass. It is further submitted that Lee *et al* demonstrates that, "The amidate prodrug GS 7340 was designed to overcome the permeability limitations of tenofovir by masking the dianion with a neutral promoity and increasing the plasma stability of the prodrug relative to its intracellular stability" (see internal page 1903, LHS para 2, lines 41-45). Lee *et al* states that "The principal advantage of this approach with nucleosides is the ability to deliver the nucleoside monophosphate into the cell, thereby removing the potential rate-limiting step in the formation of nucleoside triphosphate" (see internal page 1903, RHS column, lines 43-47). Therefore, Lee *et al* implies that (2S)-isopropyl 2- (((phenoxy) phosphoryl) amino) propanoate) is a suitable solution for the problem of cell permeability.

70. Hence a person having ordinary skill in the art, starting from compound 9 of Clark *et al* would apply the teachings of Lee *et al* to arrive at the compound of claim 1 of the Present Application. Similarly applying the teachings of WO '147 and Cahard *et al* with Lee *et al*, a person skilled in the art would arrive at the compound of claim 1 of the Present Application.

71. On comparison of GS 7340 with the compound claimed in the Present Application, one would find that both the compounds have the isopropylalaninyl monoamidate phenyl monoester moiety in common. The compound in the Present Application and GS 7340 as reproduced next to each other below:



Perrone et al (Published: November, 2006)

72. The Opponent relies on a paper titled “*Application of the Phosphoramidate ProTide Approach to 4’-Azidouridine Confers Sub-micromolar Potency versus Hepatitis C Virus on an Inactive Nucleoside*” by Perrone et al (J. Med. Chem. 2007, 50, pp. 1840-1849) (hereinafter referred as “Perrone et al” and exhibited as **Exhibit I**). This document was received for publication on November 17, 2006 and was published on web on March 17, 2007. The date of publication of Perrone et al is prior to the earliest priority date of the Present Application. Therefore, this document may be considered as prior art.
73. Perrone et al reports that “*4’-azidocytidine was discovered as a potent inhibitor of HCV replication in cell culture. The corresponding 5’-triphosphate was described as a competitive inhibitor of cytidylate incorporation by HCV polymerase and a potent inhibitor of native, membrane-associated HCV replicase in vitro...the first phosphorylation step to produce the 5’-monophosphate has often been found to be the rate-limiting step in the pathway to intracellular nucleotide triphosphate formation, suggesting that nucleoside monophosphate analogues could be useful antiviral agents...nucleoside monophosphate...also show poor membrane permeation*” (see internal page 1840, LHS para 4 and RHS para 3, lines 9-15).
74. Further, Perrone et al notes that “*the first phosphorylation step to produce 5’-monophosphate has often been found to be rate limiting step in pathway to intracellular nucleoside triphosphate formation, suggesting that nucleoside monophosphate analogues could be useful antiviral agents. However, as unmodified agents, nucleoside monophosphates are unstable in biological media and they also show poor membrane permeation because of associated negative charges at physiological pH*” (see internal page 1840, RHS column, para 3, lines 9-17).
75. Further, the amino acid ester and the aryl moiety have been identified as the two masking groups in the prodrug (see internal page 1840, RHS para 4, *placitum* 32-36). Perrone et al further states that “*L-alanine derivatives represented a series of active antiviral phosphoramidates (11-17). Low or sub-molecular activity was noted in marked contrast to the inactive nucleoside parent (1). The tert-butyl ester (16) was the least active of the series...The isopropyl ester (15) showed high potency and represented one of the most*

active phosphoramidates prepared. Similarly the 2-butyl ester (14) was highly active...in contrast to the previous observations with other nucleoside analogues. Together with benzyl analogue (17), these three esters provided the most potent compounds of the HCV replication inhibitors in the L-alanine series, all having μM inhibition of HCV. The antiviral activity of these three phosphoramidates was exceptional if compared to the parent compound 1. $EC_{50} > 100\mu\text{M}$), providing strong support for the notion of ProTide-mediated kinase bypass.” (see internal page 1842, RHS column, para 2, lines 4-16 and page 1843, LHS column, para 1, lines 1-5). Compound 1, identified in this paragraph is azidouridine. The relevant portions of table showing biological activity of the L-alanine phosphoramidates in the HCV replicon assay as reported in Perrone *et al* (see internal page 1843 LHS, table 1) is reproduced below:

Table 1. Anti HCV Activity and Cytotoxicity Data for (1) and Phenyl Phosphoramidate Nucleotide Analogues

compound	amino acid	ester	EC_{50} (μM)	CC_{50} (μM)
11	L-Ala	Me	3.1	> 100
12	L-Ala	Et	1.3	> 100
13	L-Ala	Bu	1.2	> 100
14	L-Ala	2-Bu	0.63	> 100
15	L-Ala	iPr	0.77	> 100
16	L-Ala	tBu	5.1	> 100
17	L-Ala	Bn	0.61	> 100

76. Perrone *et al* concludes by stating that “A series of phosphoramidate ProTides of 4'-azidouridine were prepared and evaluated as inhibitors of HCV replication *in vitro*. The phosphoramidate approach provided novel compounds with highly increased potency in the replicon assay when compared to the inactive parent compound, corresponding to boosts in anti-HCV potency of >450-fold. All phosphoramidates tested were non-toxic in the replicon assay ($CC_{50} > 100\mu\text{M}$)...This report demonstrates the ability of ProTide approach to successfully bypass the rate limiting initial phosphorylation of a ribonucleoside analogue and thus confer significant antiviral activity on an inactive parent nucleoside.” (see internal page 1844, lines 14-20 and 26-30)

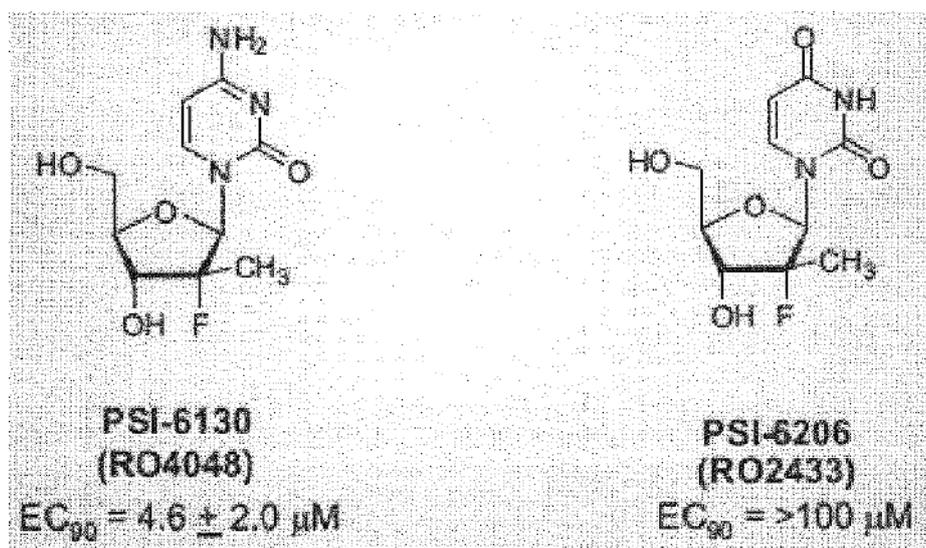
77. Thus, a PHOSITA willing to overcome the problem of cell permeability in drugs used for treating Hepatitis-C would be prompted to replace 4'-azidouridine by all available

embodiments for treating HCV. Further, a skilled person would also be motivated to use an inactive parent nucleoside such as compound 9 of Clark *et al.*

Sofia et al (Published: September, 2007)

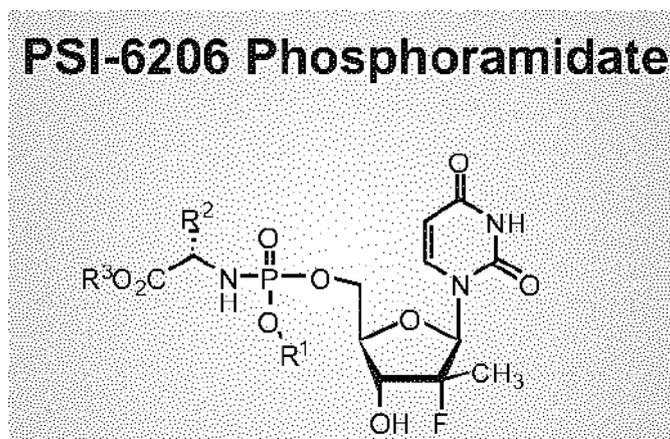
78. The Opponent further relies on a poster (P-259) presented at 14th International symposium on Hepatitis C virus held in Glasgow, Scotland on September 9-13, 2007 by Michael J. Sofia et al titled “ β -D-2'-deoxy-2'-fluoro-2'-C-methyluridine Phosphoramidates: potent and Selective inhibitors of HCV RNA” (hereinafter referred as “Sofia *et al*” and exhibited as **Exhibit J**). This document can be considered as a valid prior art considering the Applicant has wrongly claimed the priority date of March 30, 2007 and the effective date of priority should be October 24, 2007, as discussed in section titled ‘incorrect claim of priority’.

79. Sofia *et al* discloses compound PSI-6206 which is 2' deoxy-2'-fluoro-2'-C-methyluridine and compound PSI-6130 which is its corresponding 2'-methylcytidine. PSI-6206 and PSI-6130 are reproduced below:



80. Sofia *et al* further discloses that PSI-6130 is converted into PSI-6206 and that PSI-6130 is inactive *in vitro*, but its triphosphate counterpart is a potent inhibitor of the HCV NS5B polymerase. It also states that for investigation of the potential of PSI-6206 as an inhibitor of the HCV replication, the bypass of the first phosphorylation step is required. It further reports that in order to investigate the potential for utilizing PSI-6206 as an inhibitor of HCV replication required the first phosphorylation step to be bypassed (see introduction).

Phosphoramidate prodrugs of PSI-6206 are depicted in a markush formula in the last box left hand column of the poster as follows:



81. Radical R^2 is a variable defining the amino acid residue (see title of table 1). It can be appreciated that the radical R^2 of the amino acid side chain is projecting away from the viewer as is the case in compounds of claims 1-3 of the Present Application. Radical R^3 is the amino acid ester radical and R^1 is the phosphate ester (see title of table 3). No exact meanings/substitutions of radicals R^1 to R^3 are disclosed in the poster and each compound is identified by a code. Further, the EC_{90} values for the compounds disclosed in tables 1-3 are lower than the parent compound PSI-6206 thus confirming that they had better anti-HCV activity.
82. Sofia *et al* teaches that 5'-phosphoramidate derivatives of PSI-6206 are potent inhibitors of HCV, that selected phosphoramidates of PSI-6206 are as much as 100x more potent than cytidine analogue PSI-6130, that β -D-2'-Deoxy-2'-fluoro-2'-C-methyluridine phosphoramidates have potential as therapeutic agents for treatment of HCV infection and that several PSI-6206 phosphoramidates have demonstrated stability profiles that are attractive for further development (see conclusions).
83. It is submitted that starting from the teachings disclosed in Sofia *et al*, an alternative technical problem could be formulated, which is, providing an active form of PSI-6206 which is useful in the treatment of HCV. The structural variation seen in tables 3 and 5 (Sofia *et al*) would have motivated a skilled person to carefully select the amino acid part, which is known to have a potential negative impact on cytotoxicity. The data in the Sofia et

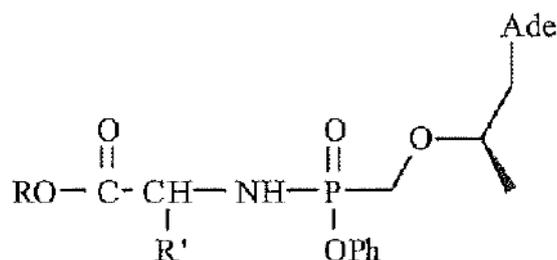
al therefore would lead a person skilled in the art to work out the groups at R¹, R², R³ with a reasonable expectation of success.

84. Also, the solution to the problem of identifying a suitable combination for the residues R¹ – R³ as discussed in Sofia et al is suggested by Perrone et al which proposes three candidates (L-alanine derivatives) as being “exceptional”. (See Perrone et al, internal page 1843, LHS para 1, placitum 2-5). There was, thus only a limited number of possible candidates of suitable phosphoramidates and in view of envisaged success promised in Sofia et al, there was no undue burden to test and identify the most suitable candidate. Therefore, in light of the teachings of Clark *et al*, WO '147, Cahard *et al* and Lee *et al* and Sofia *et al* the compound of claims 1-3 of the Present Application, lacks an inventive step and would have been obvious to a person skilled in the art.

d. THE STEREOISOMERS ARISING FROM CHIRALITY AT PHOSPHATE IN A PHOSPHORAMIDATE WERE KNOWN

Cahard *et al* (Published: 2005)

85. The Opponent also relies on Cahard *et al* (**Exhibit G**) on another aspect. This prior art reports that “*one of the notable features of all the phosphoramidate triesters, excluding some phosphoramidates, is the presence of a chiral centre at the phosphate. Due to the chirality of the nucleoside, all of the compounds prepared are thus isolated as a pair of diastereoisomers....as early as 1990 we had started to partly separate the diastereoisomers. Thus, some alkoxy phosphoramidates of AZT were partly separated by flash silica chromatography and fractions enriched in the more lipophilic ('fast') and less ('slow') isomer were separately evaluated. We found a small, ca 3-fold difference in potency, with the 'fast' isomer being less potent. A subsequent study on some mixed haloalkyl triesters of type (6) indicated 10 fold difference, with 'fast' isomer being more potent...Thus working with alanine phosphoramidates of PME A (34), researchers at Gilead found a 10-fold difference in potency, with the S-phosphate isomer being more potent. Their recent disclosure of a large scale synthesis and purification of the most active isomer clears the way towards clinical evaluation of single isomers of phosphoramidate triesters.*” (see internal page 378, RHS column, lines 5-20 and 24-29). The structure of **compound 34** is reproduced below for reference (see internal page 375, LHS bottom)



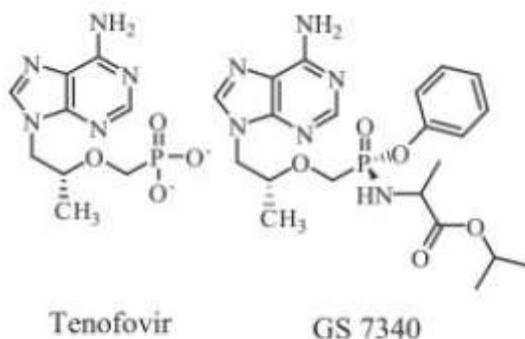
(34) R = Me, iPr; R' = H, Me, Bn

86. Cahard *et al* also teaches the “the issue of phosphate stereochemistry is worth considering” (see internal page 380, LHS column, para 3, placitum 1), thus rendering the diastereomers of claims 2 and 3 to be obvious.

87. The Opponent submits that, a PHOSITA starting with compound 9 of Clark *et al* would have considered applying the teachings of Cahard *et al* with (2S)-isopropyl 2-(((phenoxy)phosphoryl)amino) propanoate) of compound 34, and would have arrived at compounds of claims 1-3 of the Present Application with no need of inventive skill.

Lee et al (Published: 2005)

88. The Opponent again relies on Lee *et al* (**Exhibit H**). Lee *et al* has described the *in vivo* and *in vitro* characterisation of GS 7340, an isopropylalaninyl monoamidate phenyl monoester prodrug of tenofovir. This structures of Tenofovir and GS 7340 are reproduced below for reference (see internal page 1899, LHS column)



89. Lee *et al* notes that, “In this report, we describe the *in vitro* and *in vivo* characterisation of GS 7340, an isopropylalaninyl monoaminodate phenyl monoester prodrug of tenofovir (fig 1). This molecule demonstrates extremely potent *in vitro* activity and selective targeting to

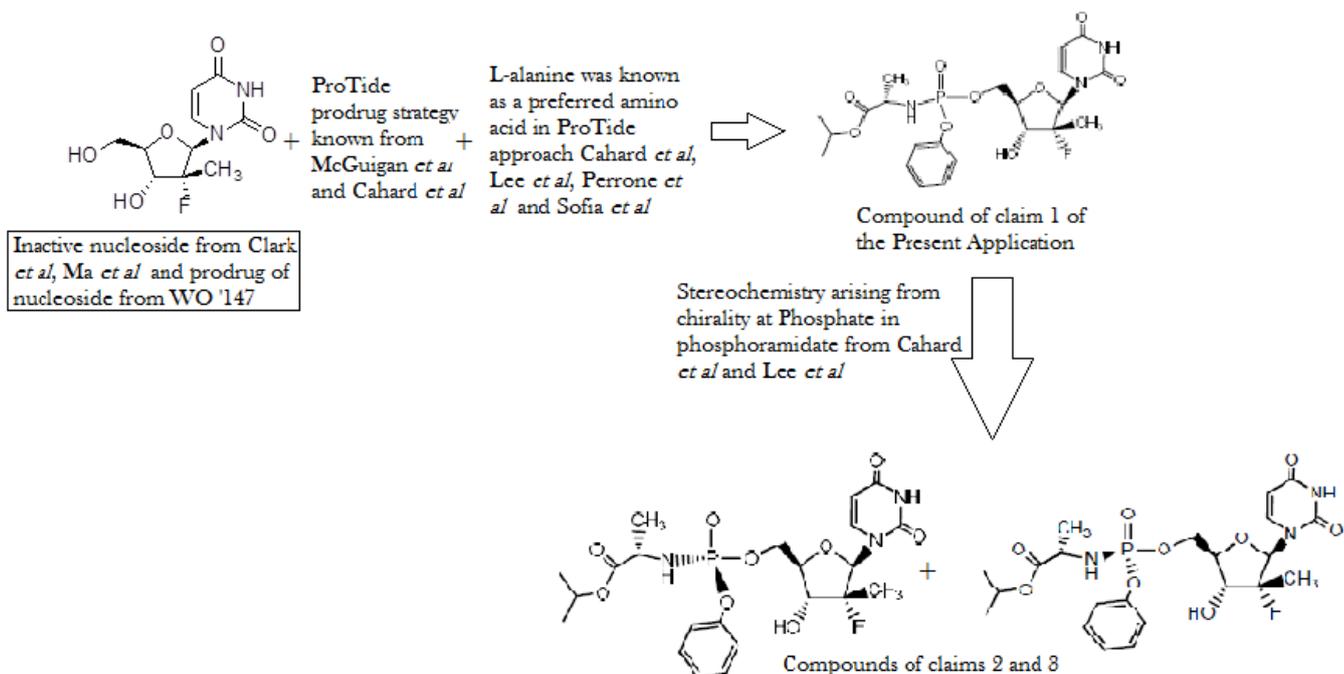
lymphoreticular tissues and PBMCs in vitro and in vivo.” (see internal page 1898, RHS column, para2, lines 29-30 and page 1899, LHS column, para 1, lines 1-4).

90. Further, it records that “*GS 7340 was synthesized from (R)-PMPA and (L)-isopropyl alanine ester in a nonstereospecific synthesis, resulting in the formation of equal amounts of two stereoisomers at phosphorus. These two diastereomers, GS 7339 and GS 7340, were subsequently separated by chromatography. To assess the ability of these prodrugs to cross the cellular membrane and undergo intracellular metabolism to tenofovir, their in vitro activities were measured against HIV-1 in MT-2 cells. The antiviral activities for the diastereomeric mixture (GS 7171), the individual diastereomers (GS 7339 and GS 7340), the diastereomeric mixture of D-alaninyl analog (GS 7485), and the alaninyl monoamidate metabolite (GS 7160) are shown in Table 1. Compared to tenofovir, the individual isomers, GS 7339 and GS 7340, were 83- and 1,000-fold more active, respectively, whereas the D-isopropyl alaninyl analog (GS 7485) and the metabolite (GS 7160) showed activity similar to tenofovir. The enhanced activities of the L-alaninyl prodrugs compared to those of tenofovir are a result of greater cellular permeability and rapid conversion to tenofovir inside the MT-2 cells. The dramatically reduced activity (1,000 fold) of the D-alaninyl analog (GS 7485), relative to the L-alaninyl analog (GS 7171) demonstrates a strong metabolic preference inside the MT-2 cells for the L-amino acid (see below). **The 12-fold-greater activity of GS 7340 compared to that of GS 7339 further suggests that intracellular metabolism is also sensitive to stereochemistry at the phosphorus.** The greater selectivity index (~10x) for GS 7340 than tenofovir DF may reflect the kinetics of cell loading; GS 7340 results in a higher initial intracellular concentration of tenofovir, which may differentially affect antiviral potency and cytotoxicity.*” (see internal page 1900, ‘Results’, LHS column and RHS column lines 1-12)

91. Hence, a person skilled in the art working towards finding a potent anti-HCV compound on reading about compound 9 of Clark *et al* or twelfth embodiment of WO '147 or RO2433 of Ma *et al* would be motivated to find means to activate the nucleoside found to be active *in vitro* but inactive *in vivo*. Cahard *et al* and McGuigan *et al* teach that further inactive nucleoside can be activated by phosphate pro-drug formation. On reading Cahard *et al*, Lee *et al*, Sofia *et al*, Perrone *et al*, PHOSITA would be motivated to use L-alanine ester in the ProTide approach to the nucleoside identified in Clark *et al* and would also be able to

identify the stereochemistry at Phosphorus. On putting together these teachings, a skilled person would arrive at the compounds of claims 1-3 without any inventive step.

92. For the sake of convenience, the ground of claims 1-3 lacking an inventive step is summarised in a pictorial representation below:



ii. S. 25(1)(f) :THE SUBJECT OF ANY CLAIM OF THE COMPLETE SPECIFICATION IS NOT AN INVENTION WITHIN THE MEANING OF THE PATENTS ACT

93. Section 25(1)(f) of the Patents Act provides a ground for opposition if the subject matter of any claim of the Complete Specification is not an invention within the meaning of the Act.

The Opponent raises this ground specifically in the ground that-

- i) That the subject matter of claims 1-3 falls within the scope of Section 3(d);
- ii) That the subject matter of claim 4 fall within the scope of 3(e);
- iii) That the subject matter of claims 2 and 3 do not have industrial applicability and hence do not qualify as an invention as per S. 2(1)(j).

THAT CLAIMS 1-3 OF THE PRESENT APPLICATION DO NOT SATISFY THE TEST OF SECTION 3(d) AND THEREFORE ARE OBJECTED TO UNDER SECTION 25(1) (f)

94. Without prejudice to other grounds raised herein, it is submitted that claims 1-3 fail under section 3(d) of the Patents Act.

95. Section 3(d) of the Patents Act states:

“the mere discovery of a new form of a known substance which does not result in the enhancement of the known efficacy of that substance or the mere discovery of any new property or new use for a known substance or of the mere use of a known process, machine or apparatus unless such known process results in a new product or employs at least one new reactant.

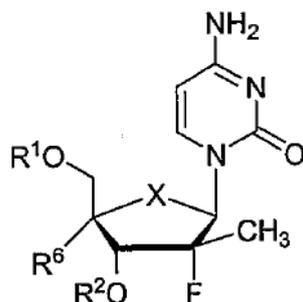
Explanation-For the purposes of this clause, salts, esters, ethers, polymorphs, metabolites, pure form, particle size, isomers, mixtures of isomers, complexes, combinations and other derivatives of known substance shall be considered to be the same substance, unless they differ significantly in properties with regard to efficacy.”

96. Section 3(d) of the Patents Act was amended in 2005 to prevent patents on modification of known substances. The statute requires product claim relating to a known substance, to satisfy the requirement of S. 3(d). It is an established position of law that S. 3(d) has to be satisfied independently of Section 2(1)(j) and S. 2(1)(ja) [see *Novartis AG versus Union of India and Others* (2013) 6 SCC 1]. This requirement under S. 3(d) is to be satisfied by the Applicant by showing efficacy (see *Novartis AG versus Union of India and Others* 2007 4 MLJ 1153, para 13). In case of pharmaceutical products this efficacy would have to be shown in terms of therapeutic efficacy. Further, such data has to be provided by the Applicant in the complete specification (see the order of the Hon'ble IPAB, *Novartis AG versus Union of India*, MIPR 2009 (2) 0345, para 9(xvii)).

WO '147

97. The Opponent relies on the disclosure of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine in WO '147 (see internal page 55). This compound is the first compound drawn in the series of compound identified in the (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine series and therefore qualifies as a preferred form because WO '147 at internal page 56 indicates the “*In*

each example above, the first drawn structure is the preferred form” (see placitum 1). The structure of the said compound is reproduced below for easy reference:



where X is O; R1 is H, R6 is H and R2 is H (substitutions as provided on internal pages 31 and 32 of WO '147).

98. From the prior art documents cited above (particularly Clark *et al*), it is clear that closest compound known to (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine was its uridine derivate, which was formed *in-vivo*. It was also known that this uridine derivative did not show any activity *in vitro* (see Clark *et al* at internal page 5506, RHS column, para 1, lines 9-10). Therefore, the closest compound to the compounds in claims 1-3 that recorded activity would be (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine.

99. WO '147 also provides the activity of the known compound- (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine in different anti-HCV replicon assays (see Table 1 at internal page 90).

Table 1 is reproduced below for reference:

Replicon	(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine
HCV-WT 1b	4.6 ± 2.0
S282T mut. 1b	30.7 ± 11.7
9-13 (subgenomic)	4.6 ± 2.3
21-5 (full-length)	1.6 ± 0.7

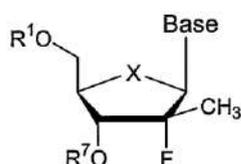
* Values represent EC₉₀ (μM)

100. Therefore, it is submitted that the Applicant has failed to comply with the requirement under Section 3(d) by not showing any enhancement therapeutic by compounds of claims 1-

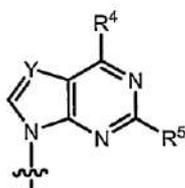
3 over the therapeutic effect (if any) of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine. The burden to show that the claimed compounds have any enhanced therapeutic efficacy over the known compound lies with the Applicant.

WO '147

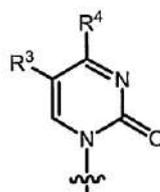
101. Without prejudice to the above, in the alternative, the Opponent relies on the embodiments provided in WO '147 under the heading “*Active Compound, and physiologically Acceptable Derivatives and Salts Thereof*” (see internal page 31). The Opponent in particular relies on the seventh embodiment (see internal page 36). Seventh embodiment is reproduced below for reference:



wherein Base is selected from



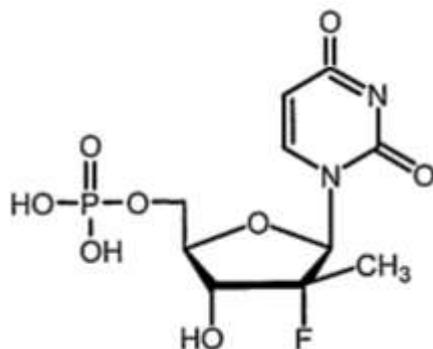
(a)



(b)

and wherein X, Y, R¹, R³, R⁴, R⁵, R⁷ and R' are as defined above

The substitutions have been defined at internal pages 31 and 34. When R¹ is a monophosphate, R³ and R⁷ are H and R⁴ is O we get the following compound-



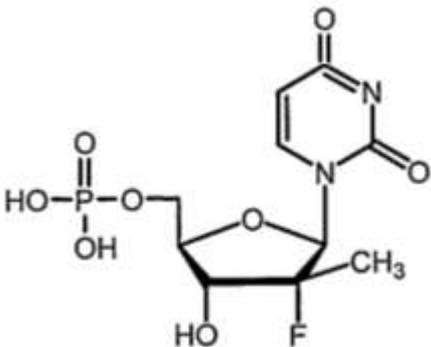
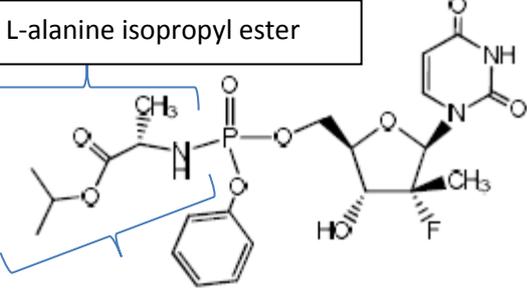
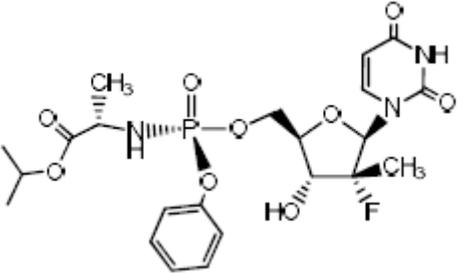
Formula II

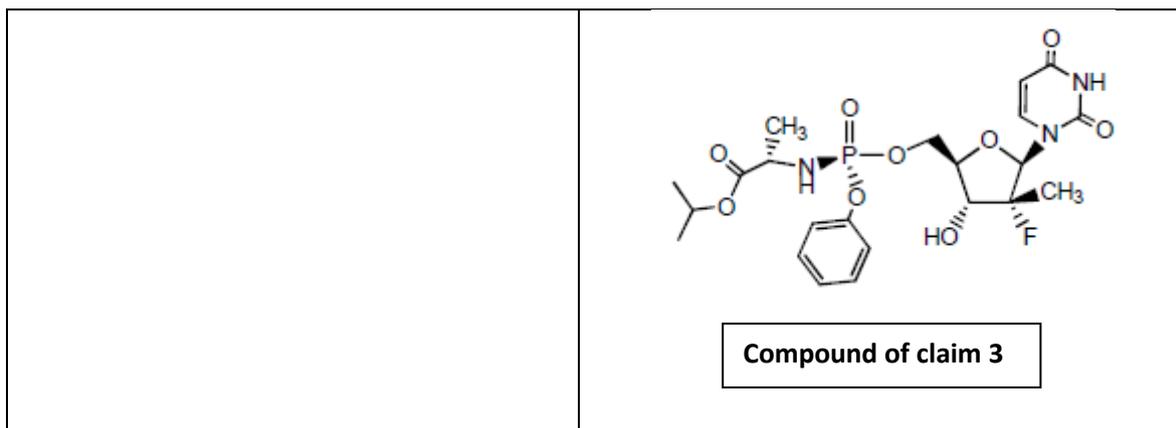
For the purpose of convenience, the compound has been labelled as Formula II.

102. It may be noted that WO '147 was an application filed by the Applicant of the Present Application. Therefore, it is reasonable to assume that the Applicant would have known the activity of any of the compounds disclosed by it in its previous patent applications.

103. It is submitted that the compounds of claims 1-3 of the Present Application are an ester derivative, particularly L-alanine isopropyl ester derivative of the compound of Formula II. The Applicant, however, has failed to provide any data that indicates that the compounds of claims 1-3 show an enhancement of therapeutic efficacy of compound of Formula II, which it was bound to show. Therefore, claims 1-3 must be rejected as they fail to comply with the standards laid down in S. 3(d).

104. The Formula II disclosed in WO '147 is compared with compounds of claims 1-3 below:

<p align="center">Formula II In WO '147</p>	<p align="center">Compounds of claims 1-3 of the Present Application</p>
	<p data-bbox="794 1131 1153 1193">L-alanine isopropyl ester</p>  <p data-bbox="895 1429 1222 1491">Compound of claim 1</p>  <p data-bbox="895 1821 1222 1883">Compound of claim 2</p>

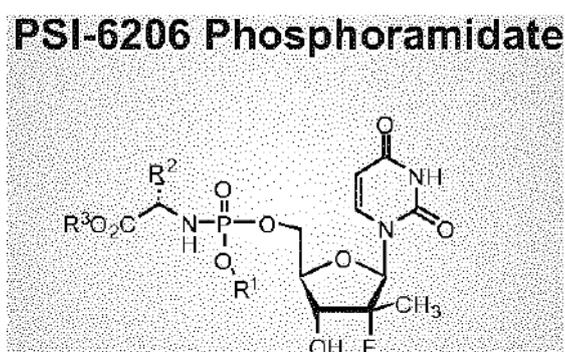


105. The Applicant of the Present Application has failed to show any data that indicates that compound of claims 1-3 result in the enhancement of the efficacy of the compound of Formula II.

Sofia et al

106. Without prejudice to the above, in the alternative, the Opponent again relies on the poster (P-259) presented at 14th International symposium on Hepatitis C virus held in Glasgow, Scotland on September 9-13, 2007 by Michael J. Sofia et al titled “β-D-2'-deoxy-2'-fluoro-2'-C-methyluridine Phosphoramidates: potent and Selective inhibitors of HCV RNA” (referred as “Sofia et al” and exhibited as **Exhibit J**). As discussed in the section related to obviousness, this document can be considered to have been known on the second priority date as claimed by the Applicant.

107. It is submitted that this poster discloses the Phosphoramidate nucleoside-PSI-6206. The structure of PSI-6206 as given in Sofia et al is reproduced below for easy reference-



108. Sofia *et al* clearly studies the structure activity relationship and records EC₉₀ of different amino acid ester substitutions at R³ (as disclosed in Table 3). Table 3 as given on the RHS column of the Sofia *et al* poster is reproduced below for easy reference:

Table 3: Amino Acid Ester (R³) SAR

Cmpd No.	EC ₉₀ CloneA Cells (μ M)
PSI-7672	0.90
PSI-7818	0.98
PSI-7838	0.09
PSI-7839	0.13
PSI-7851	0.52
PSI-7849	0.06

109. It is submitted that as on the second priority date, the activity of various compounds with amino acid ester substitution at R³ position of PSI- 6206 was known. The exact nature of these acetic acid ester substitutions were also known to the Applicant as on the second priority date of the Present Application. Therefore, as on the second priority date, the compounds disclosed in Table-3 of Sofia *et al*, were the closest compounds to the compounds claimed in claims 1-3 of the Present Application. Therefore, the Applicant ought to have shown sufficiently enhanced efficacy of the compounds of claims 1-3 over any of the compounds (PSI-7672, PSI-7818, PSI-7838, PSI-7839, PSI-7851, PSI-7849) in Table-3 in Sofia *et al*, which it has failed to do.

110. In summary it is submitted that the Applicant has failed to discharge the burden of showing an increase in therapeutic efficacy for compounds of claims 1-3. Therefore, these claims fail under Section 3(d) and ought to be rejected under Section 25(1) (f) of the Patents Act.

THAT CLAIM 4 OF THE PRESENT APPLICATION FALLS WITHIN THE SCOPE OF S. 3(e) AND THEREFORE ARE OBJECTED TO UNDER SECTION 25(1) (f)

111. Section 3(e) of the Patents Act states that:

“A substance obtained by a mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance.”

112. Without prejudice to other grounds raised herein, the pharmaceutical composition as claimed by the Applicant in Claim 4 is a substances obtained by a mere admixture. Claim 4 of the Present Application relates to a composition comprising the compound as claimed in any of the claims 1 to 3. The Applicant has not shown that the compound of claim 4 exhibits any synergistic effect, whether improved and unexpected or otherwise, over and above the aggregation of the properties of the components thereof. In other words, the Applicant has not any therapeutic effect of such pharmaceutical composition of claim 4 to be in excess of the sum of that is observed in either of the therapeutic agents individually. Thus, the Opponent states that the Patent Applicant has failed to show synergistic activity for the claimed pharmaceutical composition.

113. Therefore, Claim 4 to 8 fails under section 3(e) and should be rejected under section 25(1) (f) of the Patents Act.

THAT CLAIMS 1-3 OF THE PRESENT APPLICATION DID NOT HAVE ANY INDUSTRIAL APPLICABILITY AS ON THE DATE OF FILING OF THE APPLICATION AND THEREFORE ARE OBJECTED TO UNDER SECTION 25(1) (F)

114. It is submitted that on the date of making Present Application, the Applicant did not submit any activity or industrial applicability of compounds of claims 1-3. Further, the burden is on the Applicant to show industrial utility of the claimed compounds, on the date of filing the application. Therefore, as the Applicant has, *ex facie*, failed to disclose activity of the compounds claimed in claims 1-3, these claims should be rejected under S. 25(1)(f) as they do not qualify as an invention.

iii. S. 25(1)(g) : THE COMPLETE SPECIFICATION DOES NOT SUFFICIENTLY AND CLEARLY DESCRIBE THE INVENTION

115. The Present Application does not sufficiently and clearly describe the invention or the method by which it is to be performed. Further the claims are not appropriately supported by the specification of the alleged application. Hence, without prejudice to the grounds raised in this representation, the Opponent invokes Section 25(1) (g).

Preparation of compound of claim 1 has not been sufficiently described

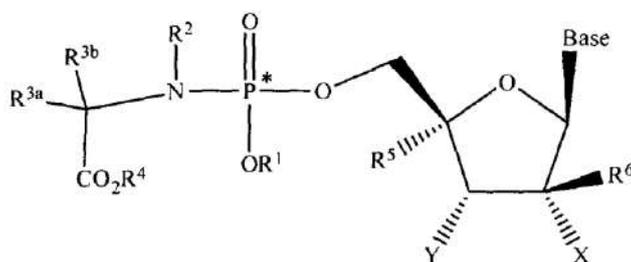
116. It is submitted that the Applicant has failed to specifically describe the process of preparation of compound of claim 1. Compound IX-25-2 in Table IX on page 251 mentions all substituents of the compound claimed in claim 1 of the Present Application. This table has to be read in combination with structure given on page 243 of the complete specification which does not mention the stereochemistry on "Phosphorus atom". In the absence of a specific method for preparation of this aspect of the embodiment, a person having ordinary skill in the art will be drawn to method used for preparing phosphoramidate as identified at example 3 (internal page 675 of the complete specification). The general preparation for making the nucleoside as in claim 1 has been given at example 4 (internal page 676 of the complete specification). However, no procedure to prepare the exact compound of claim 1 has been disclosed in the complete specification. Further, nothing in the complete specification indicates that the disclosed method for preparing example 3 and example 4 would enable making compound at IX-25-2.

117. Further, even if one refers to Examples 5-8 (on pages 677-680 of the complete specification); one finds that they only disclose a process for the preparation of methyl ester analog of the compound claimed in claim 1 of the Present Application. Also, the complete specification has provided that examples 13-54 and 56-66 on pages 684-689 may be prepared using similar procedures used for examples 5-8. It is submitted that example 25 covered in these pages (see particularly page 685 of the complete specification) covers the compound claimed in claim 1. It is submitted that the specification has only given a general method of preparation and does not enable the preparation of the compound of claim 1.

118. The Opponent submits that the compound of claim 1 contains 6 stereogenic centres giving the possibility of multiple stereoisomers (64 isomers) with a specific configuration. The specification of the alleged application has not provided any specific process for the preparation of stereoisomers. Further, the Applicant has also failed to show any activity and reason for choosing the diastereomers identified in claims 2 and 3.

Preparation of stereoisomers claimed in claims 2 and 3 has not been sufficiently described

119. In the last paragraph given at page 18 of the complete specification, the Applicant has stated that the term “P*” means that the phosphorus atom is chiral. It is contemplated that the compounds of Formula I (as given at page 8) are racemic because the chirality of phosphorus. It then states that ‘applicant contemplates use of the racemate and/or the resolved enantiomer’ (emphasis added). The use of the term ‘the’ clearly indicates that the contemplation is in relation to the racemate of the compound reproduced below-



Formula III

120. The passage at page 18 of the complete specification, then goes on to other cases mentioned in the complete specification, where the asterisk does not appear next to the phosphorus atom. It states that ‘in these instances, it is understood that the phosphorus atom is chiral and that one of the ordinary skill understands this to be so unless the substituents bound to the phosphorus exclude the possibility of chirality at the phosphorus.’ In other words, the skilled person would understand that a formula not showing P* but only P may still be chiral. It cannot be read from this passage that the patentee contemplates the use of resolved enantiomers of a compound of formula IX-25-2, since such a compound is depicted in the patent application with a P and not P*, as well as because the contemplation of using resolved enantiomers is only made in connection with compound of Formula III. This passage cannot be read to be disclosing the pure resolved diastereoisomers claimed in claim 2 and 3. Even if one is to assume that separate stereoisomers can be arrived at, the complete specification does not disclose how to assign the phosphorus atom steric configuration of the two separated diastereoisomers of claim 2 and 3.

121. Further, the compounds claimed in claims 2 and 3 depict specifically different configurations at phosphorous atom ((S) and (R) respectively). However, neither these

structures nor the IUPAC name of claims 2 and 3 are disclosed as such in the specification of the Present Application.

122. The specification of the Present Application however, fails to disclose specific preparation of stereoisomer, except for the generic procedure in Example 81 which states that certain exemplified compounds were obtained as mixture of diastereomers because of the chirality at phosphorous. The diastereomers were separated on a Chiralpak-AS-H (2 X 25 cm) column under Supercritical Fluid Chromatography (SFC) conditions using 20% methanol in carbon dioxide as solvent. The absolute stereochemistry of the P-chiral centre of the diastereomers was not determined. However, chromatographic resolution of these two diastereomers provides for isomers that are characterized as fast eluting and slow eluting isomers (see page 695 of the complete specification). The examples given are reproduced below:

Compound	EC90 (μM)
Example 15 (Diastereomeric mixture)	0.86
Fast moving isomer of Example 15	1.35
Slow moving isomer of Example 15	0.26
Example 39 (Diastereomeric mixture)	0.47
Fast moving isomer of Example 39	0.78
Slow moving isomer of Example 39	0.02
Example 49 (Diastereomeric mixture)	0.126
Fast moving isomer of Example 49	0.03
Slow moving isomer of Example 49	5.78

123. The specification of the Present Application failed to specify (R) and (S) nomenclature to the fast moving and slow moving isomer, which means the applicant did not conclude whether fast moving isomer or the slow moving isomer is the (S)-isomer or vice versa.

124. The Applicant appears to have left to the intuition of the reader to find out the stereochemistry of the fast moving and slow moving isomers. If one would assume that the compound of claim 1 can be separated into a slow and fast eluting isomer by following the

procedure in Example 81, even then it would be unknown how to determine which of the fast / slow eluting isomers is (R) isomer and (S) isomer.

125. The PHOSITA would have known that the synthesis of nucleosides is often complicated, as a result of the number of chiral centres in the sugar ring and the number of reactive functional groups attached to the sugar (which might give rise to unwanted reactions and which would therefore need masking with suitable protecting groups). Hence, it is difficult to a person skilled in the art to prepare subject matter claimed in claims 1 to 3 specifically claims 2 and 3 and their isolation as a specific diastereomers.

126. The Opponent would also like to draw attention to the Applicant's corresponding patent at the European Union, numbered – EP 2 203 462. The first three claims of this patent were identical to the claims of the Present Application. However, ten oppositions were filed against this patent.

127. The Opposition Division of the European Patent Office (EPO), in its order (**Exhibit B**), did not find claims (corresponding to claims 1-3 of the Present Application) to be sufficiently described.

128. The Opposition Division noted that the compound IX-25-2 disclosed in the complete specification of the impugned patent had 2 possible points of chirality leading to 4 possible enantiomers. It also noted that these 4 possible enantiomers were not individualised in the complete specification (Refer to internal page 15, para 19.10 of **Exhibit B**). Therefore, the Opposition Division of the EPO allowed the Applicant's amendment that retained only one claim that corresponds to claim 1 of the Present Application.

129. Therefore, the Opponent humbly requests that the Controller should particularly disallow claims 2-3, considering that EPO has found the claims, identical to claims 2-3 of the Present Application, to be not sufficiently described in the complete specification. **The Applicant itself has disclaimed the compounds corresponding to claims 2-3 of the Present Application at EPO and has amended the claim to limit its scope to one claim that corresponds to claim 1 of the Present Application.**

Reason for choosing compounds of claims 1-3 has not been disclosed

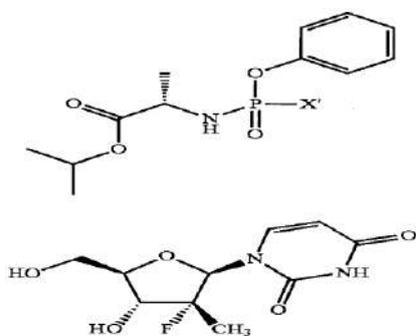
130. It is submitted that the Applicant has failed to show any activity or industrial utility of compounds of claims 1-3. In the absence of such evidence, the Applicant ought show reasons of choosing the claimed compounds.

Method to prepare compound claimed in claim 4 not disclosed sufficiently

131. It is submitted that the invention as claimed in claim 4, has not been sufficiently described. The specification of the Present Application does not support or describe a method by which the composition claimed in claim 4 can be arrived at. Since the specification fails to describe how to arrive at the compound claimed in claim 1, it can be said that the method of arrive at the composition has also not been disclosed.

The process as claimed in claim 5 has not been disclosed

132. Claim 5 of the Present Application claims “ a process for preparing the compound or a stereoisomer thereof as claimed in claim , the said process comprising reacting a compound of 4” with compound of 5””. For the sake of convenience, the compounds 4” and 5” are being disclosed as below:



Where X is a leaving group.

133. It may be noted that the claim 5 talks about a leaving group but does not identify what all groups may be included in the same. Therefore, Opponent submits that claim 5 is vague. It is further submitted that as per the requirement under Section 10, the applicant for a patent should fully and particularly describe the compound. The Applicant in the Present Application has failed to do so. Thereby a person skilled in the art would not be enabled to carry out invention as claimed in claims 1 through 5.

iv. **THAT THE APPLICANT FAILED TO DISCLOSE INFORMATION REQUIRED BY SECTION 8, HENCE THE OPPOSITION IS RAISED UNDER SECTION 25(1)(h)**

134. Section 25(1) (h) of the Patents Act provides a ground for opposition if the patent applicant has not furnished information required under Section 8 of the Patents Act, within the time prescribed by law. Without prejudice to other grounds raised herein, the present application should be rejected because the Patent Applicant has not complied with the mandatory requirements of Section 8 of the Patents Act.

135. Section 8 of the Patents Act read with rule 12(1) of the Patents Rules requires, inter alia, a patent applicant, who is prosecuting, either alone or jointly with any other person, an application for a patent in any country outside India in respect of the same or substantially the same invention, to file a statement setting out the particulars of such application (Form - 3) within six months of the date of filing of such application in India.

136. On 16.01.2017, the Applicant filed Form-3 giving details of the status of corresponding applications (of the Present Application) in other jurisdictions. This form indicates that apart from India, the corresponding application has been opposed in China, European Union and Thailand. It is to be noted that the Applicant has amended the claims in the European Union subsequent to the filing of opposition. However the Form-3 filed by the Applicant only reflects the status of the Application in the European Union as 'opposed'. Given that complete information related to the corresponding applications in other jurisdictions has not been given, the Applicant is in violation of S. 25(1) (h) of the Patents Act. The Opponent also requests the Controller to direct the Applicant to submit translated copies of the opposition proceedings in these jurisdictions to facilitate examination of the Present Application.

VII. PRAYER FOR RELIEF

In view of the above said references Opponent prays as follows:

- i) To be heard and be allowed to lead evidence (documentary and oral) before any order is passed;

- ii) To reject the claims of IN 3658/KOLNP/2009 filed by Gilead Pharmasset Inc. in *toto*;
- iii) To allow the Opponent to file further documents as evidence if necessary to support the averments;
- iv) To allow amendment of the opposition as and when the need may arise;
- v) To allow the Opponent to make further submissions in case the Applicant amends the claims;
- vi) any other relief considering the facts and circumstances that may be granted in favour of the Opponent in the interest of justice.

Dated this Sixth (06th) day of July, 2017.



Dr. S. Padmaja
Agent for the Opponent
IN/PA/883

To,
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Kolkata-700091
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(11) **EP 2 203 462 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

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- (21) Application number: **08732818.3**
- (22) Date of filing: **26.03.2008**
- (51) Int Cl.: **A61K 31/7064^(2006.01) A61K 31/7076^(2006.01)**
- (86) International application number: **PCT/US2008/058183**
- (87) International publication number: **WO 2008/121634 (09.10.2008 Gazette 2008/41)**

(54) **NUCLEOSIDE PHOSPHORAMIDATE PRODRUGS**

NUKLEOSID-PHOSPHORAMIDAT-PRODRUGS

PROMÉDICAMENTS DE PHOSPHORAMIDATE DE NUCLÉOSIDE

- | | |
|---|--|
| <p>(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MT NL NO PL PT RO SE SI SK TR</p> <p>(30) Priority: 30.03.2007 US 909315 P
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21.03.2008 US 53015</p> <p>(43) Date of publication of application:
07.07.2010 Bulletin 2010/27</p> <p>(60) Divisional application:
14151876.1</p> <p>(73) Proprietor: Gilead Pharmasset LLC
Foster City, CA 94404 (US)</p> <p>(72) Inventors:
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 WO-A-03/000713 WO-A-2005/003147
 WO-A-2005/012327 WO-A-2006/012078
 WO-A-2006/065335 WO-A-2007/020193
 WO-A-2007/095269 US-A1- 2006 241 064
 US-B1- 6 475 985 <ul style="list-style-type: none"> • GUNIC ET AL: "6-Hydrazinopurine 2'-methyl ribonucleosides and their 5'-monophosphate prodrugs as potent hepatitis C virus inhibitors" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, ELSEVIER SCIENCE, GB, vol. 17, no. 9, 14 February 2007 (2007-02-14), pages 2456-2458, XP022015324 Online ISSN: 0960-894X </p> <p><u>Remarks:</u>
The file contains technical information submitted after the application was filed and not included in this specification</p> |
|---|--|

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description**Field of Invention**

5 [0001] The present invention pertains to nucleoside phosphoramidates and their use as agents for treating viral diseases. These compounds are inhibitors of RNA-dependent RNA viral replication and are useful as inhibitors of HCV NS5B polymerase, as inhibitors of HCV replication and for treatment of hepatitis C infection in mammals. The invention provides novel chemical compounds, and the use of these compounds alone or in combination with other antiviral agents for treating HCV infection.

Background

15 [0002] Hepatitis C virus (HCV) infection is a major health problem that leads to chronic liver disease, such as cirrhosis and hepatocellular carcinoma, in a substantial number of infected individuals, estimated to be 2-15% of the world's population. There are an estimated 4.5 million infected people in the United States alone, according to the U.S. Center for Disease Control. According to the World Health Organization, there are more than 200 million infected individuals worldwide, with at least 3 to 4 million people being infected each year. Once infected, about 20% of people clear the virus, but the rest harbor HCV the rest of their lives. Ten to twenty percent of chronically infected individuals eventually develop liver-destroying cirrhosis or cancer. The viral disease is transmitted parenterally by contaminated blood and blood products, contaminated needles, or sexually and vertically from infected mothers or carrier mothers to their offspring. Current treatments for HCV infection, which are restricted to immunotherapy with recombinant interferon- α alone or in combination with the nucleoside analog ribavirin, are of limited clinical benefit. Moreover, there is no established vaccine for HCV. Consequently, there is an urgent need for improved therapeutic agents that effectively combat chronic HCV infection.

25 [0003] The HCV virion is an enveloped positive-strand RNA virus with a single oligoribonucleotide genomic sequence of about 9600 bases which encodes a polyprotein of about 3,010 amino acids. The protein products of the HCV gene consist of the structural proteins C, E1, and E2, and the non-structural proteins NS2, NS3, NS4A and NS4B, and NS5A and NS5B. The nonstructural (NS) proteins are believed to provide the catalytic machinery for viral replication. The NS3 protease releases NS5B, the RNA-dependent RNA polymerase from the polyprotein chain. HCV NS5B polymerase is required for the synthesis of a double-stranded RNA from a single-stranded viral RNA that serves as a template in the replication cycle of HCV. Therefore, NS5B polymerase is considered to be an essential component in the HCV replication complex (K. Ishi, et al, *Heptology*, 1999, 29: 1227-1235; V. Lohmann, et al., *Virology*, 1998, 249: 108-118). Inhibition of HCV NS5B polymerase prevents formation of the double-stranded HCV RNA and therefore constitutes an attractive approach to the development of HCV-specific antiviral therapies.

35 [0004] HCV belongs to a much larger family of viruses that share many common features.

Flaviviridae Viruses

40 [0005] The Flaviviridae family of viruses comprises at least three distinct genera: *pestiviruses*, which cause disease in cattle and pigs; *flaviviruses*, which are the primary cause of diseases such as dengue fever and yellow fever; and *hepaciviruses*, whose sole member is HCV. The flavivirus genus includes more than 68 members separated into groups on the basis of serological relatedness (Calisher et al., *J. Gen. Virol*, 1993,70,37-43). Clinical symptoms vary and include fever, encephalitis and hemorrhagic fever (Fields *Virology*, Editors: Fields, B. N., Knipe, D. M., and Howley, P. M., Lippincott-Raven Publishers, Philadelphia, PA, 1996, Chapter 31, 931-959). Flaviviruses of global concern that are associated with human disease include the Dengue Hemorrhagic Fever viruses (DHF), yellow fever virus, shock syndrome and Japanese encephalitis virus (Halstead, S. B., *Rev. Infect. Dis.*, 1984, 6, 251-264; Halstead, S. B., *Science*, 239:476-481, 1988; Monath, T. P., *New Eng. J. Med.*, 1988, 319, 64 1-643).

45 [0006] The pestivirus genus includes bovine viral diarrhea virus (BVDV), classical swine fever virus (CSFV, also called hog cholera virus) and border disease virus (BDV) of sheep (Moennig, V. et al. *Adv. Vir. Res.* 1992, 41, 53-98). Pestivirus infections of domesticated livestock (cattle, pigs and sheep) cause significant economic losses worldwide. BVDV causes mucosal disease in cattle and is of significant economic importance to the livestock industry (Meyers, G. and Thiel, H.J., *Advances in Virus Research*, 1996, 47, 53-118; Moennig V., et al. *Adv. Vir. Res.* 1992, 41, 53-98). Human pestiviruses have not been as extensively characterized as the animal pestiviruses. However, serological surveys indicate considerable pestivirus exposure in humans.

55 [0007] Pestiviruses and hepaciviruses are closely related virus groups within the Flaviviridae family. Other closely related viruses in this family include the GB virus A, GB virus A-like agents, GB virus-B and GB virus-C (also called hepatitis G virus, HGV). The hepacivirus group (hepatitis C virus; HCV) consists of a number of closely related but genotypically distinguishable viruses that infect humans. There are at least 6 HCV genotypes and more than 50 subtypes.

Due to the similarities between pestiviruses and hepaciviruses, combined with the poor ability of hepaciviruses to grow efficiently in cell culture, bovine viral diarrhoea virus (BVDV) is often used as a surrogate to study the HCV virus.

[0008] The genetic organization of pestiviruses and hepaciviruses is very similar. These positive stranded RNA viruses possess a single large open reading frame (ORF) encoding all the viral proteins necessary for virus replication. These proteins are expressed as a polyprotein that is co- and post-translationally processed by both cellular and virus-encoded proteinases to yield the mature viral proteins. The viral proteins responsible for the replication of the viral genome RNA are located within approximately the carboxy-terminal. Two-thirds of the ORF are termed nonstructural (NS) proteins. The genetic organization and polyprotein processing of the nonstructural protein portion of the ORF for pestiviruses and hepaciviruses is very similar. For both the pestiviruses and hepaciviruses, the mature nonstructural (NS) proteins, in sequential order from the amino-terminus of the nonstructural protein coding region to the carboxy-terminus of the ORF, consist of p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

[0009] The NS proteins of pestiviruses and hepaciviruses share sequence domains that are characteristic of specific protein functions. For example, the NS3 proteins of viruses in both groups possess amino acid sequence motifs characteristic of serine proteinases and of helicases (Gorbalenya et al., *Nature*, 1988, 333, 22; Bazan and Fletterick *Virology*, 1989, 171, 637-639; Gorbalenya et al., *Nucleic Acid Res.*, 1989, 17, 3889-3897). Similarly, the NS5B proteins of pestiviruses and hepaciviruses have the motifs characteristic of RNA-directed RNA polymerases (Koonin, E.V. and Dolja, V.V., *Crit. Rev. Biochem. Molec. Biol.* 1993, 28, 375-430).

[0010] The actual roles and functions of the NS proteins of pestiviruses and hepaciviruses in the lifecycle of the viruses are directly analogous. In both cases, the NS3 serine proteinase is responsible for all proteolytic processing of polyprotein precursors downstream of its position in the ORF (Wiskerchen and Collett, *Virology*, 1991, 184, 341-350; Bartenschlager et al., *J. Virol.* 1993, 67, 3835-3844; Eckart et al. *Biochem. Biophys. Res. Comm.* 1993, 192, 399-406; Grakoui et al., *J. Virol.* 1993, 67, 2832-2843; Grakoui et al., *Proc. Natl. Acad. Sci. USA* 1993, 90, 10583-10587; Hijikata et al., *J. Virol.* 1993, 67, 4665-4675; Tome et al., *J. Virol.*, 1993, 67, 4017-4026). The NS4A protein, in both cases, acts as a cofactor with the NS3 serine protease (Bartenschlager et al., *J. Virol.* 1994, 68, 5045-5055; Failla et al., *J. Virol.* 1994, 68, 3753-3760; Xu et al., *J. Virol.* 1997, 71:53 12-5322). The NS3 protein of both viruses also functions as a helicase (Kim et al., *Biochem. Biophys. Res. Comm.*, 1995, 215, 160-166; Jin and Peterson, *Arch. Biochem. Biophys.*, 1995, 323, 47-53; Warrenner and Collett, *J. Virol.* 1995, 69, 1720-1726). Finally, the NS5B proteins of pestiviruses and hepaciviruses have the predicted RNA-directed RNA polymerases activity (Behrens et al., *EMBO*, 1996, 15, 12-22; Lechmann et al., *J. Virol.*, 1997, 71, 8416-8428; Yuan et al., *Biochem. Biophys. Res. Comm.* 1997, 232, 231-235; Hagedorn, *PCT WO* 97/12033; Zhong et al, *J. Virol.*, 1998, 72, 9365-9369).

[0011] Currently, there are limited treatment options for individuals infected with hepatitis C virus. The current approved therapeutic option is the use of immunotherapy with recombinant interferon- α alone or in combination with the nucleoside analog ribavirin. This therapy is limited in its clinical effectiveness and only 50% of treated patients respond to therapy. Therefore, there is significant need for more effective and novel therapies to address the unmet medical need posed by HCV infection.

[0012] A number of potential molecular targets for drug development of direct acting antivirals as anti-HCV therapeutics have now been identified including, but not limited to, the NS2-NS3 autoprotease, the NS3 protease, the NS3 helicase and the NS5B polymerase. The RNA-dependent RNA polymerase is absolutely essential for replication of the single-stranded, positive sense, RNA genome and this enzyme has elicited significant interest among medicinal chemists.

[0013] Inhibitors of HCV NS5B as potential therapies for HCV infection have been reviewed: Tan, S.-L., et al., *Nature Rev. Drug Discov.*, 2002, 1, 867-881; Walker, M.P. et al., *Exp. Opin. Investigational Drugs*, 2003, 12, 1269-1280; Ni, Z.-J., et al., *Current Opinion in Drug Discovery and Development*, 2004, 7, 446-459; Beaulieu, P. L., et al., *Current Opinion in Investigational Drugs*, 2004, 5, 838-850; Wu, J., et al., *Current Drug Targets-Infectious Disorders*, 2003, 3, 207-219; Griffith, R.C., et al, *Annual Reports in Medicinal Chemistry*, 2004, 39, 223-237; Carrol, S., et al., *Infectious Disorders-Drug Targets*, 2006, 6, 17-29. The potential for the emergence of resistant HCV strains and the need to identify agents with broad genotype coverage supports the need for continuing efforts to identify novel and more effective nucleosides as HCV NS5B inhibitors.

[0014] Nucleoside inhibitors of NS5B polymerase can act either as a non-natural substrate that results in chain termination or as a competitive inhibitor which competes with nucleotide binding to the polymerase. To function as a chain terminator the nucleoside analog must be taken up by the cell and converted *in vivo* to a triphosphate to compete for the polymerase nucleotide binding site. This conversion to the triphosphate is commonly mediated by cellular kinases which imparts additional structural requirements on a potential nucleoside polymerase inhibitor. Unfortunately, this limits the direct evaluation of nucleosides as inhibitors of HCV replication to cell-based assays capable of *in situ* phosphorylation.

[0015] In some cases, the biological activity of a nucleoside is hampered by its poor substrate characteristics for one or more of the kinases needed to convert it to the active triphosphate form. Formation of the monophosphate by a nucleoside kinase is generally viewed as the rate limiting step of the three phosphorylation events. To circumvent the need for the initial phosphorylation step in the metabolism of a nucleoside to the active triphosphate analog, the preparation of stable phosphate prodrugs has been reported. Nucleoside phosphoramidate prodrugs have been shown to be pre-

cursors of the active nucleoside triphosphate and to inhibit viral replication when administered to viral infected whole cells (McGuigan, C., et al., J. Med. Chem., 1996, 39, 1748-1753; Valette, G., et al., J. Med. Chem., 1996, 39, 1981-1990; Balzarini, J., et al., Proc. National Acad Sci USA, 1996, 93, 7295-7299; Siddiqui, A. Q., et al., J. Med. Chem., 1999, 42, 4122-4128; Eisenberg, E. J., et al., Nucleosides, Nucleotides and Nucleic Acids, 2001, 20, 1091-1098; Lee, W.A., et al., Antimicrobial Agents and Chemotherapy, 2005, 49, 1898); US 2006/0241064; and WO 2007/095269.

[0016] Also limiting the utility of nucleosides as viable therapeutic agents is their sometimes poor physicochemical and pharmacokinetic properties. These poor properties can limit the intestinal absorption of an agent and limit uptake into the target tissue or cell. To improve on their properties prodrugs of nucleosides have been employed. It has been demonstrated that preparation of nucleoside phosphoramidates improves the systemic absorption of a nucleoside and furthermore, the phosphoramidate moiety of these "pronucleotides" is masked with neutral lipophilic groups to obtain a suitable partition coefficient to optimize uptake and transport into the cell dramatically enhancing the intracellular concentration of the nucleoside monophosphate analog relative to administering the parent nucleoside alone. Enzyme-mediated hydrolysis of the phosphate ester moiety produces a nucleoside monophosphate wherein the rate limiting initial phosphorylation is unnecessary.

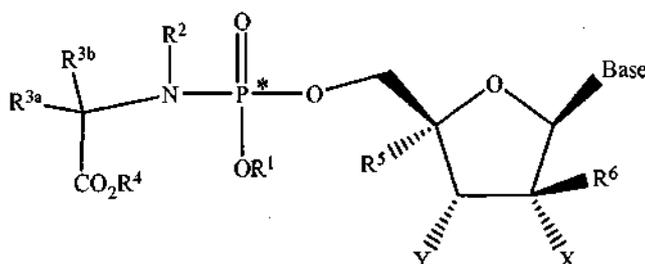
[0017] WO 2005/003147 discloses compositions and methods of treating a *Flaviviridae* infection, including hepatitis C virus.

[0018] J. Med. Chem., 2005, 48, 5504-5508, Clark et al., discloses 2'-deoxy-2'-fluoro-2'-C-methylcytidine as an inhibitor of Hepatitis C Virus.

SUMMARY OF THE INVENTION

[0019] The present invention is as set out in the claims.

[0020] The present disclosure is directed toward phosphoramidate prodrugs of nucleoside derivatives for the treatment of viral infections in mammals, which is a compound, its stereoisomers, salts (acid or basic addition salts), hydrates, solvates, or crystalline forms thereof, represented by the following structure:



I

wherein

(a) R¹ is hydrogen, n-alkyl; branched alkyl; cycloalkyl; or aryl, which includes, but is not limited to, phenyl or naphthyl, where phenyl or naphthyl are optionally substituted with at least one of C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ alkoxy, F, Cl, Br, I, nitro, cyano, C₁₋₆ haloalkyl, -N(R¹)₂, C₁₋₆ acylamino, -NHSO₂C₁₋₆ alkyl, -SO₂N(R¹)₂, COR^{1'}, and -SO₂C₁₋₆ alkyl; (R¹ is independently hydrogen or alkyl, which includes, but is not limited to, C₁₋₂₀ alkyl, C₁₋₁₀ alkyl, or C₁₋₆ alkyl, R^{1'} is -OR' or -N(R¹)₂);

(b) R² is hydrogen, C₁₋₁₀ alkyl, R^{3a} or R^{3b} and R² together are (CH₂)_n so as to form a cyclic ring that includes the adjoining N and C atoms, C(O)CR^{3a}R^{3b}NHR¹, where n is 2 to 4 and R¹, R^{3a}, and R^{3b};

(c) R^{3a} and R^{3b} are (i) independently selected from hydrogen, C₁₋₁₀ alkyl, cycloalkyl, -(CH₂)_c(NR^{3'})₂, C₁₋₆ hydroxy-alkyl, -CH₂SH, -(CH₂)₂S(O)_dMe, -(CH₂)₃NHC(=NH)NH₂, (1H-indol-3-yl)methyl, (1H-imidazol-4-yl)methyl, -(CH₂)_eCOR^{3'}, aryl and aryl C₁₋₃ alkyl, said aryl groups optionally substituted with a group selected from hydroxyl, C₁₋₁₀ alkyl, C₁₋₆ alkoxy, halogen, nitro and cyano; (ii) R^{3a} and R^{3b} both are C₁₋₆ alkyl; (iii) R^{3a} and R^{3b} together are (CH₂)_f so as to form a spiro ring; (iv) R^{3a} is hydrogen and R^{3b} and R² together are (CH₂)_n so as to form a cyclic ring that includes the adjoining N and C atoms (v) R^{3b} is hydrogen and R^{3a} and R² together are (CH₂)_n so as to form a cyclic ring that includes the adjoining N and C atoms, where c is 1 to 6, d is 0 to 2, e is 0 to 3, f is 2 to 5, n is 2 to 4, and where R^{3'} is independently hydrogen or C₁₋₆ alkyl and R^{3'} is -OR' or -N(R^{3'})₂; (vi) R^{3a} is H and R^{3b} is H, CH₃,

CH₂CH₃, CH(CH₃)₂, CH₂CH(CH₃)₂, CH(CH₃)CH₂CH₃, CH₂Ph, CH₂-indol-3-yl, -CH₂CH₂SCH₃, CH₂CO₂H, CH₂C(O)NH₂, CH₂CH₂COOH, CH₂CH₂C(O)NH₂, CH₂CH₂CH₂CH₂NH₂, -CH₂CH₂CH₂NHC(NH)NH₂, CH₂-imidazol-4-yl, CH₂OH, CH(OH)CH₃, CH₂((4'-OH)-Ph), CH₂SH, or lower cycloalkyl; or (viii) R^{3a} is CH₃, -CH₂CH₃, CH(CH₃)₂, CH₂CH(CH₃)₂, CH(CH₃)CH₂CH₃, CH₂Ph, CH₂-indol-3-yl, -CH₂CH₂SCH₃, CH₂CO₂H, CH₂C(O)NH₂, CH₂CH₂COOH, CH₂CH₂C(O)NH₂, CH₂CH₂CH₂CH₂NH₂, -CH₂CH₂CH₂NHC(NH)NH₂, CH₂-imidazol-4-yl, CH₂OH, CH(OH)CH₃, CH₂((4'-OH)-Ph), CH₂SH, or lower cycloalkyl and R^{3b} is H, where R³ is independently hydrogen or alkyl, which includes, but is not limited to, C₁₋₂₀ alkyl, C₁₋₁₀ alkyl, or C₁₋₆ alkyl, R^{3'} is -OR' or -N(R^{3'})₂;

(d) R⁴ is hydrogen, C₁₋₁₀ alkyl, C₁₋₁₀ alkyl optionally substituted with a lower alkyl, alkoxy, di(lower alkyl)-amino, or halogen, C₁₋₁₀ haloalkyl, C₃₋₁₀ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aminoacyl, aryl, such as phenyl, heteroaryl, such as, pyridinyl, substituted aryl, or substituted heteroaryl;

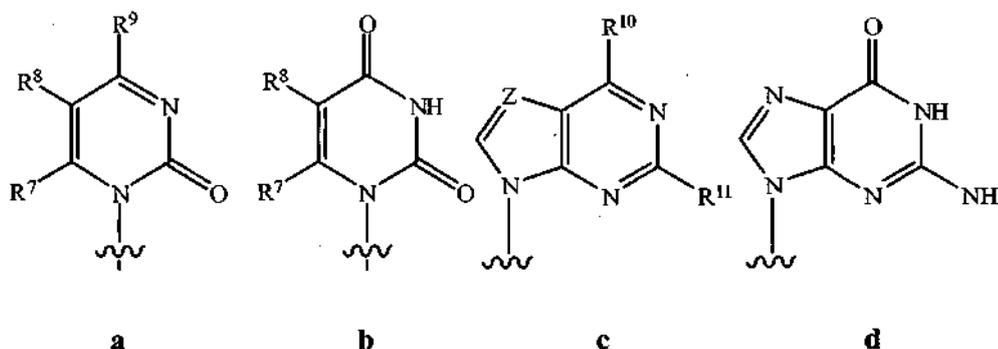
(e) R⁵ is H, a lower alkyl, CN, vinyl, O-(lower alkyl), hydroxyl lower alkyl, i.e., -(CH₂)_pOH, where p is 1-6, including hydroxyl methyl (CH₂OH), CH₂F, N₃, CH₂CN, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or halogen, including F, Cl, Br, or I, with the provisos that when X is OH, base is cytosine and R⁶ is H, R⁵ cannot be N₃ and when X is OH, R⁶ is CH₃ or CH₂F and B is a purine base, R⁵ cannot be H;

(f) R⁶ is H, CH₃, CH₂F, CHF₂, CF₃, F, or CN;

(g) X is H, OH, F, OMe, halogen, NH₂, or N₃;

(h) Y is OH, H, C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, OC(O)O(C₁₋₄ alkyl), OC(O)O(C₁₋₄ alkyl), OC(O)O(C₂₋₄ alkynyl), OC(O)O(C₂₋₄ alkenyl), OC₁₋₁₀ haloalkyl, O(aminoacyl), O(C₁₋₁₀ acyl), O(C₁₋₄ alkyl), O(C₂₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₂₋₄ alkynyl), S(C₂₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₂₋₄ alkynyl), SO(C₂₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₂₋₄ alkynyl), SO₂(C₂₋₄ alkenyl), OS(O)₂(C₁₋₄ acyl), OS(O)₂(C₁₋₄ alkyl), OS(O)₂(C₂₋₄ alkynyl), OS(O)₂(C₂₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₂₋₄ alkenyl), NH(C₂₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₂₋₄ alkynyl), C(O)O(C₂₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₂₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₂₋₄ alkynyl), S(C₂₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₂₋₄ alkynyl), SO(C₂₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₂₋₄ alkynyl), SO₂(C₂₋₄ alkenyl), OS(O)₂(C₁₋₄ acyl), OS(O)₂(C₁₋₄ alkyl), OS(O)₂(C₂₋₄ alkynyl), OS(O)₂(C₂₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₂₋₄ alkenyl), NH(C₂₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂;

the base is a naturally occurring or modified purine or pyrimidine base represented by the following structures:



wherein

Z is N or CR¹²;

R⁷, R⁸, R⁹, R¹⁰, and R¹¹ are independently H, F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁₋₆, halogenated (F, Cl, Br, I) lower alkyl of C₁₋₆, lower alkenyl of C₂₋₆, halogenated (F, Cl, Br, I) lower alkenyl of C₂₋₆, lower alkynyl of C₂₋₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂₋₆, lower alkoxy of C₁₋₆, halogenated (F, Cl, Br, I) lower alkoxy of C₁₋₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, or CH=CHCO₂R',

wherein R' is an optionally substituted alkyl, which includes, but is not limited to, an optionally substituted C₁₋₂₀

alkyl, an optionally substituted C₁₋₁₀ alkyl, an optionally substituted lower alkyl; an optionally substituted cycloalkyl; an optionally substituted alkenyl of C₂₋₆, an optionally substituted lower alkenyl of C₂₋₆, or optionally substituted acyl, which includes but is not limited to C(O) alkyl, C(O)(C₁₋₂₀ alkyl), C(O)(C₁₋₁₀ alkyl), or C(O)(lower alkyl) or alternatively, in the instance of NR'₂, each R' comprise at least one C atom that are joined to form a heterocycle comprising at least two carbon atoms; and

R¹² is H, halogen (including F, Cl, Br, I), OH, OR', SH, SR', NH₂, NHR', NR'₂, NO₂ lower alkyl of C₁₋₆, halogenated (F, Cl, Br, I) lower alkyl of C₁₋₆, lower alkenyl of C₂₋₆, halogenated (F, Cl, Br, I) lower alkenyl of C₂₋₆, lower alkynyl of C₂₋₆, halogenated (F, Cl, Br, I) lower alkynyl of C₂₋₆, lower alkoxy of C₁₋₆, halogenated (F, Cl, Br, I) lower alkoxy of C₁₋₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, or CH=CHCO₂R'; with the proviso that when base is represented by the structure c with R¹¹ being hydrogen, R¹² is not a: (i) -C≡C-H, (ii) -C=CH₂, or (iii) -NO₂.

DEFINITIONS

[0021] The phrase "a" or "an" entity as used herein refers to one or more of that entity; for example, a compound refers to one or more compounds or at least one compound. As such, the terms "a" (or "an"), "one or more", and "at least one" can be used interchangeably herein.

[0022] The phrase "as defined herein above" refers to the first definition provided in the Summary of the Invention.

[0023] The terms "optional" or "optionally" as used herein means that a subsequently described event or circumstance may but need not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. For example, "optional bond" means that the bond may or may not be present, and that the description includes single, double, or triple bonds.

[0024] The term "independently" is used herein to indicate that a variable is applied in any one instance without regard to the presence or absence of a variable having that same or a different definition within the same compound. Thus, in a compound in which R appears twice and is defined as "independently carbon or nitrogen", both R's can be carbon, both R's can be nitrogen, or one R' can be carbon and the other nitrogen.

[0025] The term "alkenyl" refers to an unsubstituted hydrocarbon chain radical having from 2 to 10 carbon atoms having one or two olefinic double bonds, preferably one olefinic double bond. The term "C_{2-N} alkenyl" refers to an alkenyl comprising 2 to N carbon atoms, where N is an integer having the following values: 3, 4, 5, 6, 7, 8, 9, or 10. The term "C₂₋₁₀ alkenyl" refers to an alkenyl comprising 2 to 10 carbon atoms. The term "C₂₋₄ alkenyl" refers to an alkenyl comprising 2 to 4 carbon atoms. Examples include, but are not limited to, vinyl, 1-propenyl, 2-propenyl (allyl) or 2-butenyl (crotyl).

[0026] The term "halogenated alkenyl" refers to an alkenyl comprising at least one of F, Cl, Br, and I.

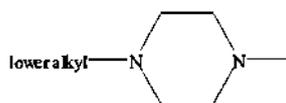
[0027] The term "alkyl" refers to an unbranched or branched chain, saturated, monovalent hydrocarbon residue containing 1 to 30 carbon atoms. The term "C_{1-M} alkyl" refers to an alkyl comprising 1 to M carbon atoms, where M is an integer having the following values: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30. The term "C₁₋₄ alkyl" refers to an alkyl containing 1 to 4 carbon atoms. The term "lower alkyl" denotes a straight or branched chain hydrocarbon residue comprising 1 to 6 carbon atoms. "C₁₋₂₀ alkyl" as used herein refers to an alkyl comprising 1 to 20 carbon atoms. "C₁₋₁₀ alkyl" as used herein refers to an alkyl comprising 1 to 10 carbons. Examples of alkyl groups include, but are not limited to, lower alkyl groups include methyl, ethyl, propyl, *i*-propyl, *n*-butyl, *i*-butyl, *t*-butyl or pentyl, isopentyl, neopentyl, hexyl, heptyl, and octyl. The term (ar)alkyl or (heteroaryl)alkyl indicate the alkyl group is optionally substituted by an aryl or a heteroaryl group respectively.

[0028] The term "cycloalkyl" refers to an unsubstituted or substituted carbocycle, in which the carbocycle contains 3 to 10 carbon atoms; preferably 3 to 8 carbon atoms; more preferably 3 to 6 carbon atoms (i.e., lower cycloalkyls). Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, 2-methyl-cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

[0029] The term "cycloalkyl alkyl" refers to an additionally unsubstituted or substituted alkyl substituted by a lower cycloalkyl. Examples of cycloalkyl alkyls include, but are not limited to, any one of methyl, ethyl, propyl, *i*-propyl, *n*-butyl, *i*-butyl, *t*-butyl or pentyl, isopentyl, neopentyl, hexyl, heptyl, and octyl that is substituted with cyclopropyl, 2-methyl-cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

[0030] The term "cycloheteroalkyl" refers to an unsubstituted or substituted heterocycle, in which the heterocycle contains 2 to 9 carbon atoms; preferably 2 to 7 carbon atoms; more preferably 2 to 5 carbon atoms. Examples of cycloheteroalkyls include, but are not limited to, aziridin-2-yl, *N*-C₁₋₃-alkyl-aziridin-2-yl, azetidiny, *N*-C₁₋₃-alkyl-azetidiny, pyrrolidin-*m*'-yl, *N*-C₁₋₃-alkyl-pyrrolidin-*m*'-yl, piperidin-*m*'-yl, and *N*-C₁₋₃-alkyl-piperidin-*m*'-yl, where *m*' is 2, 3, or 4 depending on the cycloheteroalkyl. Specific examples of *N*-C₁₋₃-alkyl-cycloheteroalkyls include, but are not limited to, *N*-methyl-aziridin-2-yl, *N*-methyl-azetidiny, *N*-methyl-pyrrolidin-3-yl, *N*-methyl-pyrrolidin-4-yl, *N*-methyl-piperidin-2-yl, *N*-methyl-piperidin-3-yl, and *N*-methyl-piperidin-4-yl. In the instance of R⁴, the point of attachment between the cycloheteroalkyl ring carbon and the oxygen occurs at any one of *m*'

[0031] The term "heterocycle" refers to an unsubstituted or substituted heterocycle containing carbon, hydrogen, and at least one of N, O, and S, where the C and N can be trivalent or tetravalent, i.e., sp²- or sp³-hybridized. Examples of heterocycles include, but are not limited to, aziridine, azetidine, pyrrolidine, piperidine, imidazole, oxazole, piperazine, etc. In the instance of piperazine, as related to R¹⁰ for NR², the corresponding opposite nitrogen atom of the piperazinyl is substituted by a lower alkyl represented by the following structure:



Preferably, the opposite nitrogen of the piperazinyl is substituted by a methyl group.

[0032] The term "halogenated alkyl" (or "haloalkyl") refers to an unbranched or branched chain alkyl comprising at least one of F, Cl, Br, and I. The term "C_{1-M} haloalkyl" refers to an alkyl comprising 1 to M carbon atoms that comprises at least one of F, Cl, Br, and I, where M is an integer having the following values: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30. "C₁₋₃ haloalkyl" refers to a haloalkyl comprising 1 to 3 carbons and at least one of F, Cl, Br, and I. The term "halogenated lower alkyl" (or "lower haloalkyl") refers to a haloalkyl comprising 1 to 6 carbon atoms and at least one of F, Cl, Br, and I. Examples include, but are not limited to, fluoromethyl, chloromethyl, bromomethyl, iodomethyl, difluoromethyl, dichloromethyl, dibromomethyl, diiodomethyl, trifluoromethyl, trichloromethyl, tribromomethyl, triiodomethyl, 1-fluoroethyl, 1-chloroethyl, 1-bromoethyl, 1-iodoethyl, 2-fluoroethyl, 2-chloroethyl, 2-bromoethyl, 2-iodoethyl, 2,2-difluoroethyl, 2,2-dichloroethyl, 2,2-dibromomethyl, 2,2-diiodomethyl, 3-fluoropropyl, 3-chloropropyl, 3-bromopropyl, 2,2,2-trifluoroethyl or 1,1,2,2,2-pentafluoroethyl.

[0033] The term "alkynyl" refers to an unbranched or branched hydrocarbon chain radical having from 2 to 10 carbon atoms, preferably 2 to 5 carbon atoms, and having one triple bond. The term "C_{2-N} alkynyl" refers to an alkynyl, comprising 2 to N carbon atoms, where N is an integer having the following values: 3, 4, 5, 6, 7, 8, 9, or 10. The term "C₂₋₄ alkynyl" refers to an alkynyl comprising 2 to 4 carbon atoms. The term "C₂₋₁₀ alkynyl" refers to an alkynyl comprising 2 to 10 carbons. Examples include, but are limited to, ethynyl, 1-propynyl, 2-propynyl, 1-butylnyl, 2-butylnyl or 3-butylnyl.

[0034] The term "halogenated alkynyl" refers to an unbranched or branched hydrocarbon chain radical having from 2 to 10 carbon atoms, preferably 2 to 5 carbon atoms, and having one triple bond and at least one of F, Cl, Br, and I.

[0035] The term "cycloalkyl" refers to a saturated carbocyclic ring comprising 3 to 8 carbon atoms, i.e. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or cyclooctyl. The term "C₃₋₇ cycloalkyl" as used herein refers to a cycloalkyl comprising 3 to 7 carbons in the carbocyclic ring.

[0036] The term "alkoxy" refers to an -O-alkyl group or an -O-cycloalkyl group, wherein alkyl and cycloalkyl are as defined above. Examples of -O-alkyl groups include, but are not limited to, methoxy, ethoxy, *n*-propyloxy, *i*-propyloxy, *n*-butyloxy, *i*-butyloxy, *t*-butyloxy. "Lower alkoxy" as used herein denotes an alkoxy group with a "lower alkyl" group as previously defined. "C₁₋₁₀ alkoxy" refers to an -O-alkyl wherein alkyl is C₁₋₁₀. Examples of -O-cycloalkyl groups include, but are not limited to, -O-*c*-propyl, -O-*c*-butyl, -O-*c*-pentyl, and -O-*c*-hexyl.

[0037] The term "halogenated alkoxy" refers to an -O-alkyl group in which the alkyl group comprises at least one of F, Cl, Br, and I.

[0038] The term "halogenated lower alkoxy" refers to an -O-(lower alkyl) group in which the lower alkyl group comprises at least one of F, Cl, Br, and I.

[0039] The term "amino acid" includes naturally occurring and synthetic α , β , γ or δ amino acids, and includes but is not limited to, amino acids found in proteins, i.e. glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartate, glutamate, lysine, arginine and histidine. In a preferred embodiment, the amino acid is in the L-configuration. Alternatively, the amino acid can be a derivative of alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaroyl, lysinyl, argininyl, histidinyl, β -alanyl, β -valinyl, β -leucinyl, β -isoleucinyl, β -prolinyl, β -phenylalaninyl, β -tryptophanyl, β -methioninyl, β -glycyl, β -serinyl, β -threoninyl, β -cysteinyl, β -tyrosinyl, β -asparaginyl, β -glutaminyl, β -aspartoyl, β -glutaroyl, β -lysinyl, β -argininyl or β -histidinyl. When the term amino acid is used, it is considered to be a specific and independent disclosure of each of the esters of α , β , γ or δ glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartate, glutamate, lysine, arginine and histidine in the D and L-configurations.

[0040] The term "aminoacyl" includes N,N-unsubstituted, N,N-monosubstituted, and N,N-disubstituted derivatives of naturally occurring and synthetic α , β , γ or δ amino acyls, where the amino acyls are derived from amino acids. The amino-nitrogen can be substituted or unsubstituted. When the amino-nitrogen is substituted, the nitrogen is either mono- or di-substituted, where the substituent bound to the amino-nitrogen is a lower alkyl or an alkaryl. In the instance of its use for Y, the expression "O(aminoacyl)" is used. It is understood that the C3' carbon of the ribose is bound to the oxygen "O", which is then bound to the carbonyl carbon of the aminoacyl.

[0041] The terms "alkylamino" or "arylamino" refer to an amino group that has one or two alkyl or aryl substituents, respectively.

[0042] The term "protected," as used herein and unless otherwise defined, refers to a group that is added to an oxygen, nitrogen, or phosphorus atom to prevent its further reaction or for other purposes. A wide variety of oxygen and nitrogen protecting groups are known to those skilled in the art of organic synthesis. Nonlimiting examples include: C(O)-alkyl, C(O)Ph, C(O)aryl, CH₃, CH₂-alkyl, CH₂alkenyl, CH₂Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-alkyl, SO₂-aryl, *tert*-butyldimethylsilyl, *tert*-butyldiphenylsilyl, and 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene).

[0043] The term "aryl," as used herein, and unless otherwise specified, refers to substituted or unsubstituted phenyl (Ph), biphenyl, or naphthyl, preferably the term aryl refers to substituted or unsubstituted phenyl. The aryl group can be substituted with one or more moieties selected from among hydroxyl, F, Cl, Br, I, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, and phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in T.W. Greene and P.G. M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999.

[0044] The terms "alkaryl" or "alkylaryl" refer to an alkyl group with an aryl substituent, such as benzyl. The terms "aralkyl" or "arylalkyl" refer to an aryl group with an alkyl substituent.

[0045] The term "di(lower alkyl)amino-lower alkyl" refers to a lower alkyl substituted by an amino group that is itself substituted by two lower alkyl groups. Examples include, but are not limited to, (CH₃)₂NCH₂, (CH₃)₂NCH₂CH₂, (CH₃)₂NCH₂CH₂CH₂, etc. The examples above show lower alkyls substituted at the terminus carbon atom with an N,N-dimethyl-amino substituent. These are intended as examples only and are not intended to limit the meaning of the term "di(lower alkyl)amino-lower alkyl" so as to require the same. It is contemplated that the lower alkyl chain can be substituted with an N,N-di(lower alkyl)-amino at any point along the chain, e.g., CH₃CH(N-(lower alkyl)₂)CH₂CH₂.

[0046] The term "halo," as used herein, includes chloro, bromo, iodo and fluoro.

[0047] The term "acyl" refers to a substituent containing a carbonyl moiety and a non-carbonyl moiety. The carbonyl moiety contains a double-bond between the carbonyl carbon and a heteroatom, where the heteroatom is selected from among O, N and S. When the heteroatom is N, the N is substituted by a lower alkyl. The non-carbonyl moiety is selected from straight, branched, and cyclic alkyl, which includes, but is not limited to, a straight, branched, or cyclic C₁₋₂₀ alkyl, C₁₋₁₀ alkyl, or lower alkyl; alkoxyalkyl, including methoxymethyl; aralkyl, including benzyl; aryloxyalkyl, such as phenoxymethyl; or aryl, including phenyl optionally substituted with halogen (F, Cl, Br, I), hydroxyl, C₁ to C₄ alkyl, or C₁ to C₄ alkoxy, sulfonate esters, such as alkyl or aralkyl sulphonyl, including methanesulfonyl, the mono, di or triphosphate ester, trityl or monomethoxytrityl, substituted benzyl, trialkylsilyl (e.g. dimethyl-*t*-butylsilyl) or diphenylmethylsilyl. When at least one aryl group is present in the non-carbonyl moiety, it is preferred that the aryl group comprises a phenyl group.

[0048] The term "lower acyl" refers to an acyl group in which the non-carbonyl moiety is lower alkyl.

[0049] The term "purine" or "pyrimidine" base includes, but is not limited to, adenine, N⁶-alkylpurines, N⁶-acylpurines (wherein acyl is C(O)(alkyl, aryl, alkylaryl, or arylalkyl), N⁶-benzylpurine, N⁶-halopurine, N⁶-vinylpurine, N⁶-acetylenic purine, N⁶-acyl purine, N⁶-hydroxyalkyl purine, N⁶-allylaminopurine, N⁶-thioallyl purine, N²-alkylpurines, N²-alkyl-6-thiopurines, thymine, cytosine, 5-fluorocytosine, 5-methylcytosine, 6-azapyrimidine, including 6-azacytosine, 2- and/or 4-mercaptopyrimidine, uracil, 5-halouracil, including 5-fluorouracil, C⁵-alkylpyrimidines, C⁵-benzylpyrimidines, C⁵-halopyrimidines, C⁵-vinylpyrimidine, C⁵-acetylenic pyrimidine, C⁵-acyl pyrimidine, C⁵-hydroxyalkyl purine, C⁵-amidopyrimidine, C⁵-cyanopyrimidine, C⁵-iodopyrimidine, C⁶-iodo-pyrimidine, C⁵-Br-vinyl pyrimidine, C⁶-Br-vinyl pyrimidine, C⁵-nitropurine, C⁵-amino-pyrimidine, N²-alkylpurines, N²-alkyl-6-thiopurines, 5-azacytidinyl, 5-azauracilyl, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, and pyrazolopyrimidinyl. Purine bases include, but are not limited to, guanine, adenine, hypoxanthine, 2,6-diaminopurine, and 6-chloropurine. Functional oxygen and nitrogen groups on the base can be protected as necessary or desired. Suitable protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, *t*-butyldimethylsilyl, and *t*-butyldiphenylsilyl, trityl, alkyl groups, and acyl groups such as acetyl and propionyl, methanesulfonyl, and *p*-toluenesulfonyl.

[0050] The term "tautomerism" and "tautomers" have their accepted plain meanings.

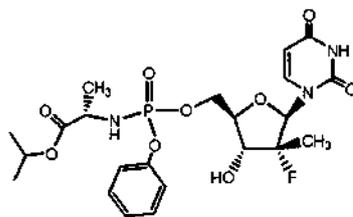
[0051] The term "P*" means that the phosphorous atom is chiral and that it has a corresponding Cahn-Ingold-Prelog designation of "R" or "S" which have their accepted plain meanings. It is contemplated that compounds of the formula I are racemic because the chirality at phosphorous. Applicants contemplate use of the racemate and/or the resolved enantiomers. In some instances, an asterisk does not appear next to the phosphoroamidate phosphorous atom. In these instances, it is understood that the phosphorous atom is chiral and that one of ordinary skill understands this to be so unless the substituents bound to the phosphorous exclude the possibility of chirality at phosphorous, such as in P(O)Cl₃.

DETAILED DESCRIPTION OF THE INVENTION

[0052] The present invention is as set out in the following clauses:

1. A compound represented by the formula

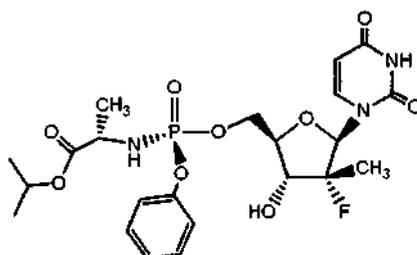
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2. A compound represented by the formula

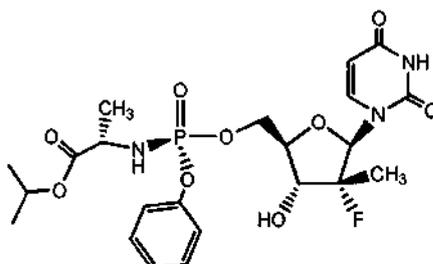
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3. A compound represented by the formula

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4. A composition comprising the compound of clause 1 and a pharmaceutically acceptable medium.

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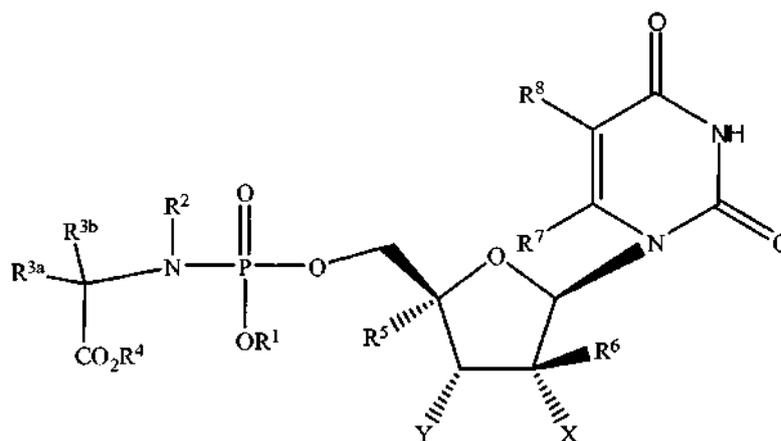
5. A composition comprising the compound of clause 2 and a pharmaceutically acceptable medium.

6. A composition comprising the compound of clause 3 and a pharmaceutically acceptable medium.

40

[0053] The following tables contain numeric identifiers associated with various substituent designators that should be viewed in light of the accompanying structure. These structures are contemplated species of the various aspects of the present disclosure. However, it is contemplated that any one of the exemplified nucleoside bases can be used in combination with any one of contemplated species that specify a particular combination of R^1 , R^2 , R^{3a} , R^{3b} , R^4 , R^5 , R^6 , X , and Y . In each of the presented tables, the phosphoramidate substituent containing the substituents R^{3a} and R^{3b} are depicted without reference to stereochemical structure. It is contemplated that the compounds recited below embody compounds in which R^{3a} projects toward the viewer while R^{3b} projects away from the viewer. Moreover, it is contemplated that the compounds recited below also embody compounds in which R^{3a} projects away from the viewer while R^{3b} projects towards the viewer. Not meant to be limiting, however, it is contemplated that preferred compounds are those in which R^{3a} projects towards the viewer and R^{3b} projects away from the viewer such that the natural L-amino acid (S)-configuration is presented. Additionally, the inventors recognize that the phosphorus atom of the phosphoramidate moiety is another source of chirality. Although the structures below do not specifically depict chirality at phosphorus, the inventors recognize that stereochemical configurations are possible such that in a staggered (or zig-zag) line structure the oxo-substituent projects towards the viewer while the OR^1 substituent projects away from the viewer, and vice versa, i.e., where the Cahn-Ingold-Prelog stereochemical designation of phosphorus is either R or S. Therefore, the structures below include all possible stereochemical configurations possible for phosphorus.

55



IX

Table IX-1.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-1-1	CH ₃	H	H	H	CH ₃	H	CH ₃	F	OH	H	H
IX-1-2	CH ₃	H	H	CH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-1-3	CH ₃	H	H	CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-1-4	CH ₃	H	H	CH ₂ CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-1-5	CH ₃	H	H	CH ₂ Ph	CH ₃	H	CH ₃	F	OH	H	H
IX-1-6	CH ₃	H	H	CH ₂ -indol-3-yl	CH ₃	H	CH ₃	F	OH	H	H
IX-1-7	CH ₃	H	H	CH ₂ CH ₂ SCH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-1-8	CH ₃	*	H	*	CH ₃	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-2.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-2-1	Et	H	H	H	CH ₃	H	CH ₃	F	OH	H	H
IX-2-2	Et	H	H	CH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-2-3	Et	H	H	CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-2-4	Et	H	H	CH ₂ CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-2-5	Et	H	H	CH ₂ Ph	CH ₃	H	CH ₃	F	OH	H	H
IX-2-6	Et	H	H	CH ₂ -indol-3-yl	CH ₃	H	CH ₃	F	OH	H	H
IX-2-7	Et	H	H	CH ₂ CH ₂ SCH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-2-8	Et	*	H	*	CH ₃	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-3.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-3-1	ⁱ Pr	H	H	H	CH ₃	H	CH ₃	F	OH	H	H
IX-3-2	ⁱ Pr	H	H	CH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-3-3	ⁱ Pr	H	H	CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-3-4	ⁱ Pr	H	H	CH ₂ CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H

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(continued)

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-3-5	^t Pr	H	H	CH ₂ Ph	CH ₃	H	CH ₃	F	OH	H	H
IX-3-6	^t Pr	H	H	CH ₂ -indol-3-yl	CH ₃	H	CH ₃	F	OH	H	H
IX-3-7	^t Pr	H	H	CH ₂ CH ₂ SCH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-3-8	^t Pr	*	H	*	CH ₃	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-4.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-4-1	^t Bu	H	H	H	CH ₃	H	CH ₃	F	OH	H	H
IX-4-2	^t Bu	H	H	CH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-4-3	^t Bu	H	H	CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-4-4	^t Bu	H	H	CH ₂ CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-4-5	^t Bu	H	H	CH ₂ Ph	CH ₃	H	CH ₃	F	OH	H	H
IX-4-6	^t Bu	H	H	CH ₂ -indol-3-yl	CH ₃	H	CH ₃	F	OH	H	H
IX-4-7	^t Bu	H	H	CH ₂ CH ₂ SCH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-4-8	^t Bu	*	H	*	CH ₃	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-5.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-5-1	Ph	H	H	H	CH ₃	H	CH ₃	F	OH	H	H
IX-5-2	Ph	H	H	CH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-5-3	Ph	H	H	CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-5-4	Ph	H	H	CH ₂ CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-5-5	Ph	H	H	CH ₂ Ph	CH ₃	H	CH ₃	F	OH	H	H
IX-5-6	Ph	H	H	CH ₂ -indol-3-yl	CH ₃	H	CH ₃	F	OH	H	H
IX-5-7	Ph	H	H	CH ₂ CH ₂ SCH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-5-8	Ph	*	H	*	CH ₃	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-6.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-6-1	p-Me-Ph	H	H	H	CH ₃	H	CH ₃	F	OH	H	H
IX-6-2	p-Me-Ph	H	H	CH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-6-3	p-Me-Ph	H	H	CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-6-4	p-Me-Ph	H	H	CH ₂ CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
IX-6-5	p-Me-Ph	H	H	CH ₂ Ph	CH ₃	H	CH ₃	F	OH	H	H
IX-6-6	p-Me-Ph	H	H	CH ₂ -indol-3-yl	CH ₃	H	CH ₃	F	OH	H	H
IX-6-7	p-Me-Ph	H	H	CH ₂ CH ₂ SCH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-6-8	p-Me-Ph	*	H	*	CH ₃	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-7.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
5	IX-7-1	p-F-Ph	H	H	H	CH ₃	H	CH ₃	F	OH	H	H
	IX-7-2	p-F-Ph	H	H	CH ₃	CH ₃	H	CH ₃	F	OH	H	H
	IX-7-3	p-F-Ph	H	H	CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
	IX-7-4	p-F-Ph	H	H	CH ₂ CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
10	IX-7-6	p-F-Ph	H	H	CH ₂ Ph	CH ₃	H	CH ₃	F	OH	H	H
	IX-7-7	p-F-Ph	H	H	CH ₂ -indol-3-yl	CH ₃	H	CH ₃	F	OH	H	H
	IX-7-8	p-F-Ph	H	H	CH ₂ CH ₂ SCH ₃	CH ₃	H	CH ₃	F	OH	H	H
	IX-7-20	p-F-Ph	*	H	*	CH ₃	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-8.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
20	IX-8-1	p-Cl-Ph	H	H	H	CH ₃	H	CH ₃	F	OH	H	H
	IX-8-2	p-Cl-Ph	H	H	CH ₃	CH ₃	H	CH ₃	F	OH	H	H
	IX-8-3	p-Cl-Ph	H	H	CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
	IX-8-4	p-Cl-Ph	H	H	CH ₂ CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
25	IX-8-5	p-Cl-Ph	H	H	CH ₂ Ph	CH ₃	H	CH ₃	F	OH	H	H
	IX-8-6	p-Cl-Ph	H	H	CH ₂ -indol-3-yl	CH ₃	H	CH ₃	F	OH	H	H
	IX-8-7	p-Cl-Ph	H	H	CH ₂ CH ₂ SCH ₃	CH ₃	H	CH ₃	F	OH	H	H
	IX-8-8	p-Cl-Ph	*	H	*	CH ₃	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-9.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
35	IX-9-1	p-Br-Ph	H	H	H	CH ₃	H	CH ₃	F	OH	H	H
	IX-9-2	p-Br-Ph	H	H	CH ₃	CH ₃	H	CH ₃	F	OH	H	H
	IX-9-3	p-Br-Ph	H	H	CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
40	IX-9-4	p-Br-Ph	H	H	CH ₂ CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
	IX-9-6	p-Br-Ph	H	H	CH ₂ Ph	CH ₃	H	CH ₃	F	OH	H	H
	IX-9-7	p-Br-Ph	H	H	CH ₂ -indol-3-yl	CH ₃	H	CH ₃	F	OH	H	H
	IX-9-8	p-Br-Ph	H	H	CH ₂ CH ₂ SCH ₃	CH ₃	H	CH ₃	F	OH	H	H
45	IX-9-20	p-Br-Ph	*	H	*	CH ₃	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-10.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
50	IX-10-1	p-I-Ph	H	H	H	CH ₃	H	CH ₃	F	OH	H	H
	IX-10-2	p-I-Ph	H	H	CH ₃	CH ₃	H	CH ₃	F	OH	H	H
	IX-10-3	p-I-Ph	H	H	CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
55	IX-10-4	p-I-Ph	H	H	CH ₂ CH(CH ₃) ₂	CH ₃	H	CH ₃	F	OH	H	H
	IX-10-5	p-I-Ph	H	H	CH ₂ Ph	CH ₃	H	CH ₃	F	OH	H	H
	IX-10-6	p-I-Ph	H	H	CH ₂ -indol-3-yl	CH ₃	H	CH ₃	F	OH	H	H

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(continued)

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-10-7	p-l-Ph	H	H	CH ₂ CH ₂ SCH ₃	CH ₃	H	CH ₃	F	OH	H	H
IX-10-8	p-l-Ph	*	H	*	CH ₃	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-11.

No	R ¹	R ¹	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-11-1	CH ₃	H	H	H	Et	H	CH ₃	F	OH	H	H
IX-11-2	CH ₃	H	H	CH ₃	Et	H	CH ₃	F	OH	H	H
IX-11-3	CH ₃	H	H	CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-11-4	CH ₃	H	H	CH ₂ CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-11-5	CH ₃	H	H	CH ₂ Ph	Et	H	CH ₃	F	OH	H	H
IX-11-6	CH ₃	H	H	CH ₂ -indol-3-yl	Et	H	CH ₃	F	OH	H	H
IX-11-7	CH ₃	H	H	CH ₂ CH ₂ SCH ₃	Et	H	CH ₃	F	OH	H	H
IX-11-8	CH ₃	*	H	*	Et	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-12.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-12-1	Et	H	H	H	Et	H	CH ₃	F	OH	H	H
IX-12-2	Et	H	H	CH ₃	Et	H	CH ₃	F	OH	H	H
IX-12-3	Et	H	H	CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-12-4	Et	H	H	CH ₂ CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-12-5	Et	H	H	CH ₂ Ph	Et	H	CH ₃	F	OH	H	H
IX-12-6	Et	H	H	CH ₂ -indol-3-yl	Et	H	CH ₃	F	OH	H	H
IX-12-7	Et	H	H	CH ₂ CH ₂ SCH ₃	Et	H	CH ₃	F	OH	H	H
IX-12-8	Et	*	H	*	Et	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-13.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-13-1	ⁱ Pr	H	H	H	Et	H	CH ₃	F	OH	H	H
IX-13-2	ⁱ Pr	H	H	CH ₃	Et	H	CH ₃	F	OH	H	H
IX-13-3	ⁱ Pr	H	H	CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-13-4	ⁱ Pr	H	H	CH ₂ CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-13-5	ⁱ Pr	H	H	CH ₂ Ph	Et	H	CH ₃	F	OH	H	H
IX-13-6	ⁱ Pr	H	H	CH ₂ -indol-3-yl	Et	H	CH ₃	F	OH	H	H
IX-13-7	ⁱ Pr	H	H	CH ₂ CH ₂ SCH ₃	Et	H	CH ₃	F	OH	H	H
IX-13-8	ⁱ Pr	*	H	*	Et	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

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Table IX-14.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-14-1	^t Bu	H	H	H	Et	H	CH ₃	F	OH	H	H
IX-14-2	^t Bu	H	H	CH ₃	Et	H	CH ₃	F	OH	H	H
IX-14-3	^t Bu	H	H	CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-14-4	^t Bu	H	H	CH ₂ CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-14-5	^t Bu	H	H	CH ₂ Ph	Et	H	CH ₃	F	OH	H	H
IX-14-6	^t Bu	H	H	CH ₂ -indol-3-yl	Et	H	CH ₃	F	OH	H	H
IX-14-7	^t Bu	H	H	CH ₂ CH ₂ SCH ₃	Et	H	CH ₃	F	OH	H	H
IX-14-8	^t Bu	*	H	*	Et	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-15.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-15-1	Ph	H	H	H	Et	H	CH ₃	F	OH	H	H
IX-15-2	Ph	H	H	CH ₃	Et	H	CH ₃	F	OH	H	H
IX-15-3	Ph	H	H	CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-15-4	Ph	H	H	CH ₂ CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-15-5	Ph	H	H	CH ₂ Ph	Et	H	CH ₃	F	OH	H	H
IX-15-6	Ph	H	H	CH ₂ -indol-3-yl	Et	H	CH ₃	F	OH	H	H
IX-15-7	Ph	H	H	CH ₂ CH ₂ SCH ₃	Et	H	CH ₃	F	OH	H	H
IX-15-8	Ph	*	H	*	Et	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-16.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-16-1	p-Me-Ph	H	H	H	Et	H	CH ₃	F	OH	H	H
IX-16-2	p-Me-Ph	H	H	CH ₃	Et	H	CH ₃	F	OH	H	H
IX-16-3	p-Me-Ph	H	H	CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-16-4	p-Me-Ph	H	H	CH ₂ CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-16-5	p-Me-Ph	H	H	CH ₂ Ph	Et	H	CH ₃	F	OH	H	H
IX-16-6	p-Me-Ph	H	H	CH ₂ -indol-3-yl	Et	H	CH ₃	F	OH	H	H
IX-16-7	p-Me-Ph	H	H	CH ₂ CH ₂ SCH ₃	Et	H	CH ₃	F	OH	H	H
IX-16-8	p-Me-Ph	*	H	*	Et	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-17.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-17-1	p-F-Ph	H	H	H	Et	H	CH ₃	F	OH	H	H
IX-17-2	p-F-Ph	H	H	CH ₃	Et	H	CH ₃	F	OH	H	H
IX-17-3	p-F-Ph	H	H	CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-17-4	p-F-Ph	H	H	CH ₂ CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-17-5	p-F-Ph	H	H	CH ₂ Ph	Et	H	CH ₃	F	OH	H	H
IX-17-6	p-F-Ph	H	H	CH ₂ -indol-3-yl	Et	H	CH ₃	F	OH	H	H
IX-17-7	p-F-Ph	H	H	CH ₂ CH ₂ SCH ₃	Et	H	CH ₃	F	OH	H	H

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(continued)

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-17-8	p-F-Ph	*	H	*	Et	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-18.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-18-1	p-Cl-Ph	H	H	H	Et	H	CH ₃	F	OH	H	H
IX-18-2	p-Cl-Ph	H	H	CH ₃	Et	H	CH ₃	F	OH	H	H
IX-18-3	p-Cl-Ph	H	H	CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-18-4	p-Cl-Ph	H	H	CH ₂ CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-18-5	p-Cl-Ph	H	H	CH ₂ Ph	Et	H	CH ₃	F	OH	H	H
IX-18-6	p-Cl-Ph	H	H	CH ₂ -indol-3-yl	Et	H	CH ₃	F	OH	H	H
IX-18-7	p-Cl-Ph	H	H	CH ₂ CH ₂ SCH ₃	Et	H	CH ₃	F	OH	H	H
IX-18-8	p-Cl-Ph	*	H	*	Et	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-19.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-19-1	p-Br-Ph	H	H	H	Et	H	CH ₃	F	OH	H	H
IX-19-2	p-Br-Ph	H	H	CH ₃	Et	H	CH ₃	F	OH	H	H
IX-19-3	p-Br-Ph	H	H	CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-19-4	p-Br-Ph	H	H	CH ₂ CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-19-5	p-Br-Ph	H	H	CH ₂ Ph	Et	H	CH ₃	F	OH	H	H
IX-19-6	p-Br-Ph	H	H	CH ₂ -indol-3-yl	Et	H	CH ₃	F	OH	H	H
IX-19-7	p-Br-Ph	H	H	CH ₂ CH ₂ SCH ₃	Et	H	CH ₃	F	OH	H	H
IX-19-8	p-Br-Ph	*	H	*	Et	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-20.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-20-1	p-I-Ph	H	H	H	Et	H	CH ₃	F	OH	H	H
IX-20-2	p-I-Ph	H	H	CH ₃	Et	H	CH ₃	F	OH	H	H
IX-20-3	p-I-Ph	H	H	CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-20-4	p-I-Ph	H	H	CH ₂ CH(CH ₃) ₂	Et	H	CH ₃	F	OH	H	H
IX-20-5	p-I-Ph	H	H	CH ₂ Ph	Et	H	CH ₃	F	OH	H	H
IX-20-6	p-I-Ph	H	H	CH ₂ -indol-3-yl	Et	H	CH ₃	F	OH	H	H
IX-20-7	p-I-Ph	H	H	CH ₂ CH ₂ SCH ₃	Et	H	CH ₃	F	OH	H	H
IX-20-8	p-I-Ph	*	H	*	Et	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

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Table IX-21.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
5	IX-21-1	CH ₃	H	H	H	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-21-2	CH ₃	H	H	CH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-21-3	CH ₃	H	H	CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-21-4	CH ₃	H	H	CH ₂ CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-21-5	CH ₃	H	H	CH ₂ Ph	<i>i</i> Pr	H	CH ₃	F	OH	H	H
10	IX-21-6	CH ₃	H	H	CH ₂ -indol-3-yl	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-21-7	CH ₃	H	H	CH ₂ CH ₂ SCH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-21-8	CH ₃	*	H	*	<i>i</i> Pr	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-22.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
20	IX-22-1	Et	H	H	H	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-22-2	Et	H	H	CH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-22-3	Et	H	H	CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-22-4	Et	H	H	CH ₂ CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
25	IX-22-5	Et	H	H	CH ₂ Ph	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-22-6	Et	H	H	CH ₂ -indol-3-yl	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-22-7	Et	H	H	CH ₂ CH ₂ SCH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-22-8	Et	*	H	*	<i>i</i> Pr	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-23.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
35	IX-23-1	<i>i</i> Pr	H	H	H	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-23-2	<i>i</i> Pr	H	H	CH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-23-3	<i>i</i> Pr	H	H	CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-23-4	<i>i</i> Pr	H	H	CH ₂ CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
40	IX-23-5	<i>i</i> Pr	H	H	CH ₂ Ph	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-23-6	<i>i</i> Pr	H	H	CH ₂ -indol-3-yl	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-23-7	<i>i</i> Pr	H	H	CH ₂ CH ₂ SCH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-23-8	<i>i</i> Pr	*	H	*	<i>i</i> Pr	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-24.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
50	IX-24-1	<i>t</i> Bu	H	H	H	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-24-2	<i>t</i> Bu	H	H	CH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-24-3	<i>t</i> Bu	H	H	CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-24-4	<i>t</i> Bu	H	H	CH ₂ CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
55	IX-24-5	<i>t</i> Bu	H	H	CH ₂ Ph	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-24-6	<i>t</i> Bu	H	H	CH ₂ -indol-3-yl	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-24-7	<i>t</i> Bu	H	H	CH ₂ CH ₂ SCH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H

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No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-24-8	<i>t</i> Bu	*	H	*	<i>i</i> Pr	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-25.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-25-1	Ph	H	H	H	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-25-2	Ph	H	H	CH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-25-3	Ph	H	H	CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-25-4	Ph	H	H	CH ₂ CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-25-5	Ph	H	H	CH ₂ Ph	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-25-6	Ph	H	H	CH ₂ -indol-3-yl	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-25-7	Ph	H	H	CH ₂ CH ₂ SCH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-25-8	Ph	*	H	*	<i>i</i> Pr	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-26.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-26-1	<i>p</i> -Me-Ph	H	H	H	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-26-2	<i>p</i> -Me-Ph	H	H	CH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-26-3	<i>p</i> -Me-Ph	H	H	CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-26-4	<i>p</i> -Me-Ph	H	H	CH ₂ CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-26-5	<i>p</i> -Me-Ph	H	H	CH ₂ Ph	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-26-6	<i>p</i> -Me-Ph	H	H	CH ₂ -indol-3-yl	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-26-7	<i>p</i> -Me-Ph	H	H	CH ₂ CH ₂ SCH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-26-8	<i>p</i> -Me-Ph	*	H	*	<i>i</i> Pr	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-27.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-27-1	<i>p</i> -F-Ph	H	H	H	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-27-2	<i>p</i> -F-Ph	H	H	CH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-27-3	<i>p</i> -F-Ph	H	H	CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-27-4	<i>p</i> -F-Ph	H	H	CH ₂ CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-27-5	<i>p</i> -F-Ph	H	H	CH ₂ Ph	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-27-6	<i>p</i> -F-Ph	H	H	CH ₂ -indol-3-yl	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-27-7	<i>p</i> -F-Ph	H	H	CH ₂ CH ₂ SCH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
IX-27-8	<i>p</i> -F-Ph	*	H	*	<i>i</i> Pr	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

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Table IX-28.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
5	IX-28-1	p-Cl-Ph	H	H	H	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-28-2	p-Cl-Ph	H	H	CH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-28-3	p-Cl-Ph	H	H	CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-28-4	p-Cl-Ph	H	H	CH ₂ CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-28-5	p-Cl-Ph	H	H	CH ₂ Ph	<i>i</i> Pr	H	CH ₃	F	OH	H	H
10	IX-28-6	p-Cl-Ph	H	H	CH ₂ -indol-3-yl	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-28-7	p-Cl-Ph	H	H	CH ₂ CH ₂ SCH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-28-8	p-Cl-Ph	*	H	*	<i>i</i> Pr	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-29.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
20	IX-29-1	p-Br-Ph	H	H	H	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-29-2	p-Br-Ph	H	H	CH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-29-3	p-Br-Ph	H	H	CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-29-4	p-Br-Ph	H	H	CH ₂ CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-29-5	p-Br-Ph	H	H	CH ₂ Ph	<i>i</i> Pr	H	CH ₃	F	OH	H	H
25	IX-29-6	p-Br-Ph	H	H	CH ₂ -indol-3-yl	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-29-7	p-Br-Ph	H	H	CH ₂ CH ₂ SCH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-29-8	p-Br-Ph	*	H	*	<i>i</i> Pr	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-30.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
35	IX-30-1	p-I-Ph	H	H	H	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-30-2	p-I-Ph	H	H	CH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-30-3	p-I-Ph	H	H	CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-30-4	p-I-Ph	H	H	CH ₂ CH(CH ₃) ₂	<i>i</i> Pr	H	CH ₃	F	OH	H	H
40	IX-30-5	p-I-Ph	H	H	CH ₂ Ph	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-30-6	p-I-Ph	H	H	CH ₂ -indol-3-yl	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-30-7	p-I-Ph	H	H	CH ₂ CH ₂ SCH ₃	<i>i</i> Pr	H	CH ₃	F	OH	H	H
	IX-30-8	p-I-Ph	*	H	*	<i>i</i> Pr	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-31.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸	
50	IX-31-1	CH ₃	H	H	H	ⁿ Bu	H	CH ₃	F	OH	H	H
	IX-31-2	CH ₃	H	H	CH ₃	ⁿ Bu	H	CH ₃	F	OH	H	H
	IX-31-3	CH ₃	H	H	CH(CH ₃) ₂	ⁿ Bu	H	CH ₃	F	OH	H	H
	IX-31-4	CH ₃	H	H	CH ₂ CH(CH ₃) ₂	ⁿ Bu	H	CH ₃	F	OH	H	H
55	IX-31-5	CH ₃	H	H	CH ₂ Ph	ⁿ Bu	H	CH ₃	F	OH	H	H
	IX-31-6	CH ₃	H	H	CH ₂ -indol-3-yl	ⁿ Bu	H	CH ₃	F	OH	H	H
	IX-31-7	CH ₃	H	H	CH ₂ CH ₂ SCH ₃	ⁿ Bu	H	CH ₃	F	OH	H	H

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(continued)

N_2	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-31-8	CH ₃	*	H	*	<i>n</i> Bu	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-32.

N_2	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-32-1	Et	H	H	H	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-32-2	Et	H	H	CH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-32-3	Et	H	H	CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-32-4	Et	H	H	CH ₂ CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-32-5	Et	H	H	CH ₂ Ph	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-32-6	Et	H	H	CH ₂ -indol-3-yl	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-32-7	Et	H	H	CH ₂ CH ₂ SCH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-32-8	Et	*	H	*	<i>n</i> Bu	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-33.

N_2	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-33-1	<i>i</i> Pr	H	H	H	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-33-2	<i>i</i> Pr	H	H	CH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-33-3	<i>i</i> Pr	H	H	CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-33-4	<i>i</i> Pr	H	H	CH ₂ CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-33-5	<i>i</i> Pr	H	H	CH ₂ Ph	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-33-6	<i>i</i> Pr	H	H	CH ₂ -indol-3-yl	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-33-7	<i>i</i> Pr	H	H	CH ₂ CH ₂ SCH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-33-8	<i>i</i> Pr	*	H	*	<i>n</i> Bu	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-34.

N_2	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-34-1	<i>t</i> Bu	H	H	H	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-34-2	<i>t</i> Bu	H	H	CH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-34-3	<i>t</i> Bu	H	H	CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-34-4	<i>t</i> Bu	H	H	CH ₂ CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-34-5	<i>t</i> Bu	H	H	CH ₂ Ph	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-34-6	<i>t</i> Bu	H	H	CH ₂ -indol-3-yl	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-34-7	<i>t</i> Bu	H	H	CH ₂ CH ₂ SCH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-34-8	<i>t</i> Bu	*	H	*	<i>n</i> Bu	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

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Table IX-35.

N_2	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-35-1	Ph	H	H	H	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-35-2	Ph	H	H	CH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-35-3	Ph	H	H	CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-35-4	Ph	H	H	CH ₂ CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-35-5	Ph	H	H	CH ₂ Ph	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-35-6	Ph	H	H	CH ₂ -indol-3-yl	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-35-7	Ph	H	H	CH ₂ CH ₂ SCH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-35-8	Ph	*	H	*	<i>n</i> Bu	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-36.

N_2	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-36-1	p-Me-Ph	H	H	H	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-36-2	p-Me-Ph	H	H	CH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-36-3	p-Me-Ph	H	H	CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-36-4	p-Me-Ph	H	H	CH ₂ CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-36-5	p-Me-Ph	H	H	CH ₂ Ph	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-36-6	p-Me-Ph	H	H	CH ₂ -indol-3-yl	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-36-7	p-Me-Ph	H	H	CH ₂ CH ₂ SCH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-36-8	p-Me-Ph	*	H	*	<i>n</i> Bu	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-37.

N_2	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-37-1	p-F-Ph	H	H	H	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-37-2	p-F-Ph	H	H	CH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-37-3	p-F-Ph	H	H	CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-37-4	p-F-Ph	H	H	CH ₂ CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-37-5	p-F-Ph	H	H	CH ₂ Ph	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-37-6	p-F-Ph	H	H	CH ₂ -indol-3-yl	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-37-7	p-F-Ph	H	H	CH ₂ CH ₂ SCH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-37-8	p-F-Ph	*	H	*	<i>n</i> Bu	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-38.

N_2	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-38-1	p-Cl-Ph	H	H	H	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-38-2	p-Cl-Ph	H	H	CH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-38-3	p-Cl-Ph	H	H	CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-38-4	p-Cl-Ph	H	H	CH ₂ CH(CH ₃) ₂	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-38-5	p-Cl-Ph	H	H	CH ₂ Ph	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-38-6	p-Cl-Ph	H	H	CH ₂ -indol-3-yl	<i>n</i> Bu	H	CH ₃	F	OH	H	H
IX-38-7	p-Cl-Ph	H	H	CH ₂ CH ₂ SCH ₃	<i>n</i> Bu	H	CH ₃	F	OH	H	H

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(continued)

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-38-8	p-Cl-Ph	*	H	*	ⁿ Bu	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-39.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-39-1	p-Br-Ph	H	H	H	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-39-2	p-Br-Ph	H	H	CH ₃	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-39-3	p-Br-Ph	H	H	CH(CH ₃) ₂	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-39-4	p-Br-Ph	H	H	CH ₂ CH(CH ₃) ₂	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-39-5	p-Br-Ph	H	H	CH ₂ Ph	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-39-6	p-Br-Ph	H	H	CH ₂ -indol-3-yl	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-39-7	p-Br-Ph	H	H	CH ₂ CH ₂ SCH ₃	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-39-8	p-Br-Ph	*	H	*	ⁿ Bu	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-40.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-40-1	p-I-Ph	H	H	H	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-40-2	p-I-Ph	H	H	CH ₃	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-40-3	p-I-Ph	H	H	CH(CH ₃) ₂	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-40-4	p-I-Ph	H	H	CH ₂ CH(CH ₃) ₂	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-40-5	p-I-Ph	H	H	CH ₂ Ph	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-40-6	p-I-Ph	H	H	CH ₂ -indol-3-yl	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-40-7	p-I-Ph	H	H	CH ₂ CH ₂ SCH ₃	ⁿ Bu	H	CH ₃	F	OH	H	H
IX-40-8	p-I-Ph	*	H	*	ⁿ Bu	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-41.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-41-1	CH ₃	H	H	H	Bz	H	CH ₃	F	OH	H	H
IX-41-2	CH ₃	H	H	CH ₃	Bz	H	CH ₃	F	OH	H	H
IX-41-3	CH ₃	H	H	CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-41-4	CH ₃	H	H	CH ₂ CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-41-5	CH ₃	H	H	CH ₂ Ph	Bz	H	CH ₃	F	OH	H	H
IX-41-6	CH ₃	H	H	CH ₂ -indol-3-yl	Bz	H	CH ₃	F	OH	H	H
IX-41-7	CH ₃	H	H	CH ₂ CH ₂ SCH ₃	Bz	H	CH ₃	F	OH	H	H
IX-41-8	CH ₃	*	H	*	Bz	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

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Table IX-42.

N ₉	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-42-1	Et	H	H	H	Bz	H	CH ₃	F	OH	H	H
IX-42-2	Et	H	H	CH ₃	Bz	H	CH ₃	F	OH	H	H
IX-42-3	Et	H	H	CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-42-4	Et	H	H	CH ₂ CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-42-5	Et	H	H	CH ₂ Ph	Bz	H	CH ₃	F	OH	H	H
IX-42-6	Et	H	H	CH ₂ -indol-3-yl	Bz	H	CH ₃	F	OH	H	H
IX-42-7	Et	H	H	CH ₂ CH ₂ SCH ₃	Bz	H	CH ₃	F	OH	H	H
IX-42-8	Et	*	H	*	Bz	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-43.

N ₉	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-43-1	ⁱ Pr	H	H	H	Bz	H	CH ₃	F	OH	H	H
IX-43-2	ⁱ Pr	H	H	CH ₃	Bz	H	CH ₃	F	OH	H	H
IX-43-3	ⁱ Pr	H	H	CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-43-4	ⁱ Pr	H	H	CH ₂ CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-43-5	ⁱ Pr	H	H	CH ₂ Ph	Bz	H	CH ₃	F	OH	H	H
IX-43-6	ⁱ Pr	H	H	CH ₂ -indol-3-yl	Bz	H	CH ₃	F	OH	H	H
IX-43-7	ⁱ Pr	H	H	CH ₂ CH ₂ SCH ₃	Bz	H	CH ₃	F	OH	H	H
IX-43-8	ⁱ Pr	*	H	*	Bz	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-44.

N ₉	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-44-1	^t Bu	H	H	H	Bz	H	CH ₃	F	OH	H	H
IX-44-2	^t Bu	H	H	CH ₃	Bz	H	CH ₃	F	OH	H	H
IX-44-3	^t Bu	H	H	CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-44-4	^t Bu	H	H	CH ₂ CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-44-5	^t Bu	H	H	CH ₂ Ph	Bz	H	CH ₃	F	OH	H	H
IX-44-6	^t Bu	H	H	CH ₂ -indol-3-yl	Bz	H	CH ₃	F	OH	H	H
IX-44-7	^t Bu	H	H	CH ₂ CH ₂ SCH ₃	Bz	H	CH ₃	F	OH	H	H
IX-44-8	^t Bu	*	H	*	Bz	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-45.

N ₉	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-45-1	Ph	H	H	H	Bz	H	CH ₃	F	OH	H	H
IX-45-2	Ph	H	H	CH ₃	Bz	H	CH ₃	F	OH	H	H
IX-45-3	Ph	H	H	CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-45-4	Ph	H	H	CH ₂ CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-45-5	Ph	H	H	CH ₂ Ph	Bz	H	CH ₃	F	OH	H	H
IX-45-6	Ph	H	H	CH ₂ -indol-3-yl	Bz	H	CH ₃	F	OH	H	H
IX-45-7	Ph	H	H	CH ₂ CH ₂ SCH ₃	Bz	H	CH ₃	F	OH	H	H

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(continued)

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-45-8	Ph	*	H	*	Bz	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-46.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-46-1	p-Me-Ph	H	H	H	Bz	H	CH ₃	F	OH	H	H
IX-46-2	p-Me-Ph	H	H	CH ₃	Bz	H	CH ₃	F	OH	H	H
IX-46-3	p-Me-Ph	H	H	CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-46-4	p-Me-Ph	H	H	CH ₂ CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-46-5	p-Me-Ph	H	H	CH ₂ Ph	Bz	H	CH ₃	F	OH	H	H
IX-46-6	p-Me-Ph	H	H	CH ₂ -indol-3-yl	Bz	H	CH ₃	F	OH	H	H
IX-46-7	p-Me-Ph	H	H	CH ₂ CH ₂ SCH ₃	Bz	H	CH ₃	F	OH	H	H
IX-46-8	p-Me-Ph	*	H	*	Bz	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-47.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-47-1	p-F-Ph	H	H	H	Bz	H	CH ₃	F	OH	H	H
IX-47-2	p-F-Ph	H	H	CH ₃	Bz	H	CH ₃	F	OH	H	H
IX-47-3	p-F-Ph	H	H	CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-47-4	p-F-Ph	H	H	CH ₂ CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-47-5	p-F-Ph	H	H	CH ₂ Ph	Bz	H	CH ₃	F	OH	H	H
IX-47-6	p-F-Ph	H	H	CH ₂ -indol-3-yl	Bz	H	CH ₃	F	OH	H	H
IX-47-7	p-F-Ph	H	H	CH ₂ CH ₂ SCH ₃	Bz	H	CH ₃	F	OH	H	H
IX-47-8	p-F-Ph	*	H	*	Bz	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-48.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-48-1	p-Cl-Ph	H	H	H	Bz	H	CH ₃	F	OH	H	H
IX-48-2	p-Cl-Ph	H	H	CH ₃	Bz	H	CH ₃	F	OH	H	H
IX-48-3	p-Cl-Ph	H	H	CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-48-4	p-Cl-Ph	H	H	CH ₂ CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-48-5	p-Cl-Ph	H	H	CH ₂ Ph	Bz	H	CH ₃	F	OH	H	H
IX-48-6	p-Cl-Ph	H	H	CH ₂ -indol-3-yl	Bz	H	CH ₃	F	OH	H	H
IX-48-7	p-Cl-Ph	H	H	CH ₂ CH ₂ SCH ₃	Bz	H	CH ₃	F	OH	H	H
IX-48-8	p-Cl-Ph	*	H	*	Bz	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-49.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-49-1	p-Br-Ph	H	H	H	Bz	H	CH ₃	F	OH	H	H
IX-49-2	p-Br-Ph	H	H	CH ₃	Bz	H	CH ₃	F	OH	H	H
IX-49-3	p-Br-Ph	H	H	CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-49-4	p-Br-Ph	H	H	CH ₂ CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-49-5	p-Br-Ph	H	H	CH ₂ Ph	Bz	H	CH ₃	F	OH	H	H
IX-49-6	p-Br-Ph	H	H	CH ₂ -indol-3-yl	Bz	H	CH ₃	F	OH	H	H
IX-49-7	p-Br-Ph	H	H	CH ₂ CH ₂ SCH ₃	Bz	H	CH ₃	F	OH	H	H
IX-49-8	p-Br-Ph	*	H	*	Bz	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

Table IX-50.

No	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	R ⁵	R ⁶	X	Y	R ⁷	R ⁸
IX-50-1	p-I-Ph	H	H	H	Bz	H	CH ₃	F	OH	H	H
IX-50-2	p-I-Ph	H	H	CH ₃	Bz	H	CH ₃	F	OH	H	H
IX-50-3	p-I-Ph	H	H	CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-50-4	p-I-Ph	H	H	CH ₂ CH(CH ₃) ₂	Bz	H	CH ₃	F	OH	H	H
IX-50-5	p-I-Ph	H	H	CH ₂ Ph	Bz	H	CH ₃	F	OH	H	H
IX-50-6	p-I-Ph	H	H	CH ₂ -indol-3-yl	Bz	H	CH ₃	F	OH	H	H
IX-50-7	p-I-Ph	H	H	CH ₂ CH ₂ SCH ₃	Bz	H	CH ₃	F	OH	H	H
IX-50-8	p-I-Ph	*	H	*	Bz	H	CH ₃	F	OH	H	H

*R² and R^{3b} joined together by (CH₂)₃ to form five-membered ring.

DOSAGE, ADMINISTRATION, AND USE

[0054] A further aspect of the present disclosure is directed to a composition for the treatment of any of the viral agents disclosed herein said composition comprising a pharmaceutically acceptable medium selected from among an excipient, carrier, diluent, and equivalent medium and a compound, that is intended to include its salts (acid or basic addition salts), hydrates, solvates, and crystalline forms can be obtained, represented by formula I.

[0055] It is contemplated that the formulation according to this aspect of the present disclosure can contain any of the compounds contemplated in any other aspect of the present disclosure or those specifically recited in the tables above or exemplified herein, either alone or in combination with another compound of the present invention.

[0056] The compounds of the present invention may be formulated in a wide variety of oral administration dosage forms and carriers. Oral administration can be in the form of tablets, coated tablets, hard and soft gelatin capsules, solutions, emulsions, syrups, or suspensions. Compounds of the present invention are efficacious when administered by suppository administration, among other routes of administration. The most convenient manner of administration is generally oral using a convenient daily dosing regimen which can be adjusted according to the severity of the disease and the patient's response to the antiviral medication.

[0057] A compound or compounds of the present invention, as well as their pharmaceutically acceptable salts, together with one or more conventional excipients, carriers, or diluents, may be placed into the form of pharmaceutical compositions and unit dosages. The pharmaceutical compositions and unit dosage forms may be comprised of conventional ingredients in conventional proportions, with or without additional active compounds and the unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed. The pharmaceutical compositions may be employed as solids, such as tablets or filled capsules, semisolids, powders, sustained release formulations, or liquids such as suspensions, emulsions, or filled capsules for oral use; or in the form of suppositories for rectal or vaginal administration. A typical preparation will contain from about 5% to about 95% active compound or compounds (w/w). The term "preparation" or "dosage form" is intended to include both solid and liquid formulations of the active compound and one skilled in the art will appreciate that an active ingredient can exist in different preparations depending on the desired dose and pharmacokinetic parameters.

[0058] The term "excipient" as used herein refers to a compound that is used to prepare a pharmaceutical composition,

and is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes excipients that are acceptable for veterinary use as well as human pharmaceutical use. The compounds of this invention can be administered alone but will generally be administered in admixture with one or more suitable pharmaceutical excipients, diluents or carriers selected with regard to the intended route of administration and standard pharmaceutical practice.

5 **[0059]** A "pharmaceutically acceptable salt" form of an active ingredient may also initially confer a desirable pharmacokinetic property on the active ingredient which were absent in the non-salt form, and may even positively affect the pharmacodynamics of the active ingredient with respect to its therapeutic activity in the body. The phrase "pharmaceutically acceptable salt" of a compound as used herein means a salt that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. Such salts include: (1) acid addition salts, formed with
10 inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as glycolic acid, pyruvic acid, lactic acid, malonic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, lauryl sulfuric acid, gluconic
15 acid, glutamic acid, salicylic acid, muconic acid, and the like or (2) basic addition salts formed with the conjugate bases of any of the inorganic acids listed above, wherein the conjugate bases comprise a cationic component selected from among Na^+ , K^+ , Mg^{2+} , Ca^{2+} , and NH_gR^{g+} , in which R^{g+} is a C_{1-3} alkyl and g is a number selected from among 0, 1, 2, 3, or 4. It should be understood that all references to pharmaceutically acceptable salts include solvent addition forms (solvates) or crystal forms (polymorphs) as defined herein, of the same acid addition salt.

20 **[0060]** Solid form preparations include powders, tablets, pills, capsules, suppositories, and dispersible granules. A solid carrier may be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the carrier generally is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component generally is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. Suitable carriers include but are not limited to magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. Solid form preparations may contain, in addition to the active component,
25 colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

30 **[0061]** Liquid formulations also are suitable for oral administration include liquid formulation including emulsions, syrups, elixirs and aqueous suspensions. These include solid form preparations which are intended to be converted to liquid form preparations shortly before use. Emulsions may be prepared in solutions, for example, in aqueous propylene glycol solutions or may contain emulsifying agents such as lecithin, sorbitan monooleate, or acacia. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or
35 synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well known suspending agents.

[0062] The compounds of the present invention may be formulated for administration as suppositories. A low melting wax, such as a mixture of fatty acid glycerides or cocoa butter is first melted and the active component is dispersed homogeneously, for example, by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and to solidify.

40 **[0063]** The compounds of the present invention may be formulated for vaginal administration. Pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

[0064] Suitable formulations along with pharmaceutical carriers, diluents and excipients are described in Remington: The Science and Practice of Pharmacy 1995, edited by E. W. Martin, Mack Publishing Company, 19th edition, Easton,
45 Pennsylvania. The compounds of the present invention can also be encapsulated in liposomes, such as those disclosed in U.S. Patent Nos. 6,180,134, 5,192,549, 5,376,380, 6,060,080, 6,132,763. A skilled formulation scientist may modify the formulations within the teachings of the specification to provide numerous formulations for a particular route of administration without rendering the compositions of the present invention unstable or compromising their therapeutic activity.

50 **[0065]** The modification of the present compounds to render them more soluble in water or other vehicle, for example, may be easily accomplished by minor modifications (e.g., salt formulation), which are well within the ordinary skill in the art. It is also well within the ordinary skill of the art to modify the route of administration and dosage regimen of a particular compound in order to manage the pharmacokinetics of the present compounds for maximum beneficial effect in patients.

[0066] A further aspect of the present disclosure is directed to a use of the compounds of the present invention in the
55 manufacture of a medicament for the treatment of any condition the result of an infection by any one of the following viral agents: hepatitis C virus, West Nile virus, yellow fever virus, dengue virus, rhinovirus, polio virus, hepatitis A virus, bovine viral diarrhea virus and Japanese encephalitis virus.

[0067] The term "medicament" means a substance used in a method of treatment and/or prophylaxis of a subject in

need thereof, wherein the substance includes, but is not limited to, a composition, a formulation, a dosage form, and the like, comprising the compounds of the present invention.

[0068] The term "subject" means a mammal, which includes, but is not limited to, cattle, pigs, sheep, chicken, turkey, buffalo, llama, ostrich, dogs, cats, and humans, preferably the subject is a human.

[0069] The term "therapeutically effective amount" as used herein means an amount required to reduce symptoms of the disease in an individual. The dose will be adjusted to the individual requirements in each particular case. That dosage can vary within wide limits depending upon numerous factors such as the severity of the disease to be treated, the age and general health condition of the patient, other medicaments with which the patient is being treated, the route and form of administration and the preferences and experience of the medical practitioner involved. For oral administration, a daily dosage of between about 0.1 and about 10 g, including all values in between, such as 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, and 9.5, per day should be appropriate in monotherapy and/or in combination therapy. A preferred daily dosage is between about 0.5 and about 7.5 g per day, more preferred 1.5 and about 6.0 g per day. Generally, treatment is initiated with a large initial "loading dose" to rapidly reduce or eliminate the virus following by a decreasing the dose to a level sufficient to prevent resurgence of the infection. One of ordinary skill in treating diseases described herein will be able, without undue experimentation and in reliance on personal knowledge, experience and the disclosures of this application, to ascertain a therapeutically effective amount of the compounds of the present invention for a given disease and patient.

[0070] Therapeutic efficacy can be ascertained from tests of liver function including, but not limited to protein levels such as serum proteins (e.g., albumin, clotting factors, alkaline phosphatase, aminotransferases (e.g., alanine transaminase, aspartate transaminase), 5'-nucleosidase, γ -glutamyltranspeptidase, etc.), synthesis of bilirubin, synthesis of cholesterol, and synthesis of bile acids; a liver metabolic function, including, but not limited to, carbohydrate metabolism, amino acid and ammonia metabolism. Alternatively the therapeutic effectiveness may be monitored by measuring HCV-RNA. The results of these tests will allow the dose to be optimized.

[0071] A further aspect of the present disclosure, is directed to a method of treatment and/or prophylaxis in a subject in need thereof said method comprises administering to the subject a therapeutically effective of a compound represented by compounds of the present invention and a therapeutically effective amount of another antiviral agent; wherein the administration is concurrent or alternative. It is understood that the time between alternative administration can range between 1-24 hours, which includes any sub-range in between including, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 23 hours. Examples of "another antiviral agents" include, but are not limited to: HCV NS3 protease inhibitors (see WO 2008010921, WO 2008010921, EP 1881001, WO 2007015824, WO 2007014925, WO 2007014926, WO 2007014921, WO 2007014920, WO 2007014922, US 2005267018, WO 2005095403, WO 2005037214, WO 2004094452, US 2003187018, WO 200364456, WO 2005028502, and WO 2003006490); HCV NS5B Inhibitors (see US 2007275947, US20072759300, WO2007095269, WO 2007092000, WO 2007076034, WO 200702602, US 2005-98125, WO 2006093801, US 2006166964, WO 2006065590, WO 2006065335, US 2006040927, US 2006040890, WO 2006020082, WO 2006012078, WO 2005123087, US 2005154056, US 2004229840, WO 2004065367, WO 2004003138, WO 2004002977, WO 2004002944, WO 2004002940, WO 2004000858, WO 2003105770, WO 2003010141, WO 2002057425, WO 2002057287, WO 2005021568, WO 2004041201, US 20060293306, US 20060194749, US 20060241064, US 6784166, WO 2007088148, WO 2007039142, WO 2005103045, WO 2007039145, WO 2004096210, and WO 2003037895); HCV NS4 Inhibitors (see WO 2007070556 and WO 2005067900); HCV NS5a Inhibitors (see US 2006276511, WO 2006120252, WO 2006120251, WO 2006100310, WO 2006035061); Toll-like receptor agonists (see WO 2007093901); and other inhibitors (see WO 2004035571, WO 2004014852, WO 2004014313, WO 2004009020, WO 2003101993, WO 2000006529).

[0072] A further aspect of the present disclosure, is directed to a method of treatment in a subject in need thereof said method comprises alternatively or concurrently administering a therapeutically effective of a compound according to the present invention and another antiviral agent to the subject. It is understood that the time between alternative administration can range between 1-24 hours, which includes any sub-range in between including, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 23 hours.

[0073] A further aspect of the present disclosure, is directed to a method of treatment and/or prophylaxis in a subject in need thereof said method comprises administering to the subject a therapeutically effective of at least one compound according to the present invention and a therapeutically effective amount of another antiviral agent; wherein the administration is concurrent or alternative. It is understood that the time between alternative administration can range between 1-24 hours, which includes any sub-range in between including, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 23 hours.

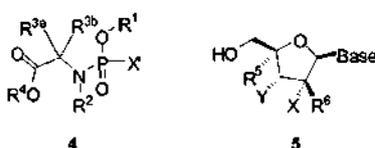
[0074] A further aspect of the present disclosure, is directed to a method of treatment in a subject in need thereof said method comprises alternatively or concurrently administering a therapeutically effective of at least one compound according to the present invention and another antiviral agent to the subject. It is understood that the time between alternative administration can range between 1-24 hours, which includes any sub-range in between including, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 23 hours.

[0075] It is contemplated that the another antiviral agent includes, but is not limited to interferon- α , interferon- β , pegylated interferon- α , ribavirin, levovirin, viramidine, another nucleoside HCV polymerase inhibitor, a HCV non-nucleoside polymerase inhibitor, a HCV protease inhibitor, a HCV helicase inhibitor or a HCV fusion inhibitor. When the active compound or its derivative or salt are administered in combination with another antiviral agent the activity may be increased over the parent compound. When the treatment is combination therapy, such administration may be concurrent or sequential with respect to that of the nucleoside derivatives. "Concurrent administration" as used herein thus includes administration of the agents at the same time or at different times. Administration of two or more agents at the same time can be achieved by a single formulation containing two or more active ingredients or by substantially simultaneous administration of two or more dosage forms with a single active agent.

[0076] It will be understood that references herein to treatment extend to prophylaxis as well as to the treatment of existing conditions. Furthermore, the term "treatment" of a HCV infection, as used herein, also includes treatment or prophylaxis of a disease or a condition associated with or mediated by HCV infection, or the clinical symptoms thereof.

PROCESS FOR PREPARATION

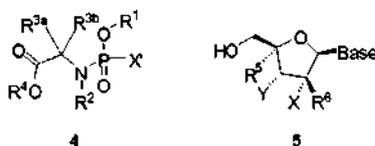
[0077] An aspect of the present disclosure is directed to a process for preparing the compounds, which comprises reacting a suitably substituted phosphochloridate compound 4 with a nucleoside analog 5



wherein the substituents R¹, R², R^{3a}, R^{3b}, R⁴, R⁵, X, Y, R⁶, and base have their meanings as disclosed in the Detailed Description of the Invention and X' is a leaving group, such as Cl, Br, I, tosylate, mesylate, trifluoroacetate, trifluorosulfonate, pentafluorophenoxide, p-NO₂-phenoxide, or other commonly used leaving groups as disclosed in Advanced Organic Chemistry by March, Fourth Edition. Leaving groups and methods that can be used to effect the formation of a phosphoramidate nucleoside conjugate are found in US 20060142238 and WO 2007095269. Preferably, the leaving group is Cl.

[0078] This reaction is performed in an anhydrous aprotic solvent such tetrahydrofuran, dioxane, or both tetrahydrofuran and dioxane, or any functional equivalent thereof, with tetrahydrofuran being the preferred solvent. The reaction is typically initiated at a temperature range from -78°C to 40°C with the preferred reaction temperature being between 0°C and room temperature. The nucleoside is first stirred with a base (5 to 12 equivalents) such as N-methylimidazole, collidine, pyridine, 2,6-lutidine, 2, 6-*t*Bu-pyridine, etc. a tertiary amine base, such as triethylamine, diisopropylethylamine, etc., or an alkyl Grignard reagent, such as *t*BuMgCl, *t*BuMgBr, MeMgCl, MeMgBr, etc. The phosphorochloridate (3-10 equivalents) is dissolved in the reaction solvent and added to the mixture of the nucleoside and base. The reaction is then allowed to stir over a period of time at a temperature between room temperature and 40°C for a period of 30 min to 24 hr. with the preferred reaction temperature being room temperature and time being 24 hr. The solvent is removed from the reaction mixture and the product is purified by chromatography on silica gel.

[0079] An aspect of the present disclosure is directed to a product obtained by a process which comprises reacting a suitably substituted phosphochloridate compound 4 with a nucleoside analog 5



wherein the substituents R¹, R², R^{3a}, R^{3b}, R⁴, R⁵, X, Y, R⁶, X', and base have their meanings as disclosed in the Detailed Description of the Invention.

[0080] This reaction can be performed in an anhydrous aprotic solvent or other suitable solvent, such as tetrahydrofuran, dioxane, or a mixture of tetrahydrofuran and dioxane, with tetrahydrofuran being the preferred solvent. The reaction is typically initiated at a temperature range from -78°C to 40°C with the preferred reaction temperature being between 0°C and room temperature. The nucleoside is first stirred with a base (5 to 12 equivalents) such as N-methylimidazole, a tertiary amine base or *t*Butyl Magnesium Chloride. A phosphorochloridate (3-10 equivalents (or suitable "phosphoro-(leaving group)-date")) is dissolved in the reaction solvent and added to the mixture of the nucleoside and base. The reaction is then allowed to stir over a period of time at a temperature between room temperature and 40°C for a period

of 30 min to 24 hr. with the preferred reaction temperature being room temperature and time being 24 hr. The solvent is removed from the reaction mixture and the product is purified by chromatography on silica gel.

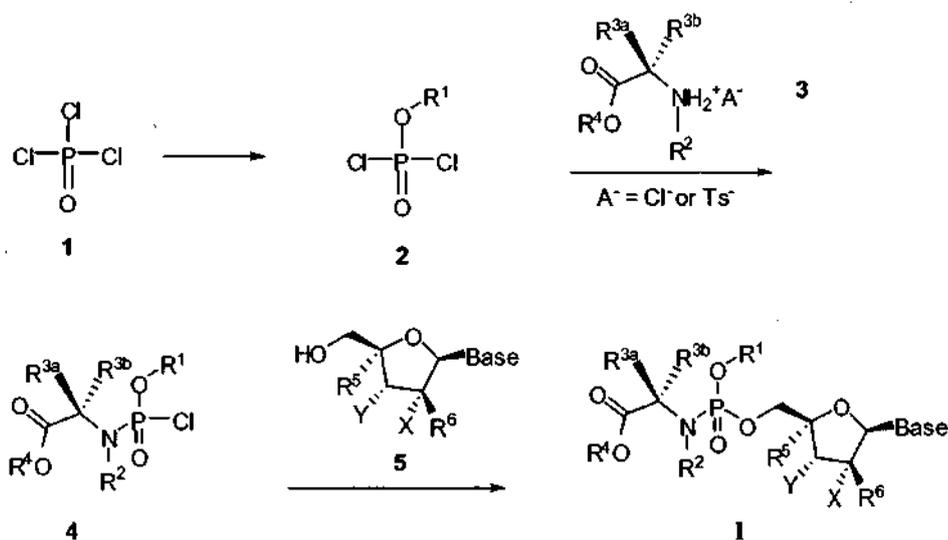
Compounds and Preparation

[0081] Phosphoramidate compounds of the present invention can be prepared by condensation of a nucleoside analog 5 with a suitably substituted phosphochloridate compound 4 (Scheme 1). The nucleoside analog is made by conventional procedures disclosed in any one of U.S. Published Application Nos. 2005/0009737, 2006/0199783, 2006/0122146, and 2007/0197463.

[0082] Disclosed ¹H-NMR values were recorded on a Varian AS-400 instrument. Mass spectral data were obtained using either a Micromass-Quattro micro API or a Waters Acquity.

[0083] Thus, by way of example only, a suitably substituted phenol can be reacted with phosphorus oxychloride (**1**) to afford an aryloxy phosphorodichloridate **2** (see Example 1) which is subsequently treated with an acid addition salt of an α -amino acid ester in the presence of TEA to afford an aryloxy phosphorochloridate **4**. This aryloxy phosphorochloridate is reacted with the nucleoside analog to provide the product **I** (for procedure see, e.g., C. McGuigan et al. *Antiviral Res.* 1992 17:311-321; D. Curley et al. *Antiviral Res.* 1990 14:345-356; McGuigan et al. *Antiviral Chem. Chemother* 1990 1(2):107-113).

Scheme 1



[0084] The preparation of nucleoside phosphoramidates requires reacting an appropriately substituted phosphochloridate with a nucleoside containing a free 5'-hydroxyl moiety. In cases where only one hydroxyl group is present, preparation of the phosphoramidate usually proceeds smoothly when the phosphochloridate is reacted with the desired nucleoside. In cases where the nucleoside contains more than one free hydroxyl group, preparation of the appropriately protected nucleoside might be required. Silyl, acetonide or other alcohol protecting groups known in the art might be warranted for protection of the sugar moiety. For protection of the nucleoside base, protecting a free amino group may require amidine protection strategy.

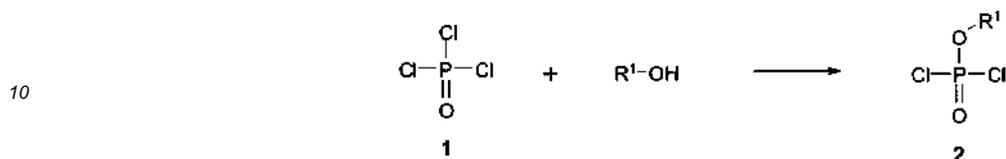
[0085] Condensation of the phosphochloridate can be carried out on the unprotected nucleoside. Since the 5'-OH group of a nucleoside is much less hindered than the 3'-OH group, selective phosphoramidation is possible under carefully controlled conditions. After condensation to form a protected phosphoramidate nucleoside, deprotection to obtain the free phosphoramidate nucleoside can be carried out using standard protocols for nucleic acid chemistry. In many cases, the desired product is readily separated from the starting material using column chromatography on silica gel. The synthetic scheme is summarized in Scheme 1.

[0086] A further understanding of the present disclosure will be appreciated by consideration of the following examples, which are only meant to be illustrative, and not limit the disclosed invention.

EXAMPLE 1

General Procedure for Preparation of phosphorodichloridates

5 [0087]

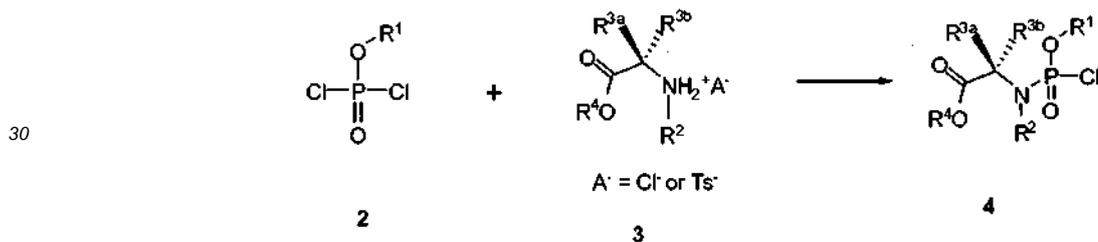


15 [0088] A solution of the appropriate phenol $\text{R}^1\text{-OH}$ (1eq) and triethylamine (1 eq.) in anhydrous ether was added dropwise to a stirred solution of phosphoryl trichloride **1** (1eq) at 0 °C over a period of 3 hours under nitrogen. Then the temperature was warmed to room temperature, and the reaction was stirred overnight. The triethylamine salt was quickly removed with suction filtration and the filtrate concentrated *in vacuo* to dryness to afford **2** as an oil which was used without further purification.

20 EXAMPLE 2

General Procedure for Preparation of phosphorochloridates

25 [0089]

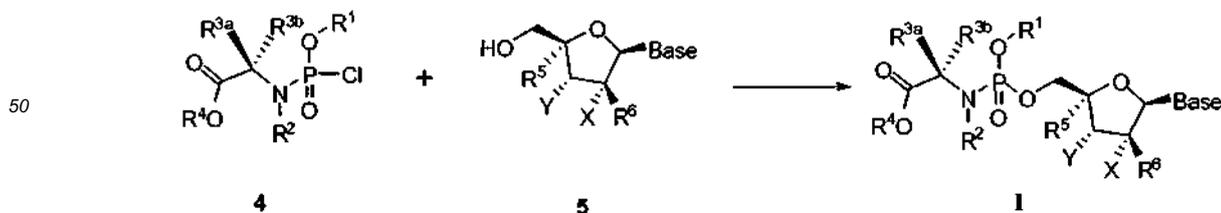


35 [0090] A solution of triethylamine (2eq) in anhydrous dichloromethane was added dropwise to a solution of aryloxyphosphodichloridate **2** (1 eq) and the appropriate amino ester **3** (1 eq) in anhydrous dichloromethane with vigorous stirring at -78 °C over a period of 30 to 120 minutes. Then the reaction temperature was allowed to warm to room temperature and stirred over night. Solvent was removed. The residue was washed with ethyl ether and filtered, the filtrate was dried over reduced pressure to give **4**.

40 EXAMPLE 3

General Procedures for nucleoside phosphoramidate derivatives

45 [0091]

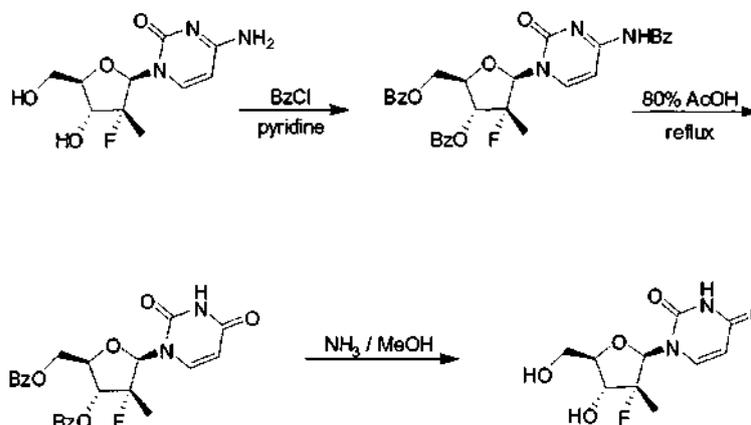


55 [0092] A solution of the appropriate phosphorochloridate **4** (6.5 equivalents) in anhydrous tetrahydrofuran (THF) was added to a mixture of nucleoside **5** (1 equivalent) and N-methylimidazole (8 equivalents) in anhydrous THF with vigorous stirring at room temperature and the reaction mixture was stirred overnight. The solvent was removed *in vacuo* and the crude was purified by column chromatography and/or preparative thin layer chromatography to give **I**.

EXAMPLE 4

Preparation of 2'-deoxy-2'-fluoro-2'-C-methyluridine

[0093]



[0094] 2'-Deoxy-2'-fluoro-2'-C-methylcytidine (1.0g, 1 eq) (Clark, J., et al., J. Med. Chem., 2005, 48, 5504-5508) was dissolved in 10 ml of anhydrous pyridine and concentrated to dryness in vacuo. The resulting syrup was dissolved in 20 ml of anhydrous pyridine under nitrogen and cooled to 0°C with stirring. The brown solution was treated with benzoyl chloride (1.63g, 3eq) dropwise over 10 min. The ice bath was removed and stirring continued for 1.5h whereby thin-layer chromatography (TLC) showed no remaining starting material. The mixture was quenched by addition of water (0.5 ml) and concentrated to dryness. The residue was dissolved in 50 mL of dichloromethane (DCM) and washed with saturated NaHCO₃ aqueous solution and H₂O. The organic phase was dried over NaSO₄ and filtered, concentrated to dryness to give N⁴,3',5'-tribenzoyl-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (2.0 g, Yield: 91%).

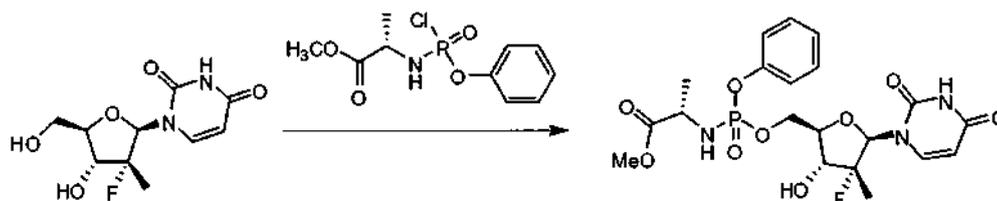
[0095] N⁴,3',5'-tribenzoyl-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (2.0g, 1 eq) was refluxed in 80% aqueous AcOH overnight. After cooling and standing at room temperature (15 °C), most of the product precipitated and then was filtered through a sintered funnel. White precipitate was washed with water and co-evaporated with toluene to give a white solid. The filtrate was concentrated and co-evaporated with toluene to give additional product which was washed with water to give a white solid. Combining the two batches of white solid gave 1.50g of 3',5'-dibenzoyl-2'-Deoxy-2'-fluoro-2'-C-methyluridine (Yield: 91%).

[0096] To a solution of 3',5'-dibenzoyl-2'-Deoxy-2'-fluoro-2'-C-methyluridine (1.5 g, 1eq) in MeOH (10 mL) was added a solution of saturated ammonia in MeOH (20mL). The reaction mixture was stirred at 0 °C for 30 min, and then warmed to room temperature slowly. After the reaction mixture was stirred for another 18 hours, the reaction mixture was evaporated under reduced pressure to give the residue, which was purified by column chromatography to afford pure compound 2'-deoxy-2'-fluoro-2'-C-methyluridine (500 mg, Yield: 60 %).

EXAMPLE 5

Preparation of 2'-Deoxy-2'-fluoro-2'-C-methyluridine 5'-(phenyl methoxy-alanyl phosphate)

[0097]

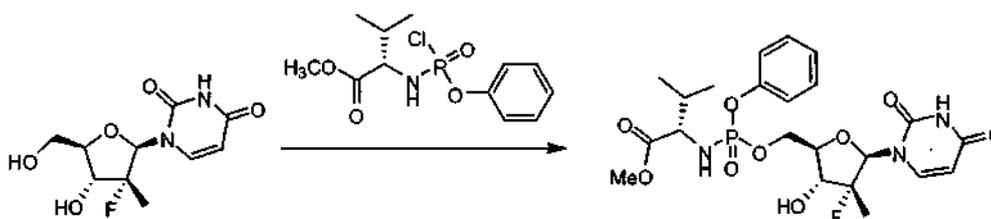


[0098] Phenyl methoxyalanyl phosphorochloridate (1 g, 6.5 eq) dissolved in 3 mL of THF was added to a mixture of 2'-Deoxy-2'-fluoro-2'-C-methyluridine (0.15 g, 1 eq) and N-methylimidazole (0.3 g, 8 eq) in 3 mL THF with vigorous stirring at room temperature, then the reaction was stirred overnight. Solvent was removed by reduced pressure. The

resulting crude product was dissolved in methanol purified by prep-HPLC on a YMC 25x30X2 mm column using a water / acetonitrile gradient elution mobile phase. The acetonitrile and water were removed under reduced pressure to give the desired product (50.1 mg, 15.6%). ¹H NMR (DMSO-*d*₆) δ 1.20-1.27 (m, 6H), 3.58 (d, *J* = 16.0 Hz, 3H), 3.75-3.92 (m, 2H), 4.015-4.379 (m, 2H), 5.54 (t, *J* = 10.2 Hz, 1H), 5.83-5.91 (m, 1H), 6.00-6.16 (m, 1H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.22 (s, 1H), 7.35 (t, *J* = 4.4 Hz, 2H), 7.55 (s, 1H), 11.52 (s, 1H); MS, *m/e* 502 (M+1)⁺.

EXAMPLE 6**Preparation of 2'-Deoxy-2'-fluoro-2'-C-methyluridine 5'-(phenyl methoxy-valyl phosphate)**

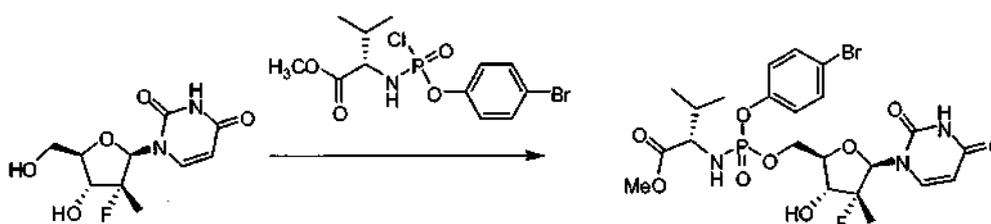
[0099]



[0100] Phenyl methoxy-valyl phosphorochloridate (0.6 g, 3.6 eq) dissolved in 3 mL of THF was added to a mixture of 2'-Deoxy-2'-fluoro-2'-C-methyluridine (0.15 g, 1 eq) and N-methylimidazole (0.44 g, 9 eq) in 3 mL THF with vigorous stirring at room temperature, then the reaction was stirred overnight. Solvent was removed by reduced pressure. The resulting crude product was dissolved in methanol purified by prep-HPLC on a YMC 25x30X2 mm column using a water / acetonitrile gradient elution mobile phase. The acetonitrile and water were removed under reduced pressure to give the desired product (60 mg, 20%). ¹H NMR (DMSO-*d*₆) δ 0.74-0.847 (m, 6H), 1.20-1.28 (m, 3H), 1.89-1.92 (m, 1H), 3.50-3.54 (m, 1H), 3.58 (d, *J* = 10.4Hz, 3H), 3.72-3.95 (m, 1H), 4.03-4.05 (m, 1H), 4.23-4.43 (m, 2H), 5.56 (t, *J* = 16.0 Hz, 1H), 5.85-5.92 (m, 1H), 6.01-6.07 (m, 1H), 7.16-7.21 (m, 3H), 7.37 (t, *J* = 8 Hz, 2H), 7.55-7.60 (m, 1H), 11.52 (s, 1H); MS, *m/e* 530 (M+1)⁺.

EXAMPLE 7**Preparation of 2'-Deoxy-2'-fluoro-2'-C-methyluridine 5'-(4-bromophenyl methoxy-valyl phosphate)**

[0101]

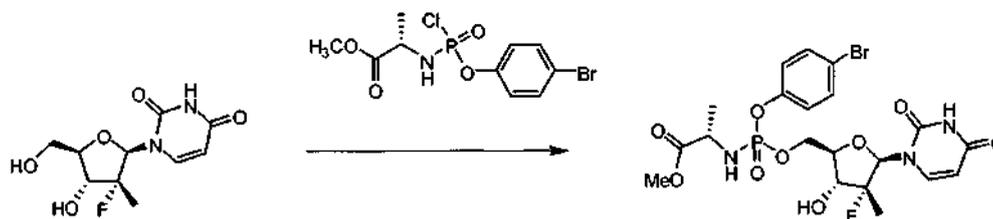


[0102] 4-Bromophenyl methoxy-valyl phosphorochloridate (1 g, 3.4 eq) dissolved in 3 mL of THF was added to a mixture of 2'-deoxy-2'-fluoro-2'-C-methyluridine (0.2 g, 1 eq) and N-methylimidazole (0.35 g, 6 eq) in 3 mL THF with vigorous stirring at room temperature, then the reaction was stirred overnight. Solvent was removed by reduced pressure. The resulting crude product was dissolved in methanol purified by prep-HPLC on a YMC 25x30X2 mm column using a water / acetonitrile gradient elution mobile phase. The acetonitrile and water were removed reduced pressure to give the desired product (120 mg, 26%). ¹H NMR (DMSO-*d*₆) δ 0.72-0.82 (m, 6H), 1.19-1.26 (m, 3H), 1.86-1.92 (m, 1H), 3.48-3.50 (m, 1H), 3.56 (d, *J* = 12.0 Hz, 3H), 3.72-3.89 (m, 1H), 3.96-4.03 (m, 1H), 4.22-4.37 (m, 2H), 5.54-5.60 (m, 1H), 5.85-5.91 (m, 1H), 5.98-6.13 (m, 1H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.49-7.56 (m, 3H), 11.53 (s, 1H); MS, *m/e* 608 (M+1)⁺.

EXAMPLE 8

Preparation of 2'-Deoxy-2'-fluoro-2'-C-methyluridine 5'-(4-bromophenyl methoxy-alanyl phosphate)

[0103]

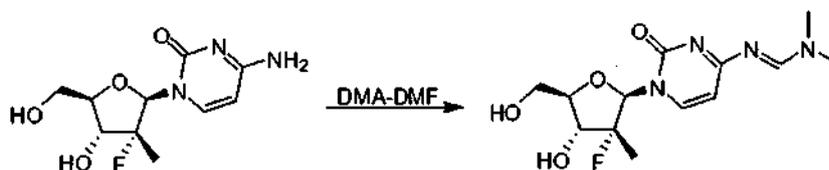


[0104] 4-Bromophenyl methoxy-alanyl phosphorochloridate (0.6 g, 5 eq) dissolved in 3 mL of THF was added to a mixture of 2'-deoxy-2'-fluoro-2'-C-methyluridine (0.15 g, 1 eq) and N-methylimidazole (0.3 g, 7.8 eq) in 3 mL THF with vigorous stirring at room temperature, then the reaction was stirred overnight. Solvent was removed by reduced pressure. The resulting crude product was dissolved in methanol purified by prep-HPLC on a YMC 25x30X2 mm column using a water / acetonitrile gradient elution mobile phase. The acetonitrile and water were removed under reduced pressure to give the desired product (40 mg, 12 %); $^1\text{H NMR}$ ($\text{DMSO-}d_6$) δ 1.20-1.26 (m, 6H), 3.57 (d, $J = 2.8$ Hz, 3H), 3.84 (s, 1H), 3.97-4.03 (m, 1H), 4.21-4.25 (m, 1H), 4.33-4.37 (m, 2H), 5.54-5.60 (m, 1H), 5.83-5.89 (m, 1H), 5.98-6.19 (m, 1H), 7.16 (t, $J = 10.2$ Hz, 2H), 7.52-7.57 (m, 3H), 11.52 (s, 1 H); MS, m/e 580(M+1) $^+$.

EXAMPLE 9

Preparation of N⁴-(N,N-dimethylformamidinyl)-2'-deoxy-2'-fluoro-2'-C-methylcytidine

[0105]

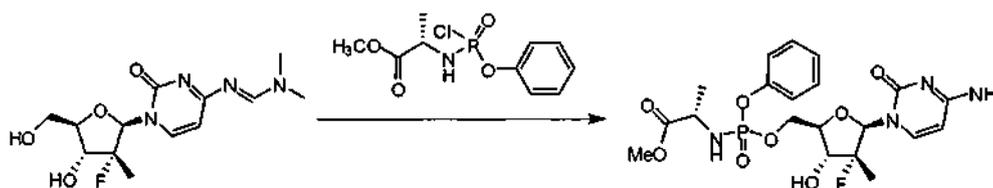


[0106] 2'-Deoxy-2'-fluoro-2'-C-methylcytidine (500 mg, 1.9 mmol) was stirred with dimethylformamide dimethyl acetal in DMF (10 mL). The resulting mixture was stirred at room temperature overnight. After solvent removal the crude product was used for next step without further purification.

EXAMPLE 10

Preparation of 2'-Deoxy-2'-fluoro-2'-C-methylcytidine 5'-(phenyl methoxy-alanyl phosphate)

[0107]



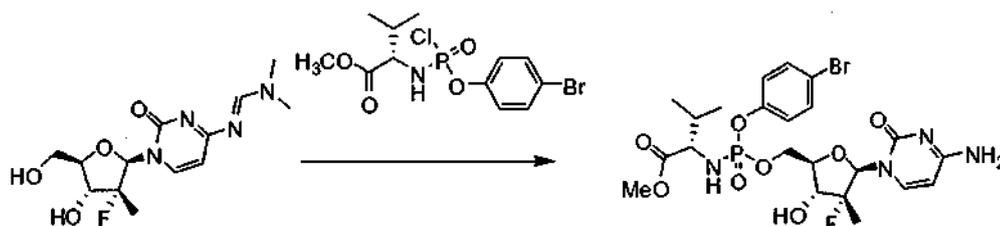
[0108] Phenyl methoxyalaninyl phosphorochloridate (0.6 g, 6 eq) dissolved in 3 mL of THF was added to a mixture of N⁴-(N,N-dimethylformamidinyl)-2'-deoxy-2'-fluoro-2'-C-methylcytidine (0.15 g, 1 eq) and N-methylimidazole (0.3 g, 7.8 eq) in 3 mL THF with vigorous stirring at room temperature, then the reaction was stirred overnight. Solvent was removed

by reduced pressure. The resulting crude product was dissolved in methanol purified by prep-HPLC on a YMC 25x30X2 mm column using a water / acetonitrile gradient elution mobile phase. The acetonitrile and water were removed under reduced pressure to give the desired product (62 mg, 20.6%). ¹H NMR (DMSO-*d*₆) δ 1.16 (d, *J* = 23.2 Hz, 3H), 1.22 (d, *J* = 7.2 Hz, 3H), 3.56 (s, 3H), 3.69-3.75 (d, *J* = 25.6 Hz, 1H), 3.82-3.86 (m, 1H), 3.96-3.98 (m, 1H), 4.21-4.34 (m, 2H), 5.68 (d, *J* = 7.2 Hz, 1H), 5.75-5.77 (m, 1H), 6.07-6.16 (m, 1H), 7.15-7.19 (m, 3H), 7.2 (d, *J* = 9.2 Hz, 2H), 7.39 (t, *J* = 7.8 Hz, 2H), 7.48 (d, *J* = 9.2 Hz, 1H); MS, *m/e* 501(M+1)⁺.

EXAMPLE 11

Preparation of 2'-Deoxy-2'-fluoro-2'-C-methylcytidine 5'-(4-bromophenyl methoxy-valyl phosphate)

[0109]

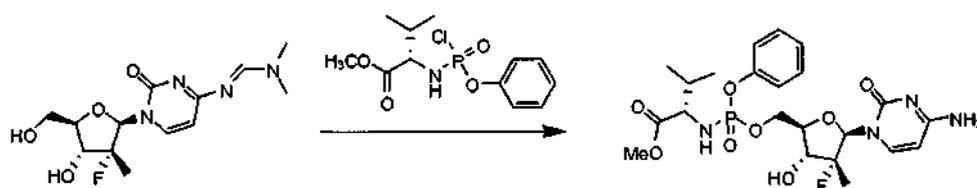


[0110] 4-Bromophenyl methoxy-valyl phosphorochloridate (1.0 g, 3.4 eq.) dissolved in 3 mL of THF was added to a mixture of N⁴-(*N,N*-dimethylformamidinyl)-2'-deoxy-2'-fluoro-2'-C-methylcytidine (0.2 g, 1 eq.) and *N*-methylimidazole (0.35 g, 6 eq.) in 3 mL THF with vigorous stirring at room temperature, then the reaction was stirred overnight. Solvent was removed by reduced pressure. The resulting crude product was dissolved in methanol purified by prep-HPLC on a YMC 25x30X2 mm column using a water / acetonitrile gradient elution mobile phase. The acetonitrile and water were removed under reduced pressure to give the desired product as a white solid (59 mg, 13%); ¹H NMR (DMSO-*d*₆) δ 0.74-0.83 (m, 6H), 1.12-1.20 (m, 3H), 1.89-1.92 (m, 1H), 3.49-3.51 (m, 1H), 3.55 (s, 3H), 3.59-3.68 (m, 1H), 3.72-3.83 (m, 1H), 4.21-4.39 (m, 2H), 5.70-5.72 (m, 1H), 5.76-5.83 (m, 1H), 6.04-6.16 (m, 1H), 7.15 (d, *J* = 13.0 Hz, 2H), 7.26 (s, 1H), 7.33 (s, 1H), 7.46-7.55 (m, 1H), 7.56 (d, *J* = 4.4 Hz, 2H); MS, *m/e* 607 (M+1)⁺.

EXAMPLE 12

Preparation of 2'-deoxy-2'-fluoro-2'-C-methylcytidine 5'-(phenyl methoxy-valyl phosphate)

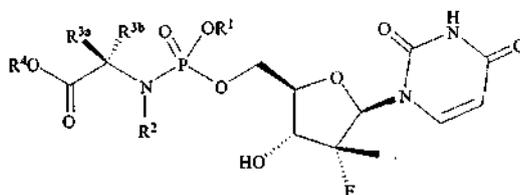
[0111]



[0112] Phenyl methoxy-valyl phosphorochloridate (0.6 g, 6 eq) dissolved in 3 mL of THF was added to a mixture of N⁴-(*N,N*-dimethylformamidinyl)-2'-deoxy-2'-fluoro-2'-C-methylcytidine (0.15 g, 1 eq) and *N*-methylimidazole (0.3 g, 7.8 eq) in 3 mL THF with vigorous stirring at room temperature, then the reaction was stirred overnight. Solvent was removed by reduced pressure. The resulting crude product was dissolved in methanol purified by prep-HPLC on a YMC 25x30X2 mm column using a water / acetonitrile gradient elution mobile phase. The acetonitrile and water were removed under reduced pressure to give the desired product as a white solid (86 mg, 42.9 %). ¹H NMR (DMSO-*d*₆) δ 0.72-0.80 (m, 6H), 1.09-1.18 (m, 3H), 1.87-1.92 (m, 1H), 3.47-3.51 (m, 1H), 3.58 (s, 3H), 3.71-3.75 (m, 1H), 3.97 (t, *J* = 11.2 Hz, 1H), 4.22-4.37 (m, 2H), 5.70 (d, *J* = 8.0 Hz, 1H), 5.76-5.84 (m, 1H), 6.01-6.15 (m, 1H), 7.13-7.18 (m, 3H), 7.27 (s, 2H), 7.34 (d, *J* = 4.0 Hz, 2H), 7.46-7.50 (m, 1H); MS, *m/e* 529 (M+1)⁺.

EXAMPLES

[0113] Example numbers 13-54 and 56-66 are prepared using similar procedures described for examples 5-8. The example number, compound identification, and NMR/MS details are shown below:



Ex.	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	NMR/MS
13	Ph	H	H	Me	Et	1H NMR (DMSO- <i>d</i> ₆) δ 1.12-1.16 (m, 3H), 1.20-1.28(m,6H), 3.70-3.90 (m, 2H), 4.00-4.08 (m, 3H), 4.18-4.45 (m, 2H), 5.52-5.58 (m, 1H), 5.85-5.98 (m, 1H), 6.00-6.20 (m, 2H), 7.16-7.23 (m, 3H), 7.37-7.40 (m, 2H), 7.54-7.60 (m, 1H), 11.54 (s.1H); MS, m/e 516.1 (M+1)+
14	1-Naphth	H	H	Me	Bn	1H NMR (DMSO- <i>d</i> ₆) δ 1.18-1.30 (m, 6H), 3.78-4.10 (m, 3H), 4.38-4.49 (m, 2H), 4.99-5.11 (m, 2H), 5.28-5.40 (m, 1H), 5.85-6.10 (m, 2H), 6.30-6.41 (m, 1H), 7.28-7.32 (m, 5H), 7.41-7.60 (m, 5H), 7.73-7.76(m, 1H), 7.94-8.11(m, 1H), 8.13-8.15(m, 1H), 11.50 (s.1H); MS, m/e 628.4 (M+1)+
15	Ph	H	H	H	Me	1H NMR (DMSO- <i>d</i> ₆) δ 1.22 (d, J=22.4 Hz, 3H), 3.59(s, 3H), 3.63-3.69 (m, 2H), 3.74-3.8(m, 1H), 4.02(d, J=11.2 Hz, 1H), 4.23-4.28(m, 1H), 4.40-4.43 (m, 1H), 5.57-5.60 (m, 1H), 5.89(d, J=6.8 Hz, 1H), 6.00-6.06(m, 2H), 7.15-7.23 (m, 3H), 7.35-7.39 (m, 2H), 7.52(d, J=8 Hz, 1H), 11.52(s, 1H); MS, m/e 487.97 (M+1)+
16	2,4-Cl-Ph	H	H	Me	Me	1H NMR (DMSO- <i>d</i> ₆) δ 1.22-1.28 (m, 6H), 3.57-3.60 (m, 3H), 3.84-3.92 (m, 2H), 4.00-4.04 (m, 1H), 4.31-4.44 (m, 2H), 5.54-5.61 (m, 1H), 5.85-6.10 (m, 2H), 6.32-6.43 (m, 1H), 7.44-7.54 (m, 3H), 7.72-7.75 (m, 1H), 11.54 (s.1H); MS, m/e 570.2 (M+1)+
17	1-Naphth	H	H	Me	Me	1H NMR (DMSO- <i>d</i> ₆) δ 1.15-1.27 (m, 6H), 3.51-3.55 (d, 3H), 3.85-3.96 (m, 2H), 4.00-4.10(m, 1H), 4.30-0.46 (m, 2H), 5.31-5.39 (m, 1H), 5.89-6.05 (m, 2H), 6.22-6.34 (m, 1H), 7.44-7.60 (m, 5H), 7.73-7.77 (m, 1H), 7.93-7.96 (m, 1H), 8.12-8.14 (m, 1H), 11.50(s.1H); MS, m/e 552.1 (M+1)+
18	Ph	*	H	*	Me	1H NMR (DMSO- <i>d</i> ₆) δ 1.19 (d, J=22.8 Hz, 3H), 1.69-1.84 (m, 3H), 1.99-2.04 (m, 1H), 3.16-3.21 (m, 2H), 3.58 (s, 3H), 3.68-3.8 (m, 1H), 4.00 (m, 1H), 4.01-4.13 (m, 1H), 4.22-4.25 (m, 1 H), 4.5 (d, J = 11.2 Hz, 1H), 5.54 (d, J = 8.0 Hz, 1H), 5.86 (s, 1H), 5.6 (d, J = 19.6 Hz, 1H), 7.15-7.2 (m, 3H), 7.34 (t, J = 8.0 Hz, 2H), 7.51 (d, J = 8.0 Hz, 1H), 11.38 (s, 1H); MS, m/e 527.93(M+1)+
19	Ph	H	H	Me	n-Bu	1H NMR (DMSO- <i>d</i> ₆) δ 0.80-0.90 (m, 3H), 1.20-1.35 (m, 8H), 1.48-1.55 (m, 2H), 3.78-3.88 (m, 2H), 3.95-0.08 (m, 3H), 4.22-4.45 (m, 2H), 5.55-5.57(t, 1H), 5.85-6.18 (m, 3H), 7.14-7.23 (m, 3H), 7.35-7.40 (m, 2H), 7.51-7.60 (d, 1H), 11.50 (s.1H); MS, m/e 544.2 (M+1)+

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(continued)

Ex.	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	NMR/MS	
5	20	Ph	H	H	Me	Bn	1H NMR (DMSO- <i>d</i> ₆) δ 1.20-1.30 (m, 6H), 3.72-4.05 (m, 3H), 4.23-4.27 (m, 1H), 4.32-4.45 (m, 1H), 5.07-5.10(t, 2H), 5.52-5.56(t, 1H), 5.86-6.10 (m, 2H), 6.13-6.21(m,1H), 7.15-7.21 (m, 3H), 7.29-7.40 (m, 7H), 7.51-7.56 (d, 1H), 11.50 (s,1H); MS, m/e 578.2 (M+1)+
10	21	4-F-Ph	H	H	Me	Me	1H NMR (DMSO- <i>d</i> ₆) δ 1.28-1.34 (m, 6H), 3.65(d, J= 4 Hz, 3H), 3.85-3.96 (m, 2H), 4.06-4.12 (m, 1H), 4.30-4.34 (m,1H), 4.40-4.47 (m, 1H), 5.62-5.67 (m, 1H), 5.94-6.01(m, 1H), 6.09 (d, J=18.8 Hz, 1H), 6.17-6.26 (m, 1H), 7.27-7.33(m, 4H), 7.62 (d, J = 7.6 Hz, 1H), 11.61 (s, 1H) ; MS, m/e 519.94(M+1)+
15	22	4-Cl-Ph	H	H	Me	Me	1H NMR (DMSO- <i>d</i> ₆) δ 1.22-1.28 (m, 6H), 3.58 (d, 2H), 3.70-3.95(m,2H), 3.95-4.08 (m,1H), 4.23-4.45 (m, 2H), 5.55-5.61(t, 1H), 5.85-6.10 (m, 2H), 6.15-6.23(m,1H), 7.20-7.26 (m, 2H), 7.43-7.46 (m, 2H), 7.54-7.57 (d, 1H), 11.50 (s,1H); MS, m/e 536.1 (M+1)+
20	23	3,4-Cl-Ph	H	H	Me	Me	1H NMR (DMSO- <i>d</i> ₆) δ 1.13 (m, 6H), 3.49 (s, 3H), 3.61-3.85 (m, 2H), 3.90-3.93 (m, 1H), 4.16-4.22 (m, 1H), 4.27-4.31 (m, 1H), 5.47-5.52 (m, 1H), 5.82 (d, J = 11.6 Hz, 1H), 5.93(d, J = 19.2 Hz, 1H), 6.15-6.25 (m, 1H), 7.13 (t, J = 9.6 Hz, 1H), 7.43 (d, J = 12Hz, 2H), 7.57 (d, J = 6.0 Hz, 1H), 11.43(s, 1H); MS, m/e 569.85 (M+1)+
25	24	Ph	H	H	Me	2-Bu	1H NMR (DMSO- <i>d</i> ₆) δ 0.83 (d, J = 6.8 Hz, 6H), 1.20-1.26 (m, 6H), 1.79-1.86 (m, 1H), 3.73-3.90 (m, 4H), 4.01 (t, J = 11.2 Hz, 1H), 4.21-4.28 (m, 1H), 4.33-4.42 (m, 1H), 5.54 (t, J = 7.6 Hz, 1H), 5.85-5.92 (m, 1H), 5.99-6.13 (m, 2H), 7.19 (t, J = 8 Hz, 3H), 7.36 (t, J = 7.6 Hz, 2H), 7.53 (d, J = 7.6 Hz, 1H), 11.52 (s, 1H); MS, m/e 544.00 (M+1)+
30	25	Ph	H	H	Me	i-Pr	1H NMR (DMSO- <i>d</i> ₆) δ 1.13-1.28 (m, 12H), 3.74-3.81 (m, 2H), 3.95-4.08 (m,1H), 4.20-0.45 (m, 2H), 4.83-4.87 (m, 1H), 5.52-5.58 (m, 1H), 5.84-6.15 (m, 3H), 7.17-7.23 (m, 3H), 7.35-7.39 (m, 2H), 7.54-7.57 (m, 1H), 11.50 (s,1H); MS, m/e 530.2 (M+1)+
35	26	4-MeOH-Ph	H	H	Me	n-Bu	1HNMR (400MHz, DMSO- <i>d</i> ₆): δ =0.78-0.82 (m, 3H), 1.29-1.47 (m, 8H), 1.49-1.54 (m, 2H), 3.66-3.87 (m, 5H), 3.96-4.02 (m, 3H), 4.21-4.39 (m, 2H), 5.57 (t, J= 12.0Hz, 1H), 5.84-6.05 (m, 3H), 6.90 (dd, J1 =8.0Hz, J2=4.0Hz, 2H), 7.09-7.14 (dd, J1=16.0Hz, J2=4.0Hz, 2H), 7.55 (d, J=8.0Hz, 1H), 11.48-11.62 (s, 1H)
40	27	4-F-Ph	H	H	Me	Et	1H NMR (DMSO- <i>d</i> ₆) δ 1.12-1.28 (m, 9H), 3.72-3.94(m,2H),3.98-4.10 (m,3H), 4.21-4.42(m,2H), 5.55-5.61 (t, 1H), 5.85-6.20 (m, 3H), 7.18-7.25 (m,4H), 7.55-7.58 (d, 1H), 11.50 (s,1H); MS, m/e 533.90 (M+1)+
45	28	4-F-Ph	H	H	Me	i-Pr	1H NMR (DMSO- <i>d</i> ₆) δ 1.13-1.30 (m, 12H), 3.74-3.85(m,2H), 3.98-4.06 (m, 1H), 4.23-4.41(m,2H), 4.83-4.87 (m, 1H), 5.55-5.61 (t, 1H), 5.85-6.12 (m, 3H), 7.18-7.24 (m,4H), 7.55-7.58 (d, 1H), 11.50 (s,1H); MS, m/e 547.91 (M+1)+
50	29	4-F-Ph	H	H	Me	Bn	1H NMR (DMSO- <i>d</i> ₆) δ 1.10-1.23 (m, 6H), 3.65-3.89(m,3H),4.10-4.30 (m,2H), 4.96-5.00(m,2H), 5.46-5.50 (t, 1H), 5.75-5.96 (m, 2H), 6.04-6.12(m,1H), 7.05-7.11 (m,4H), 7.20-7.24 (d, 5H), 7.42-7.45(d,1H), 11.50 (s,1H); MS, m/e 595.94 (M+1)+
55							

(continued)

Ex.	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	NMR/MS	
5	30	4-MeO-Ph	H	H	Me	i-Pr	¹ H NMR (400MHz, DMSO- <i>d</i> ₆): δ=1.15-1.27 (m, 12H), 3.71-3.89 (m, 5H), 3.98-4.02 (m, 1H), 4.22-4.25 (m, 1H), 4.33-4.39 (m, 1H), 4.84-4.87 (m, 1H), 5.57 (t, J= 12.0Hz, 1H), 5.91-6.03 (m, 3H), 6.90 (d, J= 8.0Hz, 2H), 7.09-7.14 (m, 2H), 7.55 (d, J= 8.0Hz, 1H), 11.51 (s, 1H)
10	31	2-Cl-Ph	H	H	Me	Bn	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.23 (m, 6 H), 3.93-4.00 (m, 3 H), 4.27-4.40 (m, 2H), 5.0(t, J= 7.2 Hz, 2 H), 5.53 (m, 1 H), 5.80-6.0(m, 2 H), 6.30(m, 1H), 7.15 (d, J= 2.4 Hz, 1 H), 7.27 (m, 6 H), 7.51 (m, 3 H), 11.5 (s, 1 H) ; MS, m/e 579.87(M+1)+/ 596.78 (M+18)+
15	32	2,4-Cl-Ph	H	H	Me	n-Bu	¹ H NMR (DMSO- <i>d</i> ₆) δ=0.82 (m, 3 H),1.23 (m, 8 H), 1.47 (m, 2 H), 3.86 (m, 2 H), 3.84 (m, 3 H),4.27-4.43 (m, 2H), 5.5 (m, 1H), 6.02 (m, 2 H), 6.35(m, 1H), 7.44 (m, 3 H), 7.77 (m, 1 H), 11.5 (s, 1 H) ; MS, m/e 611.87(M+1)+
20	33	4-Me-Ph	H	H	Me	i-Pr	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.14-1.27 (m, 12H), 2.17-2.26 (m, 3H), 3.73-3.82 (m, 1H), 3.99-4.02 (m, 1H), 4.23-4.26 (m, 1H), 4.37 -4.40 (m, 1H), 4.82-4.88 (m, 1H), 5.52-5.58 (m, 1H), 5.85-6.07 (m, 3H), 7.01-7.20 (m, 4H), 7.55 (d, J = 16Hz, 1H), 11.51 (s, 1H); MS, m/e 543.98 (M+1)+; 1108.86 (2M+23)+
25	34	4-F-Ph	H	H	Me	n-Bu	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.82-0.89 (m,3H), 1.20-1.31 (m, 8H), 1.48-1.53 (m,2H), 3.77-3.90 (m,2H) ,3.95-4.10 (m,3H), 4.21-4.45(m,2H), 5.56-5.61 (t, 1H), 5.83-6.20 (m, 3H), 7.18-7.25 (m,4H), 7.55-7.58 (d, 1H), 11.50 (s,1H); MS, m/e 584.1 (M+23)+
30	35	3,4-diCl-Ph	H	H	Me	Et	¹ H NMR (DMSO- <i>d</i> ₆) δ1.12-1.31 (m, 9H), 3.77-3.92 (m,2H), 3.95-4.08 (m,3H), 4.21-4.45(m,2H), 5.56-5.62 (t, 1H), 5.80-6.11 (m, 2H), 6.18-6.33(m,1H), 7.18-7.25 (m,1H), 7.49-7.56 (d, 2H), 7.62-7.67(m,1H), 11.50 (s,1H); MS, m/e 606.1 (M+23)+
35	36	2-Cl-Ph	H	H	Me	i-Pr	¹ H NMR (400MHz, DMSO- <i>d</i> ₆): δ =1.12-1.16 (m, 6H), 1.21-1.27 (m, 6H), 3.79-3.85 (m, 2H), 4.00-4.07 (m, 1H), 4.28-4.32 (m, 1H), 4.38-4.43 (m, 1H), 4.83-4.87 (m, 1H), 5.56 (dd, J1=16.0Hz, J2=8.0Hz, 1H), 5.85-6.12 (m, 2H), 6.20-6.33 (m, 1H), 7.19-7.22 (m, 1H), 7.33 (t, J= 16.0Hz, 1H), 7.48-7.55 (m, 3H), 11.55 (s, 1H)
40	37	4-MeO-Ph	H	H	Me	Bn	¹ H NMR(400MHz, DMSO- <i>d</i> ₆): δ=1.19-1.26 (m, 6H), 3.69-3.70 (s, 3H), 3.87 (m, 2H), 3.99 (m, 1H), 4.20-4.21 (m, 1H), 4.35 (m, 1H), 5.07-5.09 (m, 2H), 5.54 (t, J = 16.0Hz, 1H), 5.85-5.92 (m, 1H), 6.04-6.10 (m, 2H), 6.86 (d, J= 8.0Hz, 2H), 7.09 (dd, J1=16.0Hz, J2=4.0Hz, 2H), 7.30-7.34 (m, 5H), 7.53 (s, 1H), 11.52(s, 1H)
45	38	Ph	H	H	Me	n-Pen	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.79-0.81 (m, 3H), 1.17-1.23 (m, 10H), 3.74-3.81 (m, 2H), 3.94-3.96 (m, 3H), 4.19-4.36 (m, 2H), 5.49-5.54 (m, 1H), 5.87-6.08 (m,3H), 7.14-7.33 (m, 3H), 7.31-7.35 (m, 2H), 7.51 (d, J = 8Hz, 1H), 11.51 (s, 1H); MS, m/e 557.9 (M+1)+; 1136.88 (2M+23)+
50	39	4-Cl-Ph	H	H	Me	i-Pr	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.04-1.19 (m, 12H), 3.76-3.80 (m, 2H), 3.98-4.08 (m, 1H), 4.42-4.42 (m, 2H), 4.82-4.85 (m, 1H), 5.55-5.60 (m, 1H), 5.80-6.20 (m,3H),7.20-7.2 5(m, 2H), 7.43 (d, J = 8.8Hz, 1H), 7.54 (d, J = 8Hz, 1H), 11.51 (s, 1H); MS, m/e 563.88 (M+1)+; 1148.73 (2M+23)+
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Ex.	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	NMR/MS	
5	40	4-Cl-Ph	H	H	Me	n-Bu	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.85 (t, J = 7.2 Hz, 3H), 1.22-1.33 (m, 8H), 1.45-1.53 (m, 2H), 3.80-3.87 (m, 2H), 3.96-4.04 (m, 3H), 4.24-4.27 (m, 1H), 4.35-4.39 (m, 1H), 5.56-5.61 (m, 1H), 5.82-6.11 (m, 2H), 6.15-6.18 (m, 1H), 7.20-7.56 (m, 4H), 7.51-7.57 (m, 1H), 11.54 (s, 1H); MS, <i>m/e</i> 577.95(M+1)+
10	41	4-Cl-Ph	H	H	Me	Et	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.14 (t, J = 7.0 Hz, 3H), 1.20-1.28 (m, 6H), 3.77-3.88 (m, 2H), 3.99-4.07 (m, 3H), 4.24-4.28 (m, 1H), 4.34-4.43 (m, 1H), 5.56-5.61 (m, 1H), 5.86-6.13 (m, 2H), 6.15-6.24 (m, 1H), 7.20-7.26 (m, 2H), 7.44 (d, J = 7.6 Hz, 2H), 7.55 (d, J = 7.6 Hz, 1H), 11.55 (s, 1H); MS, <i>m/e</i> 549.11(M+1)+
15	42	4-Me-Ph	H	H	Me	n-Bu	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.79-0.83 (m, 3H), 1.17-1.28 (m, 8H), 1.45-1.47 (m, 2H), 2.22 (d, J = 2.8 Hz, 1H), 3.70-3.90 (m, 2H), 3.95-3.98 (m, 3H), 4.10-0.40 (m, 2H), 5.51 (t, 1H), 5.80-5.90 (m, 1H), 5.95-6.05 (m, 2H), 7.02-7.06 (m, 2H), 7.51 (t, J = 4.2 Hz, 4H), 7.51 (d, 1H), 11.51 (s, 1H); MS, <i>m/e</i> 557.99(M+1)+; 1136.84(2M+23)+
20	43	4-Me-Phe	H	H	Me	Bn	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.16-1.24 (m, 6H), 2.22 (s, 3H), 3.65-4.03 (m, 3H), 4.11-4.38 (m, 2H), 5.04-5.05 (m, 2H), 5.48-5.50 (m, 1H), 5.77-5.87 (m, 1H), 5.90-6.11 (m, 2H), 6.98-7.10 (m, 4H), 7.28-7.32 (m, 5H), 7.50 (t, 1H), 11.48 (s, 1H); MS, <i>m/e</i> 592.00 (M+1)+.
25	44	Ph	H	H	Et	Me	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.70-0.80 (m, 3H), 1.11-1.26 (m, 3H), 1.42-1.61 (m, 2H), 3.50-3.54 (m, 3H), 3.58-3.80 (m, 2H), 3.91-4.02 (m, 1H), 4.12-4.38 (m, 2H), 5.47-5.52 (m, 1H), 5.90-6.03 (m, 2H), 7.08-7.16 (m, 3H), 7.26-7.35 (m, 2H), 7.48 (t, 1H), 11.45 (s, 1H); MS, <i>m/e</i> 515.95 (M+1)+; 1052.82 (2M+23)+
30	45	Ph	H	H	Me	4-F-Bn	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆): δ 1.20-1.26 (m, 6H), 3.80-3.93 (m, 2H), 3.98 (s, 1H), 4.25-4.26 (m, 1H), 4.36-4.37 (m, 1H), 5.07 (s, 2H), 5.52-5.55 (m, 1H), 5.86-5.87 (m, 1H), 5.98-6.04 (m, 1H), 6.14-6.17 (m, 1H), 7.15-7.20 (m, 5H), 7.36 (dd, J = 20.0, 8.0 Hz, 4H), 7.54 (s, 1H), 11.55 (s, 1H)
35	46	4-Cl-Ph	H	H	Me	n-Bu	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆): δ 1.21-1.28 (m, 6H), 3.71-3.88 (m, 1H), 3.91-3.98 (m, 1H), 4.00-4.01 (m, 1H), 4.23-4.27 (m, 1H), 4.35-4.38 (m, 1H), 5.08 (d, J = 4.0 Hz, 2H), 5.57 (dd, J = 12.0, 8.0 Hz, 1H), 5.91 (d, J = 8.0 Hz, 1H), 6.01 (d, J = 8.0 Hz, 1H), 6.22-6.24 (m, 1H), 7.17-7.23 (m, 2H), 7.31-7.40 (m, 7H), 7.53 (s, 1H), 11.50 (s, 1H)
40	47	Ph	H	H	Me	3-Me-1-Bu	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.80-0.82 (m, 6H), 1.18-1.40 (m, 8H), 1.50-1.58 (m, 1H), 3.71-3.82 (m, 3H), 3.97-3.4.01 (m, 3H), 4.21-4.40 (m, 2H), 5.30 (t, J = 8.6 Hz, 1H), 5.81-6.10 (m, 3H), 7.15-7.20 (m, 3H), 7.32-7.36 (m, 2H), 7.48 (d, J = 8.4 Hz, 1H), 11.38 (s, 1H); MS, <i>m/e</i> 557.98 (M+1)+; 1136.88 (2M+23)+
45	48	3,4-diCl-Ph	H	H	Me	Bn	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.05-1.37 (m, 6H), 3.71-3.82 (m, 1H), 3.87-4.02 (m, 2H), 4.28-4.29 (m, 1H), 4.36-4.38 (m, 1H), 5.04 (d, J = 5.2 Hz, 2H), 5.55-5.64 (m, 1H), 5.85-5.94 (m, 1H), 6.00-6.05 (m, 1H), 6.29-6.40 (m, 1H), 7.17-7.24 (m, 1H), 7.30-7.41 (m, 5H), 7.45-7.58 (m, 2H), 7.61 (d, J = 4.0 Hz, 1H), 11.53 (s, 1H); MS, <i>m/e</i> 545.80(M+1)+;
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Ex.	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	NMR/MS	
5	49	Ph	H	H	Me	c-Hex	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.18-1.41 (m, 12H), 1.59-1.67 (m, 4H), 3.74-13.80 (m, 1H), 3.96-4.02 (m, 1H), 4.19-4.26 (m, 1H), 4.31-4.39 (m, 1H), 4.60 (s, 1H), 5.52 (t, J= 7.8 Hz, 1H), 5.80-6.09 (m, 3H), 7.15-7.20 (m, 3H), 7.32-7.36 (m, 2H), 7.52 (d, J = 8 Hz, 1H), 11.50 (s, 1H); MS, 569.98 (M+1) ⁺ ; 592.14 (M+23) ⁺
10	50	Ph	H	Me	H	n-Bu	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.76 (t, J = 7.2Hz, 3H), 1.10-1.22 (m, 8H), 1.38-1.43 (m, 2H), 3.72-3.75 (m, 2H), 3.87-3.93 (m, 3H), 4.14-4.21 (m, 1H), 4.23-4.33 (m, 1H), 5.46-5.54 (m, 1H), 5.84-6.11 (m, 3H), 7.09-7.14 (m, 2H), 7.27-7.32 (m, 2H), 7.34-7.51 (m, 1H), 11.47 (s, 1H); MS, m/e 543.98(M+1) ⁺
15	51	Ph	H	Me	H	i-Pr	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.39 (d, J = 7.2Hz, 6H), 1.19-1.29 (m, 6H), 3.65-3.75 (m, 2H), 3.95-4.05 (m, 1H), 4.20-4.22 (m, 1H), 4.31-4.33 (m, 1H), 4.79-4.82 (m, 1H), 5.48-5.57 (m, 1H), 5.84-5.91 (m, 1H), 5.96-6.07 (m, 2H), 7.12-7.35 (m, 5H), 7.44-7.54 (m, 1H), 11.49(s, 1H); MS, m/e 529.96 (M+1) ⁺
20	52	Ph	H	Me	H	Bn	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.18-1.28 (m, 6H), 3.70-3.83 (m, 1H), 3.87-3.94 (m, 1H), 3.99-4.01 (m, 1H), 4.23-4.26 (m, 1H), 4.33-4.37 (m, 1H), 5.03-5.12 (m, 2H), 5.51-5.59 (m, 1H), 5.87-5.90 (m, 1H), 5.95-6.07 (m, 1H), 6.10-6.27 (m, 1H), 7.15-7.23 (m, 3H), 7.31-7.38 (m, 7H), 7.47-7.56 (m, 1H), 11.50 (s, 1H); MS, m/e 577.99 (M+1) ⁺
25	53	2-Cl-Ph	H	H	Me	n-Bu	¹ H NMR (400MHz, DMSO- <i>d</i> ₆): δ 0.81-0.86 (m, 3H), 1.21-1.31 (m, 8H), 1.46-1.52 (m, 2H), 3.84-3.90 (m, 2H), 3.97-4.04 (m, 3H), 4.27-4.41 (m, 2H), 5.53-5.58 (m, 1H), 5.82-5.95 (m, 1H), 5.96-6.10 (m, 1H), 6.27-6.31 (m, 1H), 7.19-7.22 (m, 1H), 7.34 (dd, J=8.0, 4.0 Hz, 1H), 7.47-7.55 (m, 3H), 11.55 (s, 1H)
30	54	4-Br-Ph	H	H	Me	i-Pr	¹ H NMR (400MHz, DMSO- <i>d</i> ₆): δ 1.10-1.14 (m, 6H), 1.20-1.27 (m, 6H), 3.74-3.81 (m, 2H), 3.99-4.01 (m, 1H), 4.21-4.25 (m, 1H), 4.37-4.38 (m, 1H), 4.81-4.85 (m, 1H), 5.58 (dd, J=8.0, 4.0 Hz, 1H), 5.82-5.95 (m, 1H), 5.96-6.09 (m, 1H), 6.10-6.13 (m, 1H), 7.18 (dd, J = 12.0, 8.0 Hz, 2H), 7.53-7.57 (m, 3H), 11.52 (s, 1H)
35	55	4-F-Ph	H	H	Me	c-Hex	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.20-1.44 (m, 12H), 1.60-1.71 (m, 4H), 3.75-4.02 (m, 2H), 3.94-4.02 (m, 1H), 4.19-4.26 (m, 2H), 4.59-4.61 (m, 1H), 5.57 (t, J = 8.4 Hz, 1H), 5.85-6.06 (m, 3H), 7.17-7.23 (m, 4H), 7.54 (d, J = 8.4 Hz, 1H), 11.50 (s, 1H); MS, m/e 587.92 (M+1) ⁺
40	56	4-Br-Ph	H	H	Me	c-Hex	¹ H NMR (400MHz, DMSO- <i>d</i> ₆): δ =1.18-1.46 (m, 12H), 1.61-1.69 (m, 4H), 3.75-3.82 (m, 2H), 3.95-4.08 (m, 1H), 4.25-4.28 (m, 1H), 4.38 (s, 1H), 4.60-4.62 (m, 1H), 5.56-5.60 (m, 1H), 5.82-5.95 (m, 1H), 6.02-6.20 (m, 2H), 7.09-7.20 (m, 2H), 7.53-7.57 (m, 3H), 11.52 (s, 1H) MS, m/e 650.0 (M+3) ⁺
45	57	Ph	H	H	Et	i-Pr	¹ H NMR (400MHz, DMSO- <i>d</i> ₆): δ =0.75-0.82 (m, 3H), 1.12-1.26 (m, 9H), 1.52-1.59 (m, 2H), 3.55-3.68 (m, 1H), 3.72-3.85 (m, 1H), 3.95-4.08 (m, 1H), 4.18-4.28 (m, 1H), 4.32-4.41 (m, 1H), 4.83-4.86 (m, 1H), 5.55 (m, J = 7.6Hz, 1H), 5.99-6.04 (m, 2H), 6.05-6.10 (m, 1H), 7.14-7.21 (m, 3H), 7.33-7.37 (m, 2H), 7.52-7.54 (m, 1H), 11.53 (s, 1H); MS, m/e 566.07(M+23) ⁺
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Ex.	R ¹	R ²	R ^{3a}	R ^{3b}	R ⁴	NMR/MS	
5	58	Ph	H	H	Et	c-Hex	¹ H NMR (400MHz, DMSO- <i>d</i> ₆): δ 0.75-0.88 (m, 3H), 1.26-1.46 (m, 9H), 1.52-1.69 (m, 6H), 3.60-3.63 (m, 1H), 3.72-3.90 (m, 1H), 4.02-4.03 (m, 1H), 4.24-4.27 (m, 1H), 4.37-4.38 (m, 1H), 4.63-4.65 (m, 1H), 5.55 (dd, <i>J</i> = 8.0Hz, 4.4Hz, 1H), 5.80-5.95 (m, 1H), 6.00-6.07 (m, 2H), 7.15-7.22 (m, 3H), 7.34-7.38 (m, 2H), 7.54 (d, <i>J</i> = 8.0Hz, 1H), 11.55 (s, 1H); MS, <i>m/e</i> 584.01 (M+1) ⁺ ; 606.17 (M+23) ⁺
10	59	4-F-Ph	H	H	Et	c-Hex	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.75-0.84 (m, 3H), 1.24 (d, <i>J</i> = 22.8Hz, 3H), 1.29-1.47 (m, 6H), 1.51-1.70 (m, 6H), 3.59-3.66 (m, 1H), 3.77-3.84 (m, 1H), 3.98-4.04 (m, 1H), 4.21-4.27 (m, 1H), 4.34-4.41 (m, 1H), 4.60-4.65 (m, 1H), 5.56-5.60 (m, 1H), 5.84-5.90 (m, 1H), 6.00-6.08 (m, 2H), 7.20-7.24 (m, 4H), 7.56(d, <i>J</i> = 8.0Hz, 1H), 11.49 (s, 1H); MS, <i>m/e</i> 602.00(M+1) ⁺
15	60	Ph	H	H	Me	F-CH ₂ -CH ₂ -	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.18-1.25(m, 6H), 3.71-3.89 (m, 2H), 3.92-3.99 (m, 1H), 4.19-4.27 (m, 4H), 4.48-4.61 (m, 2H), 3.94-3.98 (m, 2H), 4.11-4.23 (m, 4H), 5.47-5.52 (m, 1H), 6.01-6.11 (m, 1H), 5.90-6.14 (m, 2H), 7.15-7.21 (m, 3H), 7.32-7.36 (m, 2H), 7.46-7.57 (m, 1H), 11.49 (s, 1H); MS, <i>m/e</i> 533.86 (M+1) ⁺
20	61	Ph	H	H	Me	F ₂ CH-CH ₂ -	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.17-1.24 (m, 6H), 3.67-3.81 (m, 1H), 3.89-3.98 (m, 2H), 4.21-4.36 (m, 4H), 5.48-5.53 (m, 1H), 5.82-6.05 (m, 2H), 6.18-6.22 (m, 2H), 7.15-7.20 (m, 3H), 7.32-7.36 (m, 2H), 7.51 (s, 1H), 11.50 (s, 1H); MS, <i>m/e</i> 551.92 (M+1) ⁺ ;
25	62	Ph	H	H	Me	(CF ₃) ₂ -CH-	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.13-1.29 (m, 6H), 3.67-3.81 (m, 1H), 3.94-4.32 (m, 4H), 5.47 (t, <i>J</i> = 8 Hz 1H), 5.82-6.01 (m, 2H), 6.33-6.36 (m, 1H), 6.70-6.78 (m, 1H), 7.09-7.15 (m, 3H), 7.28-7.32 (m, 2H), 7.43-7.46 (m, 1H), 11.44 (s, 1H); MS, <i>m/e</i> 637.90 (M+1) ⁺
30	63	Ph	H	H	Me	(CH ₂ F) ₂ - -CH-	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.20-1.29 (m, 6H), 3.70-3.90 (m, 1H), 3.91-4.12 (m, 2H), 4.20-4.33 (m, 1H), 4.35-4.48 (m, 1H), 4.52-4.55 (m, 2H), 4.63-4.67 (m, 2H), 5.20-5.35 (m, 1H), 5.56 (t, <i>J</i> = 8.4 Hz, 1H), 5.80-5.95 (m, 1H), 5.95-6.10 (m, 1H), 6.18-6.21 (m, 1H), 7.18-7.23 (m, 3H), 7.35-7.39 (m, 2H), 7.54 (s, 1H), 11.55 (s, 1H); MS, <i>m/e</i> 565.98 (M+1) ⁺
35	64	Ph	H	H	Me	c-Pr- CH ₂ -	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.20-0.24(m, 2H), 0.47-0.48(m, 2H), 0.76-0.84(m, 3H), 1.03-1.05(m, 1H), 1.23(dd, <i>J</i> = 22.4 Hz 3H), 1.55-1.60(m, 2H), 3.61-3.68(m, 1H), 3.81-3.89 (m, 3H), 3.98-4.03(m, 1H), 4.23-4.29(m, 1H), 4.35-4.41(m, 1H), 5.56-6.00(m, 1H), 5.88-5.91(m, 1H), 6.04-6.10(m, 2H), 7.20-7.24(m, 4H), 7.55 (d, <i>J</i> = 7.6 Hz 1H), 11.53 (s, 1H); MS, <i>m/e</i> 573.17 (M+1) ⁺
40	65	Ph	H	H	Et	c-Pen	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.75-0.83 (m, 3H), 1.20-1.28 (m, 3H), 1.49-1.63 (m, 8H), 1.76-1.80 (m, 2H), 3.58-3.60 (m, 1H), 3.70-3.82 (m, 1H), 3.98-4.05 (m, 1H), 4.24-4.26 (m, 1H), 4.37-4.42 (m, 1H), 5.03 (s, 1H), 5.54-5.57 (m, 1H), 5.90-6.00 (m, 1H), 6.02-6.07 (m, 2H), 7.15-7.22 (m, 3H), 7.35-7.39 (m, 2H) 7.55 (d, <i>J</i> = 8.0 Hz, 1H), 11.55 (s, 1H); MS, <i>m/e</i> 570.03 (M+1) ⁺

*R² and R^{3b} together are -(CH₂)₃- as derived from L-proline

The purification procedure by Prep-HPLC:

[0114] Crude products were dissolved in methanol. Injection volumes of these solutions were 5 mL.

[0115] The preparative HPLC system including 2 sets of Gilson 306 pumps, a Gilson 156 UV/V is detector, a Gilson 215 injector & fraction collector, with Unipoint control software. A Ymc 25×30×2 mm column was used. The mobile phase was HPLC grade water (A), and HPLC grade acetonitrile (B). Fractions were collected into 100*15mm glass tubes.

[0116] HPLC gradient is shown in Table 1. Once the gradient was selected, acetonitrile solution was injected into HPLC system, and then fractions collected according to UV peaks. After the separation, each glass tubes were run MS test to collect the desired compounds. The fractions with target MS were combined in a well-weighted flask. Most of acetonitrile was removed under reduce pressure and the remaining solution was freeze-dried to give desired compound.

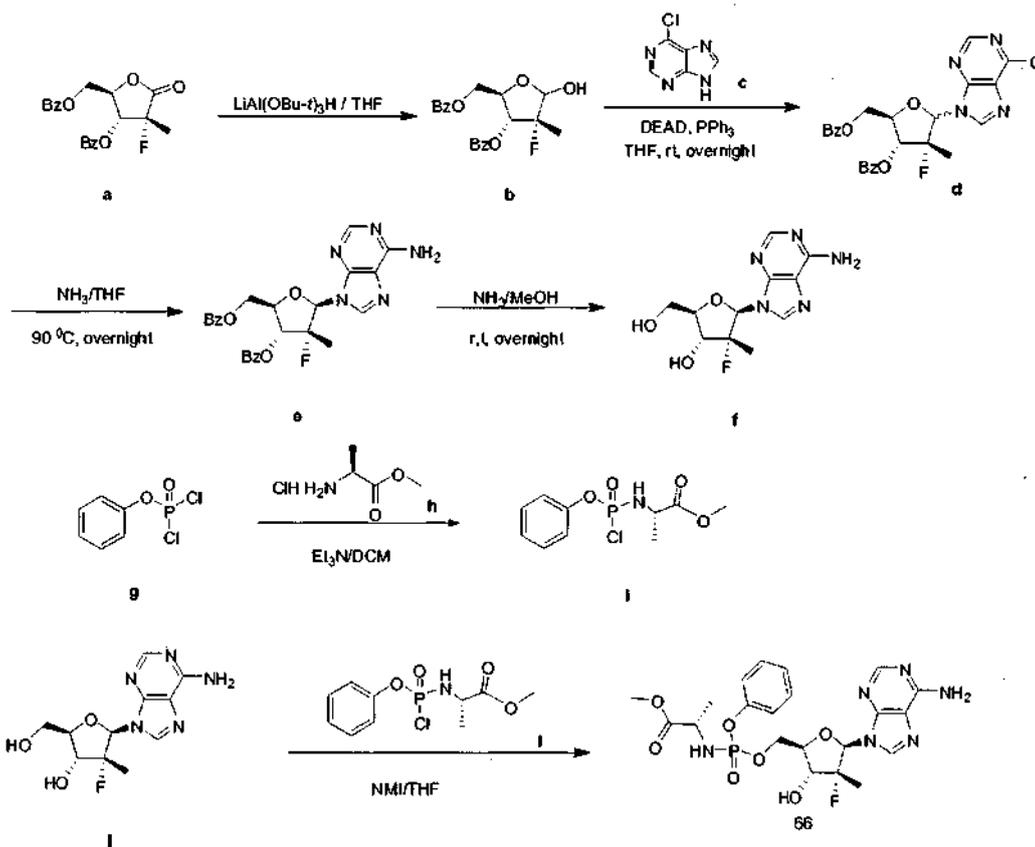
Table 1:

Preparative HPLC gradient			
Time (min)	Flow rate (mL/min)	% A	% B
0	15	90	10
30	15	60	40

Preparation of Example 66

[0117]

Scheme



Preparation of compound (b)

[0118] To a solution of compound **a** (1 g, 2.69 mmol) in anhydrous THF (30 mL) was added dropwise 1 M solution of $\text{LiAl}(\text{OBu-}t)_3\text{H}$ in THF (2.69 mL, 2.69 mmol) at $-20\text{ }^\circ\text{C}$. The reaction mixture was stirred for 2-3 h at the same temperature.

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EtOAc (100 mL) was added followed by saturated NH_4Cl solution (10 mL) and reaction mixture was slowly brought to room temperature. Reaction mixture was extracted with EtOAc and washed with 1N HCl and water. Combined organic phase was evaporated to give 0.8 g of crude compound **b** as transparent oil, which was used directly for next reaction.

5 Preparation of compound (d)

[0119] To a solution of compound **b** (0.8 g, 2.1 mmol), compound **c** (0.45 g, 2.5 mmol) and Ph_3P (0.56 g, 2.1 mmol) in anhydrous THF (20 mL) under nitrogen atmosphere was added DEAD (1.8 mL). The reaction mixture was stirred at room temperature overnight. The reaction solution was concentrated under reduce pressure. The residue was separated by preparative layer chromatography (hexanes:EtOAc = 3:1) to give crude compound **d** (0.8 g). The crude compound **d** was used to the next step without further purification.

Preparation of compound (e)

15 [0120] Compound **d** (0.8 g, 1.57 mmol) was dissolved in THF (2 mL) and THF saturated with ammonia (5 mL) was then added to this solution. The reaction mixture was heated to 90 °C overnight. After 18 hours, the solution was cooled to room temperature by ice water, then the solvent was removed under reduced pressure and the residue was purified by column to give compound **e** (0.75 g) for the next step.

20 Preparation of compound (f)

[0121] Compound **e** (0.5 g, 1.01 mmol) was dissolved in methanol (2 mL) and methanol was saturated with ammonia (5 mL) was then added to this solution. The reaction mixture was stirred at room temperature overnight. After 18 hours, the solvent was removed under reduced pressure and the residue was purified by column to give crude compound **f** (0.15 g) for the next step.

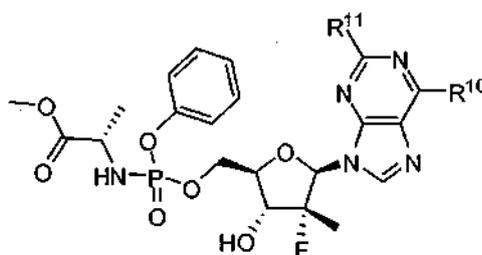
Preparation of compound (i)

30 [0122] A solution of triethylamine (1.07 g, 10.6 mmol) in anhydrous dichloromethane (15 mL) was added dropwise to a solution of compound **g** (1.16 g, 5.3 mmol) and compound **h** (1.31 g, 5.3 mmol) in dichloromethane (10 mL) with vigorous stirring at -78 °C over a period of 2 hours. After completion of addition, the reaction temperature was allowed to warm to room temperature gradually and stirred over night. Then the solvent was removed under vacuum and anhydrous ether 20 mL was added and the precipitated salt was filtered and the precipitate was washed with ether. The combined organic phase was concentrated to give the colorless oil of compound **i** (1.0 g).

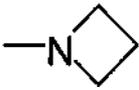
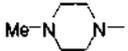
35 Preparation of Compound 66

[0123] To a solution of compound **j** (0.1 g, 0.35 mmol) dissolved in 10 mL of anhydrous THF, stirred and added 0.4g NMI till the solution became clear, added compound **i** (0.8 g, 2.89 mmol) in 10 mL THF dropwise, stirred at r.t. overnight. Compound purity and identification was confirmed by LCMS. The solvent was evaporated and purified by Prep-HPLC to afford **66**. (25 mg, Yield: 13.6%). ^1H NMR ($\text{DMSO}-d_6$) δ 1.08 (d, $J = 22.8$ Hz, 3H), 1.17-1.24 (m, 3H), 3.50-3.52 (m, 3H), 3.78-3.83 (m, 1H), 4.10-4.13 (m, 1H), 4.24-4.44 (m, 2H), 5.85-5.92 (m, 1H), 6.01-6.11 (m, 1H), 6.2.-6.27 (m, 1H), 7.08-7.19 (m, 4H), 7.31-7.38 (m, 3H), 8.15 (s, 1H), 8.26 (s, 1H); MS, m/e 525 ($\text{M}+1$) $^+$.

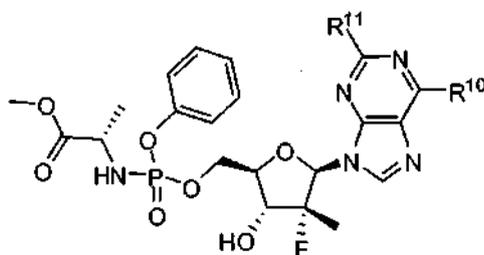
[0124] Example numbers **67-74**, identified below, were prepared using similar procedures disclosed for Example **66**, above.



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Example	R ¹¹	R ¹⁰	NMR/MS
67	OH	NH ₂	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.06-1.13 (m, 3H), 1.20-1.24 (m, 3H), 3.27-3.33(m, 3H), 3.56 (s, 1H), 3.82-3.88 (m, 1H), 4.07-4.13 (m, 1H), 4.25-4.40 (m, 2H), 5.85-5.87 (m, 1H), 5.98-6.09 (m, 2H), 6.59 (s, 32H), 7.14-7.37 (m, 3H), 7.35-7.37 (m, 2H), 7.79 (d, <i>J</i> =7.2 Hz, 1H), 10.69 (s, 1H); MS, <i>m/e</i> 541 (M+1) ⁺ ;
68	NH ₂	NH ₂	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.07 (d, <i>J</i> =22.8 Hz, 3H), 1.19 (d, <i>J</i> =7.2 Hz, 3H), 3.51 (s, 3H), 3.62 (s, 1H), 3.75-3.81 (m, 1H), 4.05-4.11 (m, 1H), 4.27-4.42 (m, 2H), 5.79-5.83 (m, 1H), 5.92 (s, 2H), 6.00-6.09 (m, 2H), 6.75 (s, 2H), 7.08-7.17 (m, 3H), 7.31-7.35 (m, 2H), 7.78 (s, 1H); MS, <i>m/e</i> 540 (M+1) ⁺ ;
69	NH ₂	c-Pentyl-NH-	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.05 (d, <i>J</i> =22.8 Hz, 3H), 1.09-1.19 (m, 3H), 1.48 (s, 4H), 1.66 (s, 1H), 1.86 (s, 1H), 3.54 (d, <i>J</i> =14 Hz, 3H), 3.65 (s, 1H), 4.25-4.43 (m, 4H), 5.71-5.82 (m, 1H), 5.94-6.04 (m, 4H), 7.11-7.24 (m, 3H), 7.26-7.34 (m, 2H), 7.77(d, <i>J</i> =3.6 Hz, 1H); MS, <i>m/e</i> 608(M+1) ⁺
70	NH ₂		¹ H NMR (DMSO- <i>d</i> ₆) δ 1.07 (d, <i>J</i> =22.4 Hz, 3H), 2.35-2.38 (m, 2H), 3.54 (d, <i>J</i> =9.2 Hz, 3H), 3.59-3.62 (m, 2H), 3.65 (s, 1H), 3.75-3.82 (m, 1H), 4.01-4.13 (m, 2H), 4.22-4.40 (m, 6H), 5.75-5.85 (m, 1H), 6.00-6.07 (m, 4H), 7.15-7.21 (m, 3H), 7.32-7.35 (m, 2H), 7.79 (d, <i>J</i> =4.0 Hz, 1H); MS, <i>m/e</i> 580 (M+1) ⁺
71	NH ₂	Et ₂ N-	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.06-1.28 (m, 12H), 3.55 (d, <i>J</i> =4.8 Hz, 3H), 3.79-3.87 (m, 4H), 4.07-4.12 (m, 2H), 4.29-4.42 (m, 3H), 5.75-5.82 (m, 1H), 5.94 (s, 2H), 6.04-6.10 (m, 2H), 7.14-7.22 (m, 3H), 7.31-7.37 (m, 2H), 7.82 (d, <i>J</i> =4.4 Hz, 1H); MS, <i>m/e</i> 596 (M+1) ⁺
72	NH ₂	n-Propyl-NH-	¹ H NMR (DMSO- <i>d</i> ₆) δ 0.84 (t, <i>J</i> =7.2 Hz, 3H), 1.01-1.01 (m, 3H), 1.09-1.12 (m, 3H), 1.51-1.56 (m, 2H), 3.48 (d, <i>J</i> =15.2 Hz, 3H), 3.79-3.82 (m, 1H), 4.04-4.05 (m, 1H), 4.27-4.38 (m, 3H), 5.72-5.79 (m, 1H), 5.98-6.04 (m, 4H), 7.13-7.20 (m, 3H), 7.26-7.32 (m, 2H), 7.76 (d, <i>J</i> =5.2 Hz, 1H); MS, <i>m/e</i> 582 (M+1) ⁺
73	NH ₂	c-Butyl-NH-	¹ H NMR (DMSO- <i>d</i> ₆) δ 1.02-1.08 (m, 3H), 1.18 (d, <i>J</i> =4.8 Hz, 3H), 1.44-1.61 (m, 2H), 2.02-2.17 (m, 4H), 3.51 (d, <i>J</i> =10.8 Hz, 3H), 3.78-3.83 (m, 1H), 4.03-4.06 (m, 1H), 4.27-4.38 (m, 2H), 4.53-4.62 (m, 1H), 5.68-5.79 (m, 1H), 5.95-6.04 (m, 4H), 7.11-7.18 (m, 3H), 7.29-7.35 (m, 2H), 7.51-7.58 (m, 1H), 7.78 (d, <i>J</i> =5.2 Hz, 1H); MS, <i>m/e</i> 594 (M+1) ⁺
74	NH ₂		¹ H NMR (DMSO- <i>d</i> ₆) δ 0.97-1.20 (m, 6H), 2.18 (s, 3H), 2.19 (s, 4H), 3.43-3.47(m, 3H), 3.75 (s, 1H), 4.01-4.06 (m, 4 H), 4.22-4.35 (m, 3H), 5.69-5.75 (m, 1H), 5.98-6.05 (m, 3H), 7.09-7.15 (m, 3H), 7.25-7.29 (m, 2H), 7.77 (d, <i>J</i> =3.6 Hz, 1H); MS, <i>m/e</i> 623 (M+1) ⁺

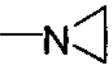
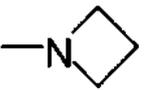
[0125] Example numbers 75-80 are prepared using similar procedures disclosed for Example 66, above.



Example	R ¹¹	R ¹⁰
75	H	n-propyl-NH-

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(continued)

Example	R ¹¹	R ¹⁰
76	H	c-Butyl-NH-
77	H	c-Pentyl-NH-
78	H	
79	H	
80	H	

EXAMPLE 81

[0126] Certain exemplified compounds were obtained as mixture of diastereomers because of the chirality at phosphorous. The diastereomers were separated on a Chiralpak-AS-H (2 X 25 cm) column under Supercritical Fluid Chromatography (SFC) conditions using 20% methanol in carbon dioxide as solvent. The absolute stereochemistry of the P-chiral center of the diastereomers were not determined. However, chromatographic resolution of these two diastereomers provides for isomers that are characterized as fast eluting and slow eluting isomers. Some examples are shown below.

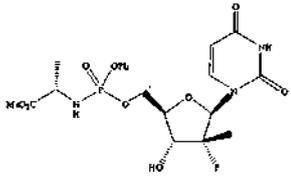
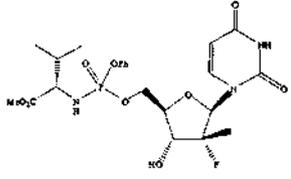
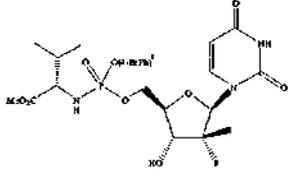
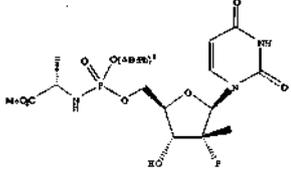
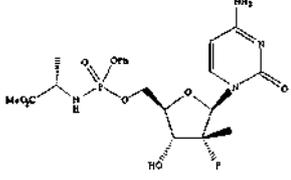
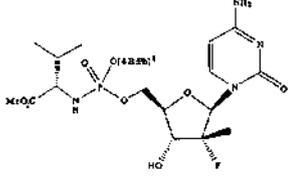
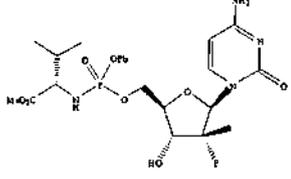
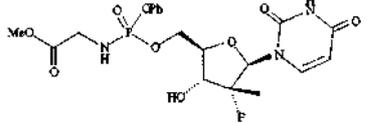
Compound	EC ₉₀ (μM)
Example 15 (Diastereomeric mixture)	0.86
Fast Moving isomer of Example 15	1.35
Slow Moving isomer of Example 15	0.26
Example 39 (Diastereomeric mixture)	0.47
Fast Moving isomer of Example 39	0.78
Slow Moving isomer of Example 39	0.02
Example 49 (Diastereomeric mixture)	0.126
Fast Moving isomer of Example 49	0.03
Slow Moving isomer of Example 49	5.78

EXAMPLE 82

[0127] HCV replicon assay. HCV replicon RNA-containing Huh7 cells (clone A cells; Apath, LLC, St. Louis, Mo.) were kept at exponential growth in Dulbecco's modified Eagle's medium (high glucose) containing 10% fetal bovine serum, 4 mM L-glutamine and 1 mM sodium pyruvate, 1×nonessential amino acids, and G418 (1,000 μg/ml). Antiviral assays were performed in the same medium without G418. Cells were seeded in a 96-well plate at 1,500 cells per well, and test compounds were added immediately after seeding. Incubation time 4 days. At the end of the incubation step, total cellular RNA was isolated (RNeasy 96 kit; Qiagen). Replicon RNA and an internal control (TaqMan rRNA control reagents; Applied Biosystems) were amplified in a single-step multiplex RT-PCR protocol as recommended by the manufacturer. The HCV primers and probe were designed with Primer Express software (Applied Biosystems) and covered highly conserved 5'-untranslated region (UTR) sequences (sense, 5'-AGCCATGGCGTTAGTA(T)GAGTGT-3', and antisense, 5'-TTCCGCAGACCACTATGG-3'; probe, 5'-FAM-CCTCCAGGACCCCCCTCCC-TAMRA-3').

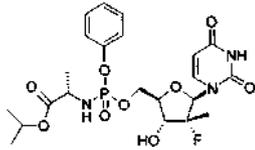
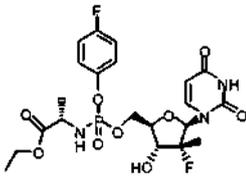
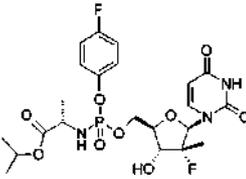
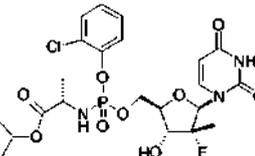
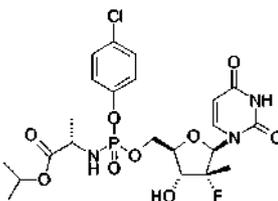
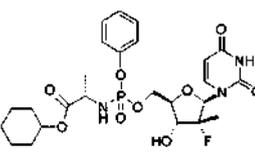
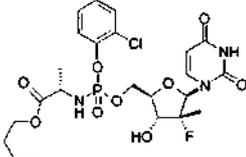
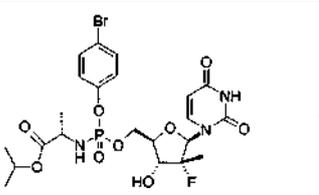
[0128] To express the antiviral effectiveness of a compound, the threshold RT-PCR cycle of the test compound was subtracted from the average threshold RT-PCR cycle of the no-drug control (ΔCt_{HCV}). A ΔCt of 3.3 equals a 1-log 10 reduction (equal to the 90% effective concentration [EC₉₀]) in replicon RNA levels. The cytotoxicity of the test compound could also be expressed by calculating the ΔCt_{rRNA} values. The $\Delta\Delta Ct$ specificity parameter could then be introduced ($\Delta Ct_{HCV} - \Delta Ct_{rRNA}$), in which the levels of HCV RNA are normalized for the rRNA levels and calibrated against the no-

drug control.

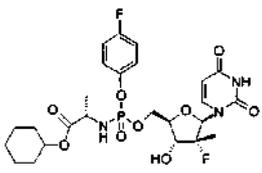
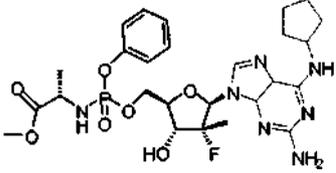
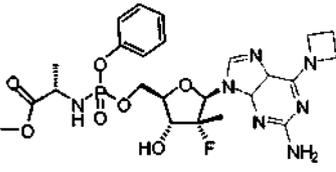
Ex #	Compound	Log10 Reduction at 50 μ M	EC90 (μ M)
5		-1.21	3.0
6		-0.45	ND
7		0.31	ND
8		-1.48	2.11
10		-1.25	19.15
11		-0.55	ND
12		0.31	ND
15		ND	0.86

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(continued)

Ex #	Compound	Log10 Reduction at 50 μ M	EC90 (μ M)
25		-2.22	0.39
27		-2.25	0.66
28		-2.16	0.75
36		-1.64	21.9
39		-1.78	0.47
49		-2.69	0.126
53		-1.33	<0.3
54		-1.55	0.57

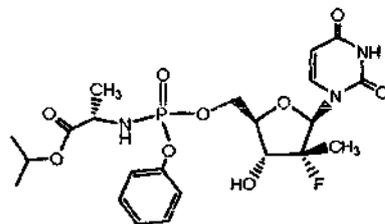
(continued)

Ex #	Compound	Log10 Reduction at 50 μ M	EC90 (μ M)
55		-2.38	<0.3
69		-2.25	< 0.3
70		-2.25	<0.3

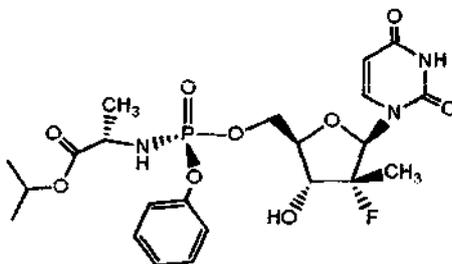
¹(4-BrPh): 4-bromo-phenyl.

Claims

1. A compound represented by the formula

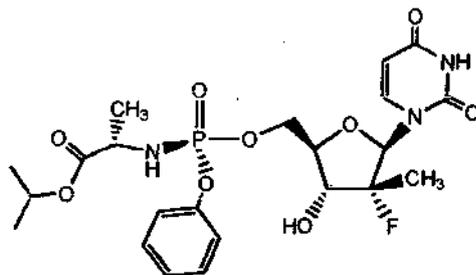


2. A compound represented by the formula



3. A compound represented by the formula

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4. A composition comprising the compound of claim 1 and a pharmaceutically acceptable medium.

5. A composition comprising the compound of claim 2 and a pharmaceutically acceptable medium.

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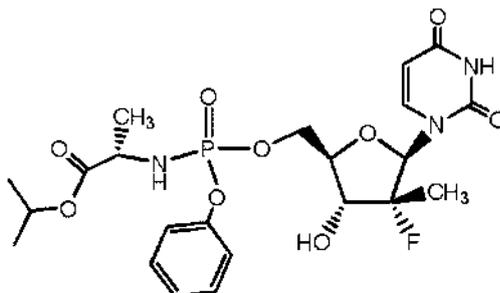
6. A composition comprising the compound of claim 3 and a pharmaceutically acceptable medium.

Patentansprüche

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1. Eine Verbindung , repräsentiert durch die Formel

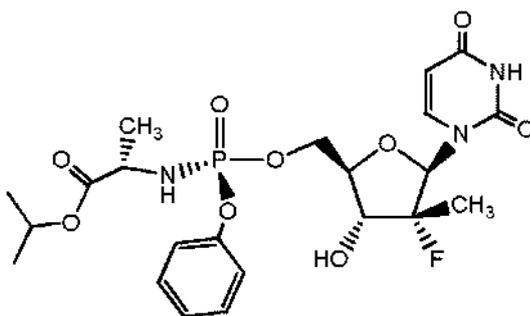
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2. Eine Verbindung , repräsentiert durch die Formel

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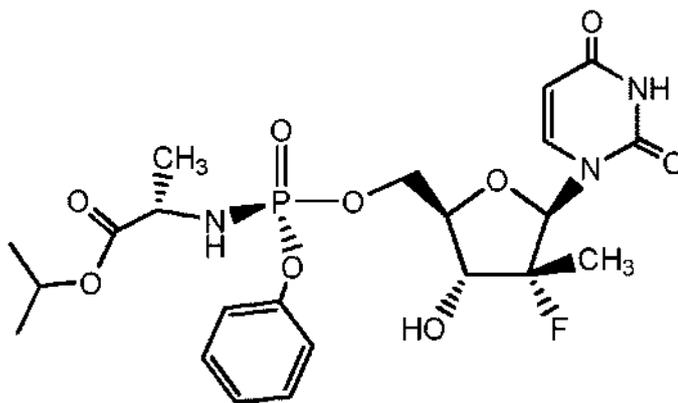
3. Eine Verbindung , repräsentiert durch die Formel

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4. Eine Verbindung, welche die Verbindung nach Anspruch 1 und ein pharmazeutisch akzeptables Medium umfasst.

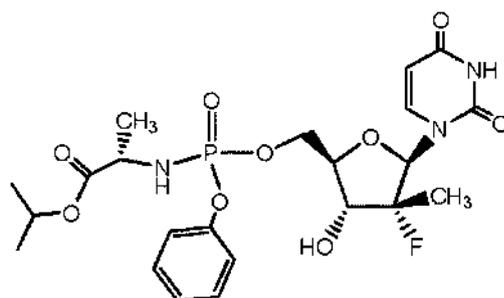
5. Eine Verbindung, welche die Verbindung nach Anspruch 2 und ein pharmazeutisch akzeptables Medium umfasst.

20 6. Eine Verbindung, welche die Verbindung nach Anspruch 3 und ein pharmazeutisch akzeptables Medium umfasst.

Revendications

25 1. Composé représenté par la formule

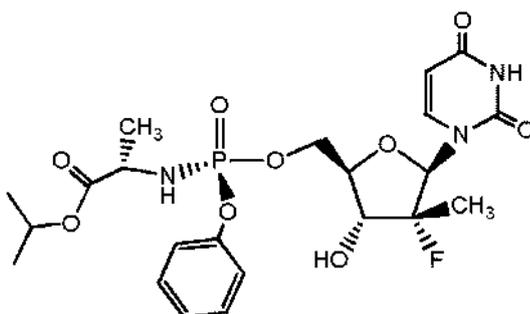
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2. Composé représenté par la formule

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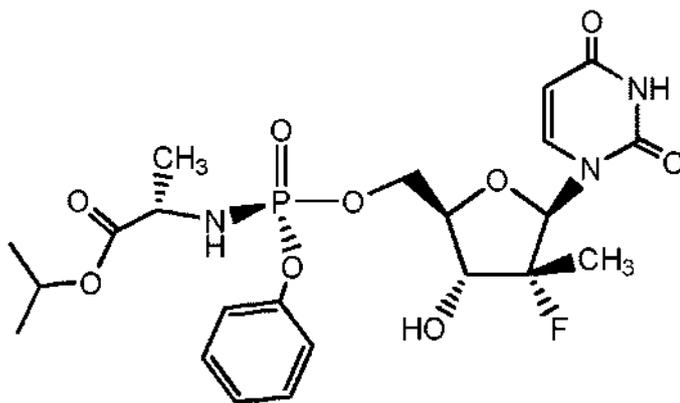
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3. Composé représenté par la formule

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4. Composition comprenant le composé selon la revendication 1 et milieu pharmaceutiquement acceptable.

5. Composition comprenant le composé selon la revendication 2 et milieu pharmaceutiquement acceptable.

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6. Composition comprenant le composé selon la revendication 3 et milieu pharmaceutiquement acceptable.

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REFERENCES CITED IN THE DESCRIPTION

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Application No.

08732818.3

INFORMATION

The oral proceedings of: 04.10.16
& 05.10.16

Resulted in:

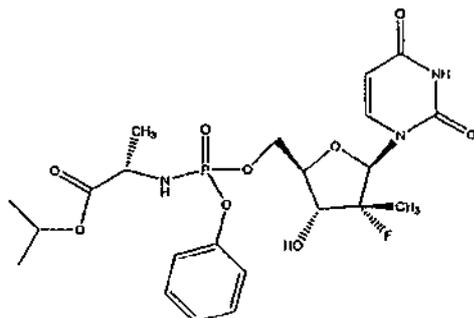
- The European patent is revoked because at least one ground for opposition prejudices the maintenance of the European patent (Art. 101(2) EPC)
- The European patent is revoked because, account being taken of the amendments made by the patent proprietor during opposition proceedings, the patent and the invention to which it relates were found not to meet the requirements of the EPC (Art. 101(3)(b) EPC).
- The opposition(s) is/are rejected (Art. 101(2) EPC).
- Account being taken of the amendments made by the patent proprietor during the opposition proceedings, the patent and the invention to which it relates are found to meet the requirements of European Patent Convention (Art. 101(3)(a) EPC).
- Amendments filed during oral proceedings are annexed.
- The proceedings were ordered to be continued in writing. The patent can be maintained in amended form, provided the amendments are filed in a form compliant with R. 49(8), 50(1), 86 EPC.
- Other:

05/10/16
Date

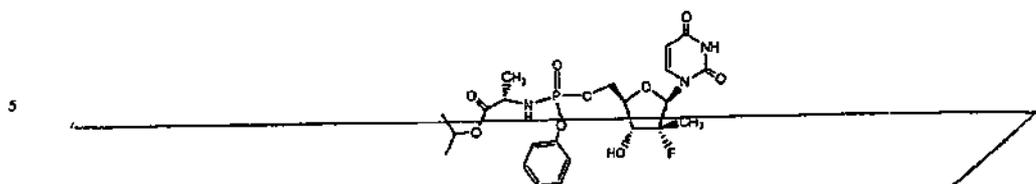
Ulrich Götting
Signature

NB: this form is provided for the sake of information only. The written decision prevails.

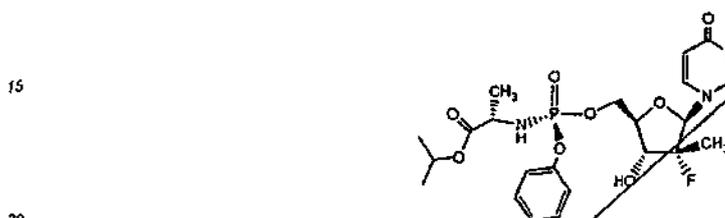
INSERT SHEET



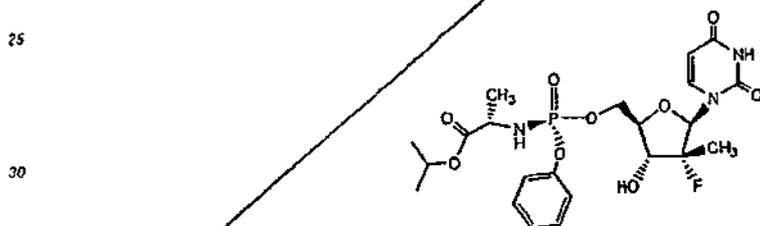
2. A composition comprising the compound of ^{clause} claim 1 and a pharmaceutically acceptable medium.



10 2. A compound represented by the formula



20 3. A compound represented by the formula



30 4. A composition comprising the compound of clause 1 and a pharmaceutically acceptable medium.

35 5. A composition comprising the compound of clause 2 and a pharmaceutically acceptable medium.

40 6. A composition comprising the compound of clause 3 and a pharmaceutically acceptable medium.

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40 [0053] The following tables contain numeric identifiers associated with various substituent designators that should be viewed in light of the accompanying structure. These structures are contemplated species of the various aspects of the present disclosure. However, it is contemplated that any one of the exemplified nucleoside bases can be used in combination with any one of contemplated species that specify a particular combination of R^1 , R^2 , R^{3a} , R^{3b} , R^4 , R^5 , R^6 , X , and Y . In each of the presented tables, the phosphoramidate substituent containing the substituents R^{3a} and R^{3b} are depicted without reference to stereochemical structure. It is contemplated that the compounds recited below embody compounds in which R^{3a} projects toward the viewer while R^{3b} projects away from the viewer. Moreover, it is contemplated that the compounds recited below also embody compounds in which R^{3a} projects away from the viewer while R^{3b} projects towards the viewer. Not meant to be limiting, however, it is contemplated that preferred compounds are those in which R^{3a} projects towards the viewer and R^{3b} projects away from the viewer such that the natural L-amino acid (S)-configuration is presented. Additionally, the inventors recognize that the phosphorus atom of the phosphoramidate moiety is another source of chirality. Although the structures below do not specifically depict chirality at phosphorus, the inventors recognize that stereochemical configurations are possible such that in a staggered (or zig-zag) line structure the oxo-substituent projects towards the viewer while the OR^1 substituent projects away from the viewer, and vice versa, i.e., where the Cahn-Ingold-Prelog stereochemical designation of phosphorus is either R or S. Therefore, the structures below include all possible stereochemical configurations possible for phosphorus.

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The oral proceedings were opened at 9:08 on 4 October 2016.

Opponent 3 (O3) and opponent 9 (O9) were not represented. The chairman confirmed that both opponents had been duly summoned. They had informed the Office that they would not attend the oral proceedings. In two cases, opponents 4 and 5 (O4/5) and opponents 6 and 10 (O6/10), two opponents were represented by a single representative. The chairman made a short statement concerning the legal framework of the opposition procedure before the EPO. He informed the parties that the EPO and the opposition division (OD) are well aware of the social and economic aspects underlying the case. The parties were then invited to confirm their requests.

O1 confirmed the request to revoke the patent in its entirety. He requested a possibility to make a presentation from his computer at a later time. All other opponents confirmed their requests as put forward in writing. Additionally, O7 questioned if two names for the patentee are indicated in the register. The proprietor (P) confirmed the requests on file (main request and 11 auxiliary requests). P reserved the right to alter the order of auxiliary requests. Additionally, P requested O1 not to be allowed to provide a presentation, and furthermore that a decision be taken about the admissibility of late filed documents. P confirmed that the name of the patent proprietor is correctly indicated in the patent register. O7 did not see a legal problem linked to the presentation of the patentee's name in the register and did not wish to comment further.

The discussion of the main request started with the issue of article 123(2). O1 argued that the claimed compounds have not been sufficiently characterised and that only post-published data would disclose their merits. He considered that the filing of the patent took place before the invention had actually been made. This did not mean that the patent system is flawed but rather that the applicant made several mistakes which gave rise to the opposition. The compounds according to claims 2 and 3 show different configurations at the phosphorus atom. However, these structures were neither depicted nor described as such in the application as filed. O1 considered the disclosure on p.20, l.8 to be only a general statement. Example 81 relates to the separation of a fast and a slow moving isomer. This example not only leaves undetermined the nature of the separated diastereoisomers, but also none of the separated examples in Example 81 relates to the compounds of claims 2 and 3. The claimed compounds were therefore seen as a selection from several lists, which require a selection from a list of structures, the selection of one configuration as R and S, the selection of the configuration at the phosphorus atom, and the selection of a set of substituents. The same considerations apply to claims 5 and 6. O1 did not consider the claimed matter to be unambiguously and directly derivable from the

application as originally filed. O2 agreed with O1 and argued that the application as filed merely acknowledged that phosphorus is a chiral atom but does not individualise its stereoisomers.

O4/5 agreed with the previous opponents. Claim 1 as filed related to a broad general formula which makes it necessary to consult the description in order to find information for stereoisomers. Original claim 2 relates to a list of compounds. The compound 25 became subject-matter of claim 1 in the granted patent. But original claim 2 did not mention the compounds of claims 2 and 3 of the patent. The application as filed (p.683) discloses a general formula which left the stereochemistry at the phosphorus undefined. The opponent discussed the disclosure on p.99-100 and the information contained in the tables following thereafter. According to O4/5 this passage is to be seen as a general statement similar to the disclosure on p.20 which recognises that the phosphor atom is another source of chirality. He referred to the case law in section I.C.6.2.3 of the EPO Case Law book, p.127 of 8th Edition, dealing with the novelty of enantiomers. He concluded that the application as filed does not disclose unambiguously and directly any particular configuration of the compounds listed in the tables following p.99-100. The original application discloses on p.692 that certain examples had been obtained as a mixture of stereoisomers and only some of them were separated. The application does not disclose exactly which stereoisomer are obtained and also does not disclose that compound 25 is one of the compounds chosen for the separation, and therefore the application does not individualise any specific isomer as claimed in claims 2 and 3. O7 agreed with the previous submission and stressed that the disclosure on p.99-100 would need to be considered as a whole. Even when some configurations are disclosed as preferred embodiments there is no disclosure found for the specific combination of structural features present in the compounds of claims 2 and 3. The opponent referred to T658/91 in support of his argumentation. O8 and O6/10 had nothing to add at this stage.

P pointed to three separate places in the original application which each alone provided a disclosure of the claimed matter. The compound IX.25.2 on p.254 exhibits all substituents of the structure of granted claim 1. The disclosure has to be read in combination with the structure on p.246 which discloses in a single line all substituents of the claimed compound. Additionally, p.99 discloses from line 22 onwards preferred options. On pp.694-697 a list of all compounds is given that had actually been made. These compounds are again preferred compounds where every single compound has the required stereoconfiguration. These passages are particular pointers and no selection needs to be done. Example 25 needs to be read in conjunction with the said passages. For the subject-matter of claim 2 also several places had been identified (e.g. p.20) which can be combined with other parts in the application, such as Formula 1 and the information given for the resolution of

enantiomers. P considered that p.99 to disclose all possible enantiomers and describes in words what can be seen from the chemical structures disclosed on the following pages. Compound IX.25.2 is an individual disclosure of claim 1 which also includes the two diastereoisomers, which are specifically and individually disclosed in the identified parts of the description. P admitted that Example 25 produced the racemate. Due to the reference to each of the possible isomers at the phosphor atom the application also discloses the isomers of Example 25. Example 81 tells how one could separate the isomers. P argued that the matter of claims 2 and 3 does not present the skilled person with new technical information because it is legitimate to combine the disclosure of Example 25 with the general disclosure in the original application. There is also no selection from lists involved because all substituents are set out in single line. P referred to case law and stressed that the mention of enantiomers is not limited to their conceptual existence. The decision T691/91 was seen to be applicable given the similarities with the situation in patent while other case law discussed in the written procedure is not pertinent.

O2 underlined that p.99-100 refer to a formula which does not depict the two specific stereocenters at the carbon and phosphor atoms. O2 argued that this passage is still a conceptual reference to the existence of isomers which does not unambiguously disclose the specific stereoisomers. O4/5 added that for the issue of article 123(2) it is not the right test of whether it would have been obvious to choose a specific isomer and it is also irrelevant whether original claim 2 covered the compounds in claims 2 and 3 of the patent. Also the disclosure in Example 81 on how to separate isomers rather is an issue of sufficiency of disclosure which is not a relevant argument for article 123(2). It is equally irrelevant if the claimed compounds had actually been synthesized as the individual isomers are not disclosed in the application as filed. O7 endorsed the comments of the opponents.

P stressed that p.99 of the application does not make the claimed compound obvious but is to be seen as an unambiguous disclosure because the passage discloses the preference for the carbon chirality and also that of the phosphorus centre. When reading the table both configurations for the phosphorus centre have to be read into the table, thereby providing a simple and direct disclosure. As there are only two possibilities, R and S, the compounds of claims 2 and 3 are also not just covered but also disclosed. P. referred to original claim 2 where Example 25 is combined with "stereoisomer". Finally, P referred to the case law which sees the requirement of article 123(2) satisfied in the light of an individualised disclosure for the specific stereochemical forms.

The chairman interrupted the oral proceedings from 10:16 to 10:54. After the break the chairman announced the opinion of the OD that claims 2, 3, 5 and 6 of the patent granted (main request) do not meet the requirement of article 123(2). He also highlighted that the same defect applies to the auxiliary requests 2, 4, 6, 8 and 10 because these requests also contain at least one claim which is directed to a specific stereoisomer as claimed in claim 2 or 3 of the main request. These auxiliary requests are therefore not allowable.

P requested to renumber previous auxiliary request 3, which was filed on 3 August 2016, as the new auxiliary request 1 and to deal, if necessary, with previous auxiliary request 1 as the new auxiliary request 2.

The opponents did not raise objections under article 123(2) against the new auxiliary request 1. Therefore the chairman invited the opponents to present the arguments against article 83.

O1, O4/5, O7, O8, O6/10 had nothing to add to the submission previously made in writing. O2 held that claim 1 embraces two stereoisomers and therefore it would be a mandatory for the patent to disclose how each of them were obtained and what method is used to assign the configuration to the stereoconfigurations at the phosphor atom. The patent does not disclose this information. If one would assume that the product of Example 25 could be separated into a slow and a fast eluting isomer by following the instructions of Example 81, then it would still be unknown how to determine which of the isomers is the R form and which the S form. Since nothing is disclosed on how to determine the phosphorus configuration the skilled person would have to turn to the common general knowledge about suitable analytical methods. Contrary to the written submissions by P the use of X-ray cannot be considered to be a routine method for determining the configuration at the phosphor atom. D59 and D60 were cited as evidence of potential problems linked with such a method. O2 considered critical to obtain a single crystal in a good quality. The patent is silent on how to obtain such a suitable crystal. With reference to D21, which was published two years after the filing date of the patent, O2 argued that at the time of filing the common general knowledge was insufficient to produce a suitable crystal of the compounds embraced in claim 1. Furthermore, D15 shows that X-ray measurements were done on a specific crystal form II which proved to be unstable. To find the correct single crystal was difficult and would amount in undue burden. Alternative methods for determining the phosphorus configuration are NMR methods. The patent

is also silent in regard of such method. Common general knowledge, such as reflected by D7, suggested this method to be successful only for compounds which meet specific structural requirements (p.1844, Figure 2, compounds 34 or 35) which are not met by the claimed compound due to the absence of a comparable pi-pi interaction.

The proceedings were interrupted at 11:20 for a 5 minute break. P stressed that claim 1 does not embrace individual stereoisomers. Article 83 requires that the skilled person is enabled to reproduce the invention. P noted that none of the opponents suggested that it would not be able to make the claimed product (Example 25) or would see a problem with a chromatographic separation of isomers. It is therefore not at question that the claimed product can be made and purified. However, the structure of individual diastereoisomers is not a feature of claim 1, and therefore there is no legal justification for requiring that the skilled person must also be able to assign the individual diastereoisomers. Still the skilled person would not have difficulty to assign the absolute phosphorus configuration based on known and generally available techniques discussed in D59, D60, D61. D19 (p.6; p.32) and D21 (p.7214) were seen as further accounts of standard methods which were available before the filing date of the patent. P considered such methods also useful for whatever polymorph is analysed because each crystal form has the same configuration at chiral centre. Also the growing of bigger crystals is within the prior art or common general knowledge.

The discussion of O2 returned to D21 which mentions on p.7209 that this document is the first demonstration of a crystalline phosphoramidate. O2 insisted that the phosphorus configuration is a feature of the claim even when it is not depicted as true 3D structure. Since the invention needed completeness in its disclosure, but this requirement is not met, an undue burden is placed on the public in searching conditions which allow the crystallisation and analysis of the claimed product. O8 concluded from the fact that claim 2 of the granted patent was not drafted as a claim which is dependent on claim 1 that the later was directed to a racemic compound. In his understanding, claim 1 of the first auxiliary request does not embrace anything else but the racemate. Should however intermediate isomers also be covered by the claim then claim 1 is seen to infringe article 83 for essentially the same reasons as presented by O2.

P held that chromatographic separation methods were commonly applied techniques at the relevant time. One could therefore make and separate the individual isomers. They are within the scope of claim 1.

After a 15 minute break starting at 12:04 the chairman announced the opinion of the OD that the requirement of article 83 is met by the first auxiliary request. Then the discussion on the novelty was opened and the parties were specifically asked for further arguments in regard of the document D4. All parties referred to the written proceedings. The chairman called for a break which lasted from 12:30 to 13:30. Thereafter he announced that in the opinion of the OD the auxiliary request 1 is novel under article 54.

O1 wished to make a presentation of slides which he had prepared on a legal issue. P requested that O1 is not allowed to make this live demonstration. Asked by the chairman about the subject of the presentation and the reason for presenting late filed evidence, O1 responded that the presentation is very simple and that the presentation had been prepared the previous night. P referred to the Guidelines which would classify such presentation as written evidence. Rule 116 requires that copies must be provided in good time before the oral proceedings. O1 argued that the presentation would merely avoid going through the documents cited in the written procedure in the numerous inventive step attacks. After a brief consultation of the OD the chairman said that the OD would like to proceed with the discussion of the inventive step without the live presentation.

O1 did not challenge further this position and turned to the issue of priority. P intervened by requesting that late filed documents are not admitted in the proceedings. P referred to the written submissions. D87 was considered to be filed late and not relevant. A second declaration D90 was also late filed and would not add anything to D87. If, however, D87 is allowed then P requested also to allow the second submission by the proprietor's expert. O1 clarified that D87 was obtained in August 2016 and was filed before the deadline under Rule 116 and was a direct reply to the declaration of Prof Thomas. D87 could not be submitted within the nine month opposition period because at that time O1 had no access to what the proprietor's expert had said. D87 is not a surprise filing since P was able to file a second declaration. O1 would consider his right to be heard violated if D87 was not admitted. O1 requested that if D90 is not admitted then also the second declaration of Prof Thomas (D89) should be excluded from the proceedings. P argued that O1 should have presented within the nine month opposition period all their evidence as the question of priority assignment is a matter of US law. The chairman announced that all documents are admitted in the proceedings.

The discussion continued with the first and second priority applications, D1 and D2. O1 observed that the earlier applications had been filed in the name of the inventors while the patent was filed in name of Pharmasset. A1 and A2 are assignments from the inventors to the applicant. O1 highlighted that the legal experts of the proprietor and the opponent arrive at contradicting conclusions as to whether an effective transfer of the priority right to the applicant had occurred (D44 and D89, D90). O1 did not consider sufficient the language of the assignments in that it does not address the priority right. O1 argued that the priority right is a separate right. A1 and A2 have to be assessed on the basis of the legal framework at the time of assignment. Applications had to be filed in the name of the inventors at the relevant time of filing. Accordingly, the assignment must comply with US practice. This means that the right to assign the invention remains in the hands of the inventors, and also the right to priority must stay in the hands of the inventor. O7 considered relevant the provisions in article 4a of the Paris convention. He considered the priority right in the light of article 89 EPC and argued that this right gives a privilege to the patentee that prior art published in the priority interval is not held against the patent. It is not a right to the invention. Priority has its own right. It can be abandoned or can be used with different applicants. O7 held that the term "invention" as used in A1 and A2 is not the priority right. There is no indication in the assignments that also the priority had been assigned. The procedural aspects have to obey local law which is the law of the country where the priority application had been filed. But the content of what has been assigned is matter of the EPC. O7 concluded that Pharmasset is not entitled to the priorities D1 and D2.

P held that A1 and A2 would be sufficient to qualify the applicant as the successor in title. Reference was made to the Guidelines Part A-III, 6. Evidence on file established that the priority right had been transferred as a matter of fact and also in consideration of the true intention of the parties to A1 and A2 (D44). P commented on the counter declaration, D87, and held that the alleged failure to adhere to any kind of "best practice" is not decisive for answering the question of whether a transfer of the priority right had taken place at the relevant time. D89 explains the relevant aspects. One is that the parties wanted to achieve full assignment.

O1 held that the intent of assignment was not to assign the priority right but to file a patent. O4/5 added that according to case law the right to the priority is transferred independently, which was seen as clear evidence that the right to the priority is a separate right. The benefit of doubt should not go to the patentee as P should show that he owned the right. O7 pointed to the Guidelines Part H which discusses the transfer of the application and the transfer of the priority as alternatives. He concluded the transfer of the priority does not automatically go with the transfer of the application. The decision T62/05 would teach that the assignment of the priority right is independently of the assignment of the application. If the priority right is not

mentioned in A1 and A2 then it was not transferred. O8 referred to the decision T205/14 which considers a generally accepted principle that the right of priority is an independent right which is to be distinguished from the right to assign a patent application.

P agreed that the right to the priority can be transferred separately. But any transfer must be valid under the applicable national law. As evidenced by Prof Thomas it is a feature of US law to identify the intention of the parties. The intention was to assign also the priority right to the applicant.

After a break (14:13 to 14:32) the chairman announced that the OD considers sufficiently proven that the right to claim priority from D1 and D2 was validly transferred before the filing date. The discussion continued on the question whether the claimed matter of the first auxiliary request 1 is disclosed and unambiguously and directly derivable from the earlier applications.

O1 argued that the invention of the auxiliary request is not the same invention as disclosed in D1. The claimed compound is not disclosed as such in the oldest priority application. Also the example of the patent which shows the synthesis is not present in the priority application. O1 concluded that the claims are not enabled by D1. O2 agreed with this position. O4/5 added that the patent cannot rely on the general formula, nor on compound 25 or on any specific stereochemistry. O8 referred to the written submission. He considered the stereochemistry of the amino acid not to be disclosed in D1. The oldest priority application is solely based on a list of 12 400 named compounds in 1550 tables. O8 has summarised this disclosure in the form of a Markush formula. When compared with the prior art document D4, which was found as a not novelty destroying prior art, then the same conclusion had to be arrived when considering D1 and the subject-matter of claim 1 of the auxiliary request. He referred to case law (G2/98, T939/92, T22/82) that the priority claim is not acknowledged if a selection invention would be considered novel. Article 87(1) establishes the concept of the same invention. However, the priority application does not identify the subject-matter of claim 1, because the 12 400 structures in D1 do not contain the technical teaching for individual structures.

P requested a 5 minute break at 14:43 which was granted. Thereafter, P identified the passages in D1 which disclose the subject-matter of claim 1 (p.195, compound IX 25.2, p.63, Example 13). The Guidelines Part VI were referred to in support of the argument that a selection from a single list does not confer novelty. As a selection of one compound from a single list is required to arrive at the claimed subject-matter, P held that D1 is a direct and unambiguous disclosure of the invention. P also argued that preferred aspects can be combined. It is of no relevance if the list is a single table

or a table separated in several parts. P was against the idea of O8 that the list or lists could be described as a Markush formula. Case law repeatedly confirmed that these representations are not equivalent. Therefore the issue is different from deciding on the novelty of a selection from the Markush formula in D4. Case law presented by the opponents, such as T903/05, was not regarded as being relevant.

O4/5 argued that the discussion on the priority issue is essentially the same as done for article 123(2) in main request because the same legal principles apply. The identification of stereoisomers in D1 is a conceptual one. Therefore, the critical issues resides not in the selection of features. Instead, the case law about isomers has to be considered. The test is one of direct and unambiguous disclosure, and not whether one is able to synthesize the compounds, nor would the plausibility of any technical effect matter as this is also not an aspect to be considered for priority. The crucial point is that the formula depicted in paragraph 113 of the patent indicated the stereochemistry in specific form whereas the formula in D1 has no defined stereochemistry. These formulas are not equivalent and therefore the disclosure is not the same. O7 and O10 agreed with this position. Only the second priority, D2, had the defined stereochemistry disclosed. Asked by the chairman whether there is an objection against the entitlement of the claims to the second priority, D2, none of the opponents raised an objection.

A break from 15:13 until 15:35 took place. The chairman announced that the subject-matter of claim 1 is not entitled to first priority application, D1, and that the OD considers the effective filing date of the claims to be 24 October 2007, which is the date of filing of D2. The discussion on the inventive step was opened. The first aspect to be considered was the identification of the closest prior art.

O1 requested a decision on the admissibility of D5, which is a PhD thesis bearing the date of February 2007 on its cover page and the information on page 3 that the author gives his consent that the thesis becomes available for photocopying and inter-library loans after expiry of a bar on access approved by the Graduate development Committee. P highlighted the importance of knowing the date when D5 had actually become available to public. P considered D86a and D86b as late filed and requested not to admit this evidence. O1 did not file evidence of the public availability of D5 during the nine month opposition period. Even when O1 obtained in August 2015 supportive evidence he did not introduce it into the proceedings, and hence P had no opportunity to contest the findings. The evidence was not considered relevant as it does not address the bar to accessibility of D5. Therefore, these documents should not be admitted. But if they were admitted, then also the evidence provided by P

(D48a-c) is relevant and should be considered. O1 submitted that he first believed that the publication date of D5 is February 2007 as indicated in the cover page. There was no intent to deprive P of the opportunity to respond. O1 held that the submissions were done in reply to a submission of P. After a short deliberation with the OD the chairman agreed that all documents are admitted in the procedure.

The discussion continued with a dispute between P and O1 about the date when O5 became available to the public. According to O1 the date is shown in D86a and D86b as the date when the thesis was entered in library system on 6 March 2007. D68b was regarded an affirmative reply by the library staff. P contested this view as D48b is superseded by the information in D48c. While the first of these documents explained that the availability started after the bar on access has expired, the later explains that no full text had been made available during the bar period, which ended only in 2009. O1 furthermore pointed to the email correspondence and the confirmation of a two year bar lasting until 26 March 2009. As the first publication was on 6 March 2007, O1 concluded that D5 was available to the public at least for 20 days, namely from 6 March 2007 to 26 March 2007, the later date would be confirmed through the two year period of bar to access. P stressed that normal practice would rule out that D5 became available in this short period. O1 had not discharged his burden of proving that the situation was different.

The legal member of the OD raised the question why the email D48b was addressed to the author of D5 but the submitted evidence does not contain any evidence of the questions asked. P confirmed the lacking trace to the initial email and explained that the only parts were included which P considered relevant.

The chairman interrupted at 15:56 for 10 minutes. Thereafter he announced that the OD is of the opinion that D5 had not become public before the earliest valid priority date of the patent. D5 was therefore not citable as prior art in the context of the inventive step discussion.

The discussion on the inventive step was continued with the identification of the closest prior art. O1 considers D7 as the closest prior art. The compound 15 is the structurally closest compound in D7. D53 would not qualify as the closest prior art because of too many differences. O2, O4/5, O7 and O8 considered D8 as the closest prior art which was made available to the public on 9 September 2007, which date is prior to the effective filing date of 24 October 2007, the date of filing of D2. Starting from D8 the objective technical problem was seen in provision of mere alternative compounds which inhibit HCV. D8 provided the complete background on the biological activity and teaches the relevant details of the mechanism of action and metabolism. D8 proposes phosphoramidates as potent and selective inhibitors of

HCV RNA replication. The purpose and effect of the compounds are therefore the same as in the patent. The only difference to the patent was that the groups R1, R2 and R3 for the phosphorus ester, amino acid side chain and amino acid ester are not stated in the document. The tables suggest that a structural variation of these residues results in a compound which has the desired properties. The level of evidence in the tables 1 to 6 of D8 is such that the skilled person would have elaborated suitable groups R1-R3 with a reasonable expectation of success. O6/10 considered D6 as the closest prior art because it disclosed the base nucleoside and its active triphosphate derivative. D6 is directed to the same purpose as the patent and suggests the administration route through mono-phosphorylation.

In the further discussion the opponents expressed their opinion why the claims lack an inventive step when departing from the chosen closest prior art. O1 did not see any technical effect linked with the structural modifications. Since the claimed compound presented similar HCV inhibitory activity the solution was considered to be obvious for the skilled person. O8 referred to the EC90 data in D8 and considered that the claimed compound is active at a comparable level. Since D8 establishes the potency, selectivity and stability of the phosphoramidate prodrug PSI-6206 it was obvious for the skilled person to find alternatives for the phosphorus ester R1, the amino acid side chain R2 and the amino acid ester group R3. Other documents in the same field provided clear pointers for these alternative groups, in particular the L-alanine, the phenyl phosphorus ester and the isopropyl ester (D6: Table 1, compound 15, Figure 1, p.1841; D7: compound 15; p.1843; D18: p.1899, Tables 1 and 5; D37: p.3585; D20: p.7219, Table 2). O4/5 focussed on the clear teaching in D8 about the need to bypass the first phosphorylation step and the aspects linked to the drug potency and safety. O6/10 considered that in the closest prior art, D6, the strategies of aiming at the problem of getting high amounts of the active substance into the cell is fully disclosed. The last but one page of D6 pointed to the previously existing problem to transfer the inhibiting compound into the cell and to the solution. A clear pointer to a prodrug as the solution is found in the reference to D7, which described in detail the nature of the prodrug, and also describes as exceptional those compounds having the phenyl, methyl and isopropyl residues shown at the places in the claimed compound of the patent. Any additional effect achieved was considered as a bonus effect which would not establish an inventive activity. All opponents held that the combination of the chosen closest prior art with one of the before secondary documents, preferably with D6 or D7, results directly in the claimed matter.

P requested a 15 minute break at 17:22 which was granted. The discussion of P focussed on the three sets of documents identified by the opponents when departing from D6, D7 or D8 as the closest prior art. P explained that a matrix approach was taken in D7 (p.1843, conclusion section) in regard of finding effective phosphoramidates with low toxicity. Compounds of interest were 4'-substituted derivatives which differ significantly from the fluoro/methyl-substituted nucleoside of the invention. Not only that a different nucleoside was combined with a specific phosphoramidate, also the best compounds, for instance as measured by the least toxicity of the compound, was a phosphoramidate prodrug which had a benzyl ester and not the isopropyl ester group of the compound claimed in the patent. The technical problem arising from the difference was seen in a compound which is effective in delivering the drug into the cell and has low toxicity. P referred to the Guidelines Part VII-11 and argued that toxicity is always a relevant property which is an inherent requirement of drugs and therefore it is allowable that toxicity and activity aspects are contemplated together. P referred to D45 and D68 and highlighted aspects related to the toxicity problems linked to off-target effects, like mitochondrial toxicity. P held that D45 (table 3.8, p.7) discloses a relatively new assay looking at the same phenomenon. The triphosphate derivative of the compound claimed in the patent proved to be less toxic than its cytidine analogous. P defined the objective technical problem as to find a combination of a phosphoramidates and a nucleoside which is capable of delivering the drug into liver cells in large quantity with a lower toxicity than the compounds in D7. But nothing in D7 suggested which phosphoramidates to use. D7 rather teaches away which becomes apparent from what is disclosed in D38 (reference 6 on the first page of D7). The skilled person would be taught by D38 (discussion section) to keep the 4'-substituted nucleoside derivative as other nucleosides were said to have limited selectivity, which would be seen as a warning to toxicity. Because of the reference to D38, the skilled person would not consider methyl/fluoro-substituted nucleosides as promising drug candidates. None of the other documents referred to by the opponents suggested to achieve lower toxicity than the compounds of D7.

P turned to D8 and stressed that it does not disclose a specific compound. Three unknowns (R1, R2 and R3) have been varied in the measurement of EC90 values but the authors of D8 have not defined one with particular combination. The structural variation shows massive differences in activity. Compounds indicated in Table 3 as the more active may be more toxic according to Table 5. P stressed that each of the moieties R1, R2 and R3 affects differently the potency and toxicity. The skilled person could not simply select a combination as the tables in D8 do not inform on which chemical compounds had what properties. The difference of the claimed compound in the patent opposed and D8 is that the claimed compound is specific. The technical

effect is that high amounts of the drug are transferred into cells. P turned to documents which are said to deliver phosphoramidates to cells (D4: p.2; D16: p.372; D21: compounds 12 and 14, p.7208, Figure 4). P derived from the activity data contained in these documents the objective technical problem as to identify a compound which is more effective than the methyl ester phosphoramidate prodrug. P argued that every time a phosphoramidate is put on a nucleoside a very separate and specific structure-activity-relationship (SAR) applied. The skilled person could not just use the phosphoramidate prodrug to make it work. P referred to the declarations D43 and D42 and highlighted the scepticism of experts at the relevant time and the belief that the phosphoramidate prodrug route would not work. P held that the prior art told that one cannot extrapolate the SAR of phosphoramidates (D7: p.1843, "...depends on different SARs", "...quite distinct SARs emerge from this family"; D37: first page and p.3506, "SAR may not fully apply to..."; D20: p.7219, "very significant differences...", "complex metabolic SAR"). P acknowledged that the compounds in D18 had the same phosphoramidate of the claimed compound in the patent opposed. But this moiety was attached to a completely different parent compound. Already the title indicates a different medical target and the data show that the phosphoramidates also get a high distribution in cells different from liver cells, such as in lymphocytes, which observation would deter the skilled person to consider pertinent the teaching of this document.

P turned to D6 and commented on the metabolism of cytidine nucleosides (Figure 7). The cytidine derivatives were disclosed as highly potent drugs which efficiently enters the cell. The data did not suggest a particular problem and therefore the difference between D6 and invention was in the specific way in which the compound was delivered to cells. P referred to the data in D55 (Table 1; Figure 2) to show that a huge difference existed in the generation of triphosphates when comparing the phosphoramidate of the patent opposed with the compounds of D6. The technical problem was therefore to provide a more active compound. D6 (Table 1) did not hint to the solution since cytidine was presented as about 2-fold more active compound than the uridine derivative. Also two different active species were obtained as uridine and cytidine would bind at different areas. Starting from D6, the skilled person would thus have looked at cytidine but would not go to uridine derivatives. There was no reason for the skilled person to combine D6 and D7 because D6 already teaches compounds which were sufficiently good. Even if he did, he would still have to work out the specific methyl, phenyl and isopropyl groups in the phosphoramidate moiety for which no pointer existed.

The chairman adjourned the oral proceedings at 19:04 until 9:00 on the next day.

The chairman opened oral proceedings at 9:00 with a brief summary of the inventive step arguments presented so far. The parties were invited to concentrate in their further submissions on the technical effects which are achieved by the differences and what conclusion the skilled person would derive therefrom.

O1 contested that the effect of a lower toxicity because such effect could not be evidenced at effective date of filing. O1 referred to D4, D45, D35, D38 and D81 and discussed aspects related to the toxicity. O1 highlighted the passages in D8 which disclose the compounds as potent inhibitors which are also selective and do not exhibit mitochondrial toxicity. From the Tables 1, 2 and 3 it was concluded that the compounds of D8 were more potent than known inhibitors. O1 maintained that combining D8 and D7 renders obvious the claims.

O2 argued that the skilled person knew before the effective date of filing that the active form is a nucleotide in triphosphate form. There was no need to refer back to cytidine in the older literature as other nucleosides had been proposed. The problem of finding a way in which the nucleoside enters the cells in a form that leads to triphosphate also had been solved by a phosphoramidate prodrug. Since it is a common problem in treating HCV, HIV or cancer to bring high amounts of the active compound into the cell it made sense for the skilled person to consider a reference to a nucleotide even which it had been used to treat diseases which are different from HCV. Starting from D8 as the closest prior art, an alternative technical problem could be formulated which is the provision of an active form of PSI-6206 which is useful in the treatment of HCV. The structural variety seen in Tables 5 and 3 of D8 would be a resource of information which made the skilled person carefully select the amino acid part, which is known from D8 to have a potential negative impact on cytotoxicity. O2 referred to prior art which already suggested that L-alanine and phenyl phosphorus ester worked best in phosphoramidates (D16: p.372-374). O2 argued that the isopropyl moiety was suggested by D7 (p.1843, Table 1) as the isopropyl group results in one of the most potent compounds. The possibility that other obvious solutions may become apparent to the skilled person is not relevant because any of such alternatives would be considered as an obvious alternative solution. O2 rejected the argument of P that the methyl ester was presented in the prior art as the most active ester because the reference to a lead compound does not exclude the possibility to further modify the ester moiety contained in such a compound. O2 referred to the definition of a lead compound given in wikipedia. O2 pointed to the disclosure of isopropyl esters in the prior art (D18, D20, D21). O2 did not consider relevant the content of the declaration D42 because the reported discussion of

experts was done in confidentiality and also is in contradiction to the reported efficacy of the phosphoramidate approach in the prior art. This position was endorsed by other opponents.

O4/5 and also O7 insisted that D8 is the closest prior art because this document already proved the success of the phosphoramidate prodrug approach in the specific nucleoside of the patent opposed. The skilled person was merely left with filling in the missing parts of groups R1, R2 and R3. The question of what substituents to be used was already answered in D7. O4/5 considered wrong the objective technical problems as set out by P because there is no legal and technical basis for redefining the scope of closest prior art and to argue that the closest prior art was limited to methyl ester derivatives. The teaching in D8 is clearly broader than just the methyl ester in phosphoramidates. O4/5 held that the availability of comparative data for methyl ester derivatives cannot justify to artificially limit the teaching of D8 to methyl ester derivatives. Therefore the objective technical problem resides not in an improvement but merely in finding the appropriate substituent groups R1-R3 as D8 already promised the benefits of what is claimed. While it might be true that without experimentation it remained uncertain that isopropyl is a suitable group it is wrong to state a skilled person would have stopped at D8. The data in D8 led the skilled person to workout the groups with a reasonable expectation of success. The skilled person would look to closely related compounds (D7: p.1842-1843, Table 1, compound 15; D16; D20). D45 raised the issue of mitochondrial toxicity. This was however not a disincentive for taking this approach of phosphoramidates (D84, D33) because any prejudice had already been overcome before the effective filing date. O8 added that the test used in D84 for assessing mitochondrial toxicity is the same test as used in D8. Therefore also an improved cytotoxicity would have been obvious when departing from D8 as the closest prior art. The solution to the problem of identifying a suitable combination for the residues R1-R3 is suggested in D7 which proposes three candidates as being "exceptional" (p.1843). There was thus only a limited number of possible candidates of suitable phosphoramidates, and in view of envisaged success promised in D8, there was no undue burden to test and identify the most suitable candidate.

O6/10 rejected the argument of P that D6 (p.29819, p.29895, Table 1) was primarily concerned with the metabolism because the inhibition of the HCV RNA synthesis and the chemical nature of inhibitors is discussed throughout the document. D6 also would not hint the skilled person only to cytidine derivatives in the light of the discussed balance of activity and cellular stability aspects. The very good results achieved in D7 (p.1843) for a compounds having methyl, phenyl and isopropyl groups in the phosphamide moiety would have generated a reasonable expectation of

success to consider this prodrug as the modification of the compounds of D6. In the light of the data in D55, O6/10 considered the claimed compounds no better than a skilled person would have expected on the basis of D7 (p.1843).

P requested a 30 minute break at 10:24 which was granted. Thereafter the discussion of P centred on D8 where the formulas do not tell what the groups R1-R3 are, and hence was considered to be an infinite Markush formula. The skilled person would need to start anew. Not a single document on file discloses a SAR for a phosphoramidate put on the nucleoside present in the claimed compound of the patent opposed. D4, D7, D20 were analysed with regard to the compounds that had been made and tested. P argued that every variation of a group corresponding to R1, R2 or R3 in D8 would lead the skilled person to believe that different moieties are the best candidates. The prior art suggested that at least 10 different residues for R1, at least 25 different moieties for R2 and at least 17 possibilities for R3 would need to be tested. Even if each of those choices would have been considered the skilled person would still not know what the result is because D8 made clear that every time one of R1, R2 or R3 is modified, significant differences were obtained for the activity and toxicity of the compounds.

P rejected the allegation of O6/10 that the SAR found for 4'-aziduridine nucleosides can be applied to a different nucleoside on the basis of the evidence on file which speaks against such presumption. P offered a submission of the expert Dr. Ray in this regard. O4/5 objected that Dr. Ray is given the floor. P referred to the request made in the submission of 3 August 2016 (paragraph 1.6). O4/5 held that the announcement did not fulfill the requirements set out in G4/95. The same principle why O1 was not allowed to present in this oral proceedings should apply to P because the opponent sees the risk to introduce new evidence into the proceedings at a very late stage of the proceedings. P argued that the case of O1 was different because the expert submission would specifically respond to what had been said and specifically why it is not possible to place a given SAR in a different technical context. O2 noted that the expert was announced to talk about D45 which is a different matter.

After a break from 11:21 to 11:27 the chairman announced the discretionary conclusion of the OD not to allow the expert to address the OD. P went on in explaining the infinite Markush formula in D8 and the matrix approach used to find the "best". The change of R1, R2 and R3 was considered as independent variables because different "best" combinations arise from a permutation. The finding of the absolute best would require to conducting a research program. P highlighted the opposing views among the opponents of whether the skilled person could rely on HIV

assays. P considered it scientifically not correct to apply the teaching of e.g. D7 to the type of compounds of D8. A redefined objective technical problem would be to find the best HCV RNA inhibitor in terms of potency, toxicity and pharmacokinetics. The prior art was discussed in regard of what had been presented as best candidate (D20: p.7219; D21: figure 4, compounds 14 and 47, Table 5, p.7205; D7: Table 1). All documents showed the necessity to conduct a research program with a wide variety of tested residues and cross-combinations. This would be the kind of approach for the skilled person when faced with infinite Markush formula taught by D8. Finding the "best" would be far from being straight forward. Finally, P commented on the choice of D6 as the closest prior art. P held that this document lacked a particular pointer to uridine (p.29818, Table 7). P held that the half-life of a compound does not tell how much of the active compound is transferred into the cell but rather how quickly. This measure would not be relevant in the context of the problem and solution approach when starting from D6, and therefore the skilled person would be taught by D6 that the cytidine nucleoside is better than other nucleoside derivatives.

A break took place from 11:50 to 13:00. Thereafter the chairman announced that the OD came to the conclusion that the auxiliary request 1 meets the requirements of inventive step. Two pages of amended description were submitted by P in order to adapt the description to the claims. None of the opponents raised an objection against these amendments. The chairman therefore announced that, taking into consideration the amendments made by P during the opposition proceedings, the patent and the invention to which it relates meet the requirements of the EPC.

The oral proceedings were closed at 13:10 on 5 October 2016.

Application No.:

08 732 818.3

Patent No.:

EP-B-2 203 462

Direct decision:

yes no

Interlocutory decision in opposition proceedings (Art. 101(3)(a) and 106(2) EPC)

The Opposition Division - at the oral proceedings dated 05.10.2016 - has decided:

Account being taken of the amendments made by the patent proprietor during the opposition proceedings, the patent EP-B-2 203 462 and the invention to which it relates are found to meet the requirements of the Convention.

The currently valid documents are:

Auxiliary Request 1

Description, Pages

2-46 of the patent specification

Description, Paragraphs

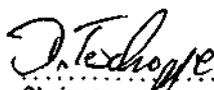
52 handwritten amendments filed during Oral Proceedings on 05-10-2016

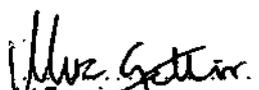
Claims, Numbers

1, 2 received on 03-08-2016 with letter of 03-08-2016

The Grounds for the decision (Form 2916) are enclosed.

24/10/16
Date


Chairman
Tzschoppe, Dieter

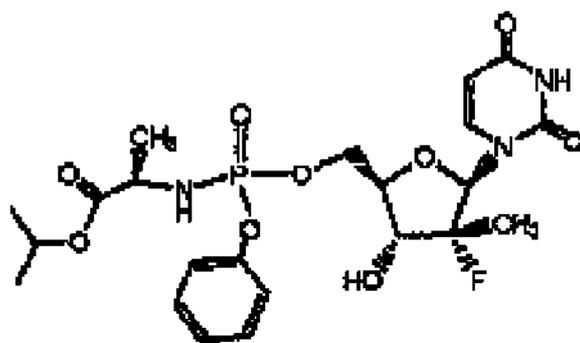

1st Examiner
Gettins, Marc


2nd Examiner
Härtinger, Stefan

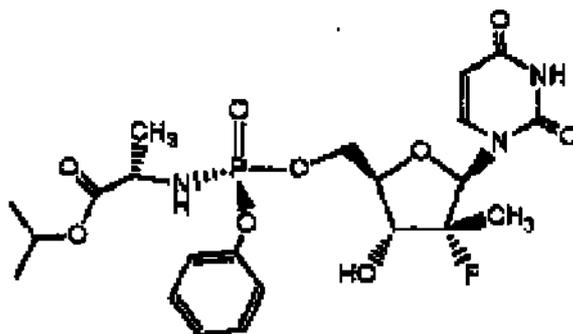

Legally qualified member
Schauwecker, Marko

Facts and Submissions

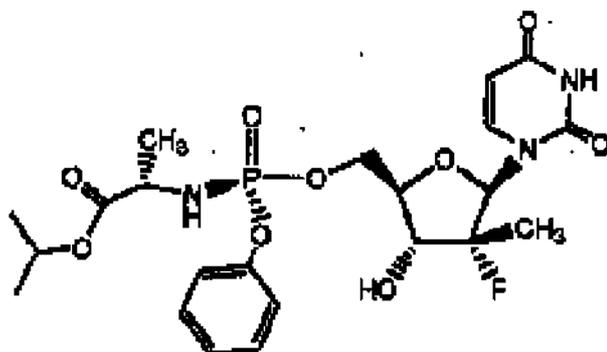
- 1 The contested patent has the patent number EP2203462 and is based upon the application number 08732818 with the title "NUCLEOSIDE PHOSPHORAMIDATE PRODRUGS". The patent proprietor is Gilead Pharmasset LLC. The patent has 3 priority dates namely: 30.03.2007; 24.10.2007 and 21.03.2008.
- 2 Claim 1 of the patent in suit is directed to the following racemate



Claims 2-3 are directed to the enantiomers of this compound and have the following formulae:



claim 2



claim 3

Claims 4-6 relate to compositions corresponding to claims 1-3 respectively.

3 A total of ten oppositions have been filed by the following opponents:

- 3.1 Médecins du Monde (Opponent 1) on 10.02.2015;
- 3.2 Mr H. H. Fleischer (Opponent 2) on 19.02.2015;
- 3.3 Ellis IP Ltd. (Opponent 3) on 19.02.2015;
- 3.4 Pharmaceutical Works Polpharma S.A. (Opponent 4) on 20.02.2015;
- 3.5 Generics [UK] Ltd (Opponent 5) on 20.02.2015;
- 3.6 Teva Pharmaceutical Industries Ltd; (Opponent 6) on 20.02.2015;
- 3.7 Intellectual Property Services (IPS) (Opponent 07) on 23.02.2015;
- 3.8 Stada Arzneimittel AG (Opponent 8) on 23.02.2015;
- 3.9 ZBM Patents S.L. (Opponent 9) on 23.02.2015;
- 3.10 Actavis Group PTC ehf (Opponent 10) on 23.02.2015

4 The following documents have been cited:

- A1 assignment of D1
- A2 assignment of D2
- A3 assignment of D3
- D1 US prov. appl. 60/909315 = 1st priority document of contested patent

- D2 US prov. appl. 60/982309 = 2nd priority document of contested patent
- D3 US prov. appl. 12/053015 = 3rd priority document of contested patent
- D4 WO2005/012327
- D5 Thesis entitled "Design, Synthesis and Biological Evaluation of Novel Nucleotide Prodrugs as Potential Anti-Hepatitis C Virus Agents" submitted by Plinio Perrone
- D6 Ma et al 2007, J. Biol. Chem. 282: pp29812-29820
- D7 Perrone et al 2007 J. Med. Chem. 50, pp1840-1849
- D7a Extract from website of J. Med. Chem. to confirm publication date of document D7
- D8 Poster presented at the 14th International Symposium on Hepatitis C Virus and Related Viruses which was held in Glasgow (Scotland) on 9-13 September 2007
- D9 Murakami et al 2008 Antimicrob. Agents Chemother. 52:458-464
- D10 Clark et al 2005, J. Med. Chem. 48, pp5504-5508
- D11 Zemlicka 2002 Biochemica Acta 1587:276-286
- D12 WO2005/003147
- D13 WO2008/121634 application document of contested patent
- D14 Lehsten et al, Org. Proces Res. Dev. 2002, 6, 819-822
- D15 WO2011/123645
- D16 Cahard et al, Mini-Reviews in Medicinal Chemistry, 2004, 4, pp:371-381
- D17 Jones et al., Minireview: Nucleotide Prodrugs. Antiviral Research 27 (1995), pp. 1-17
- D18 Lee et al, Antimicrobial Agents and Chemotherapy 2005. pp 1898-1906
- D19 WO 02/08241
- D20 McGuigan et al, J. Med. Chem, 2006, 49, pp 21 5-7226
- D21 Sofia et al. J, Med. Chem, 2010, 53, pp7202-7218
- D22 Abstract 100 "Design, synthesis, and Biological evaluation of Novel Nucleoside PPAs as Potential Anti-HCV Agents' presented at the 14th international Conference on Antiviral Research May 7-11, 2006
- D23 WO2004/002999

- D24 W02006/121820
- D25 W02006/012078
- D26 WO2006/065335
- D27 Gunic et al Bioorg & Med Chem. Letts 17(9) pp2456-2458 14.02.2007
- D28 US6475985
- D29 WO03/000713
- D30 WO2007/020193
- D31 US2006/0241064
- D32 WO2007/095269
- D33 Ma et al "Characterization of the Intracellular metabolism of 3-D-2'-deoxy-2'-fluoro-2'-C-methylcytidine." Antiviral Research 2007 74 A36 Abstract 23
- D34 Evidence as to the priority date of D33 and D35
- D35 McGuigan et al "Sub Micromolar Inhibitors of HCV Generated from Inactive Nucleosides by Application of ProTide Technology" Antiviral Research 2007 74 A36 Abstract 24
- D36 McGuigan et al FEBS Letter (1994) 11-14
- D37 McGuigan et al, J. Med. Chem. 2005, pp3504-3515
- D38 Klumpp et al, J. Biol. Chem., 2006, vol. 281, pp3793-3799
- D40 US12/053015
- D42 Declaration of Dr Michael Sofia
- D43 Declaration of Dr Valentino Stella
- D44 Declaration of Professor John R. Thomas
- D45 Declaration of Dr Adrian Ray
- D45A Second Declaration of Dr Adrian Ray
- D46 Declaration of Dr Phillip Furman
- D47 Declaration of Dr William Delaney
- D48A Email exchange dated 14 May 2015 regarding publication date of D14
- D48B Email exchange dated 12 November 2015 regarding the period

of the Bar on Access of D14

D48C Email exchange with Cardiff University Library

D49 Ghany et al., AASLD Practice Guidelines, Hepatology, 49(4), 2009, 1335-1374

D50 North et al., General Hospital Psychiatry, 2013, 35. 122-128

D51 Klebl et al., Antiviral Chemistry and Chemotherapy, 2005, 16, 69-90

D52 De Francesco et al, Nature, 2005. 436, 953-960

D53 Kaplan, Drug Discovery Today: Disease Mechanisms, 2006, 3(4), 471-477

D54 Wagner et al., Medical Research Reviews, 2000, 20(6), 417-451

D55 Poster presented at 59th Annual Meeting for the American Association for the Study of Liver Diseases, 31 October to 4 November 2008, Furman et al.

D56 Pharmasset, Inc., Press Release of 31 July 2009

D57 FDA Consumer Health Information Leaflet, July 2014, "Faster Easier Cures for Hepatitis C"

D58 EMA Press release, 22 November 2013 "European Medicines Agency recommends approval of sofosbuvir for the treatment of chronic hepatitis C"

D59 Organic Stereochemistry, Michael J T Robinson, OUP, 2005, cover page and Sections 1.4-1.8

D60 Organic Chemistry, Clayden et al., OUP, 2005, cover page and pages 48-49

D61 Inorganic Chemistry, Huheey et al, HarperCollins College Publishers, 1993, cover page and pages 233-234

D62 Roberts et al., Hepatology, 2006, 44 (S 2), S269

D63 Strader et al., Hepatology, 2004, 39(4), 1147-1171

D64 Furman et al., Antiviral Drugs: From Basic Discovery Through Clinical Trials, First Ed, 2011 , 305-315

D65 Cole et al., Drugs of the Future, 2009, 34(4), 282-290

D66 Lawitz et al., Antimicrobial Agents and Chemotherapy, 2013, 57(3), 1209-1217

D67 Dykens et al., Future Drugs, 2007, 7(2), 161-175

D67A Letter from the British Library confirming publication date of D67

D68 FDA publication, June 2006, "Guidance for industry: Antiviral Product Development - Conducting and submitting virology studies to the agency"

D69 Communication dated 15 July 2015 from Karen Winestock at the FDA

D70 Feng et al., Antimicrobial Agents and Chemotherapy, 2015, published online 23 November 2015, ref no. AAC01922-15R1

D71 Murakami et al., The Journal of Biological Chemistry, 2010, 285(45), 34337-34347

D72 Wedemeyer et al., Hepatology, 2013, 58(2), 524-537

D73 Pockros et al., Hepatology, 2013, 58(2), 514-523

D74 Pharmasset Press Release, 6 September 2011, "Pharmasset Announces 91% SVR12 from the PROTON Trial in Subjects with Hepatitis C Genotype 1"

D75 Nelson et al., Journal of hepatology, 2011, 54, S544, Abstract 1372

D76 Lalezari et al, Journal of Hepatology, 2011, 54, S28, Abstract 61

D77 Soriano et al, Expert Opinion on Pharmacotherapy, 2013, 14(9), 1161-1170

D78 Kowdley et al., The Lancet, 2013, 381, 21002107

D79 Lawitz et al., The New England Journal of Medicine, 2013, 368, 1878-1887

D80 World Health Organisation - Model List of Essential Medicines, August 2015

D81 Arnold et al, PLOS Pathogens, 2012, 8(11), e1003030

D82 Dickinson et al, Chemical Principles, 1984, 4th Ed, pp9-10

D83 T. L. Brown "Chemistry: The Central Science", 1994, 6th Ed, p50

D84 Stuyver et al, Antiviral Chemistry & Chemotherapy, 2006, 17 (2), pp79-87

D85 Wikipedia excerpt on "18s ribosomal RNA"

- D86A Bibliographic record of D5 from Cardiff University Library
- D86B Email exchange with Cardiff University Library about the public availability of D5
- D87 Declaration of Prof. Ravicher
- D88 T0106/07
- D89 Second Declaration of Prof. John R. Thomas
- D90 Second Declaration of Prof. Ravicher

5 **Opponent 1**

Opponent 1 (O1) has cited the documents D1-D12 in the notice of opposition. In the notice of opposition the patent was opposed for lack of novelty, lack of inventive step, insufficient disclosure and added subject matter. Objections were made against claims 2-3, 5-6 under Art. 123 (2) EPC. An objection under Art. 83 was made against claims 2-3 and 5-6 since the opposed patent does not disclose any stereospecific method of synthesis and/or of purification of the compounds of claims 2-3. The question of priority was discussed. At the time of filing of the application the Patentee was Pharmasset Inc. Opponent 1 considers that Pharmasset cannot be considered to be the successor in title of the inventors and that accordingly none of the claimed matter is entitled to the priority data of any of D1-D3. A novelty objection was made against claim 1 based on D4 - this objection is not linked to the entitlement of the priority date. An inventive step objection was made against claims 1-6 using D5 or D7 as the closest prior art and combining this with D6 or D8 or D9. If the priority date is held to be valid an inventive step objection is made based on D4 or D10 in combination with D5 or D7. O1 filed further arguments with the letter of 04.08.2016 and cited D86A, D86B and D87. O1 disagreed with the choice of D53 as the closest prior art and objected to the auxiliary requests under Art. 84 EPC. With the letter of 29.09.2016 O1 commented on the Patentee's letter of 02.09.2016 and submitted D90.

6 **Opponent 2**

Opponent 2 (O2) has cited the documents D1, D4, D6-D8 D10, D12-D13, D16-D25 in the notice of opposition. Claims 1-6 are said to not be entitled to the priority of D1. Claims 2-3 and 5-6 are attacked under Art. 123 (2) and Art. 83 EPC. An inventive step objection is made against claims 1-6 using D10 as

the closest prior art. The objection uses D10 on its own or in combination with any one of D7, D16, D18-D20. A further objection uses D8 as the closest prior art. For claims 2-3 D4 is also used. O2 filed additional arguments under Art. 123 (2), 83, inventive step and priority with the letter of 03.08.2016 and submitted D84-D85.

7 **Opponent 3**

Opponent 3 (O3) has cited the documents D6, D7, D9, D10 and D13-D15 in the notice of opposition. In the notice of opposition the patent was opposed for lack of inventive step, added subject matter and Art. 83 EPC. Objections were made against claims 2-3, 5-6 under Art. 123 (2) EPC. An objection under Art. 83 EPC was made against claims 1-6 since the opposed patent does not disclose enough details of how to obtain the compound of claim 1 or the compounds of claims 2-3. The question of priority was discussed. Opponent 2 considered that claims 1 and 4 are not entitled to the priority of D1 and that claims 2, 3, 5-6 are not entitled to the priority of any of D1-D3. For claims with an invalid priority claim D7 (closest prior art) and D6 were used in an inventive step objection or D9 (closest prior art) and D7. If the priority claim is held to be valid an inventive step objection is made using D10 (closest prior art) in combination with D7. A lack of inventive step objection was also made based on the fact that the alleged antiviral activity of claims 2-3 has not been substantiated and that these claims do not solve any problems, but merely represent compounds without any technical effect. O3 confirmed that he would not attend the O.P. in the letter of 19.07.2016.

8 **Opponent 4**

Opponent 4 (O4) has cited the documents D4, D7, and D25-D32 in the notice of opposition. Objections are made against the enantiomers under Art. 123 (2) and Art. 83 EPC. Claims 1-6 are attacked as lacking an inventive step. The objection makes particular reference to D7 and D12. O4 filed further arguments with the letter of 04.08.2016 and submitted D88.

9 **Opponent 5**

The text in the notice of opposition is by the same representative as for O4 and appears to be identical. The same objections as for O4 therefore apply. O5 filed arguments with the letter of 04.08.2016 and submitted D88. The text appears to be the same as that filed by Opponent 4 on the same day.

10 **Opponent 6**

Opponent 6 (O6) has cited i.a. the documents D4, D7, D10, D12, D16, D18 and D25-D36. An inventive step objection has been made against claims 1-6 using D7 or D33 as the closest prior art. O6 filed arguments Re inventive step with the letter of 03.08.2016 and submitted D7a. Auxiliary requests were objected to.

11 **Opponent 7**

Opponent 7 (O7) has cited the documents D1-D3, D6, D7, D9, D10, D30 and D38. An objection was made against claims 2-3 and 5-6 under Art. 123 (2) and Art. 83 EPC. Claims 1-6 of the contested patent are said to not be entitled to any of the priorities D1-D3 for formal (as already outlined by O1) and substantive reasons. An inventive step objection against claims 1-6 was made on the basis of combinations of D6, D7, D9, D10, D30 and D38.

12 **Opponent 8**

Opponent 8 (O8) has cited the documents A1, D4, D6-D11, D18 and D37. An objection was made against claims 2-3 and 5-6 under Art. 123 (2) and Art. 83. Claims 1-6 of the contested patent are said to not be entitled to any of the priorities D1-D3 for formal (as already outlined by O1) and substantive reasons. Claims 2-3 are considered to lack an inventive step since the problem to be solved- provision of anti-HCV agents has not been shown to have been solved. An inventive step objection against claims 1-6 is made on the basis of combinations of D4, D6-D11, D18 and D37. O8 filed arguments with the letter of 04.08.2016 Re novelty vis-à-vis D4 and inventive step using D4, D6, D9, D10 with D7, D18, D37.

13 **Opponent 9**

Opponent 9 (O9) has cited the documents D1-D3, D6-D10 and D16. An objection was made against claims 2-3 and 5-6 under Art. 123 (2) and Art. 83. Claims 2-3 and 5-6 are said to not be entitled to any of the priorities D1-D3 for formal (as already outlined by O1) and substantive reasons while claims 1 and 4 are not entitled to the first priority. Claims 2-3 are considered to lack an inventive step since the problem to be solved- the provision of anti-HCV agents has not been shown to have been solved. If D1 is a valid priority an inventive step objection is made based on D7 and D10. If it is considered that the patent is not entitled to the priority of D1, an inventive step objection against claims 1-6 is made on the basis of D7 in combination with one of D8, D9 or D16. O9 confirmed that he would not attend the O.P. with the letter of 07.09.2016.

14 **Opponent 10**

Opponent 10 (O10) has cited the documents A1-A3, D4, D6-D8, D10-D12, D16 and D37. An objection was made against claims 2-3 and 5-6 under Art. 123 (2) and Art. 83 EPC. Claims 1-6 of the contested patent are said to not be entitled to any of the priorities D1-D3 for formal (as already outlined by O1) and substantive reasons. A novelty objection against claims 1 and 4 was made based on D4. If D1 is a valid priority an inventive step objection is made based on D10 and D12 and the lack of evidence of the anti-HCV activity with particular reference to D11 and D16. If it is considered that the patent is not entitled to the priority of D1, D8 is considered to be the closest prior art and, in combination with any of D7, D16 or D37, is the basis for an inventive step objection against claims 1-6.

15 **Patentee**

The Patentee replied on 11.12.2015 and submitted nine auxiliary requests. The Patentee cited documents D42-D81. The Patentee considered that D5 was not made available until March 2009 and therefore considers that D5 is not prior art. Support for this is said to be provided by D48B. The claimed compound is said to have a unique combination of structural properties leading to a nucleotide analogue NS5B inhibitor with potent HCV activity, low toxicity and can be administered orally. Re Art. 123 (2) EPC claims 2-3 are said to be supported by entry IX-25-2 on page 254 which should be interpreted in the light of page 99, line 10 to page 100, line 5. T1046/97 and T658/91 were evaluated. The issues of sufficiency and entitlement to priority

were addressed. Novelty and inventive step were also discussed with D53 as the closest prior art. The patent is said to be inventive because of its unexpected anti-HCV activity or on account of its improved anti-HCV activity. Arguments were submitted on 03.08.2016 as were further auxiliary requests. New auxiliary requests 2-11 were filed which replaced the previous auxiliary requests and whereby only auxiliary requests 2 and 8 differ from the previous auxiliary requests. Documents D45a, D82 and D83 were cited. The main arguments related to inventive step and considered D4, D7, D10 and D12. For subject matter entitled to the priority of D1 D53 is the closest prior art. If the current application is not entitled to the priority of D1, D8 and D7/D16/ D18-20 and D37 have been considered. If priority D1 and D2 are not valid D9 or D9 and D18/D30/D37 are considered. The Patentee considered that D86A, D86B and D87 were late filed and should not be allowed into the proceedings. Further arguments on the citability of D5 and priority were filed on 02.09.2016 and D48C and D89 were cited. A consolidated list of documents was filed.

- 16 A brief summary of the Auxiliary Requests prior to the O.P. is as follows. The numbering 1 to 3 relates to the racemate of claim 1 (of the main request) and the enantiomers of claims 2-3 respectively.
- 16.1 Aux Req 1: 1 is claimed. The enantiomers are not claimed in structural terms any more, but are now the "slow eluting isomer" and the "fast eluting isomer" obtainable under specific chromatographic conditions.
- 16.2 Aux Req 2: 1 and 2 are claimed
- 16.3 Aux Req 3: Only 1 is claimed
- 16.4 Aux Req 4: Only 2 is claimed
- 16.5 Aux Req 5: Only slow eluting isomer is claimed.
- 16.6 Aux Req 6: 1-3 claimed and pharmaceutically acceptable medium specified.
- 16.7 Aux Req 7: 1 claimed as are slow and fast eluting isomers and pharmaceutically acceptable medium specified
- 16.8 Aux Req 8: 1 and 2 claimed and pharmaceutically acceptable medium specified
- 16.9 Aux Req 9: 1 claimed and pharmaceutically acceptable medium specified

16.10 Aux Req 10: 2 claimed and pharmaceutically acceptable medium specified

16.11 Aux Req 11 slow eluting isomer and pharmaceutically acceptable medium defined

17 The parties were summoned to Oral Proceedings. In accordance with Rule 116 (1) EPC all parties were informed that any written submissions (new documents, new claims etc) should be submitted at the very latest two months before the oral proceedings.

18 Oral proceedings were held on 04-05.10.2016. The opposition division decided that the main request did not meet the requirements of Art. 123(2) EPC. The auxiliary request 1 was found to meet the requirements of the European Patent Convention.

Reasons for the Decision

Main Request

Art 123 (2) EPC amendments

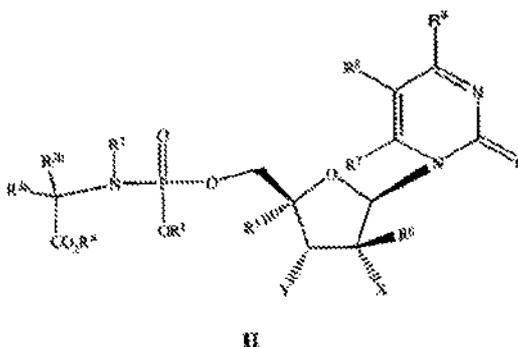
19 No objections were made against the racemate of claim 1 and its composition in claim 4. All objections were directed against the enantiomers of claims 2-3 and their compositions in claims 5-6.

19.1 The racemate of claim 1 is disclosed as example 25 - see e.g. page 683 or claim 2 of the original application (D13).

19.2 The Patentee indicated the following passages in D13 as disclosure for the two enantiomers: Formula IX-25-2 on page 683 in combination with pages 99-100, example 25 and claim 2 which discloses example 25 and "its stereoisomer". The relevant passage referred to in pages 99-100 reads:

- 19.3 " In each of the presented tables, the phosphoramidate substituent containing the substituents R^{3a} and R^{3b} are depicted without reference to stereochemical structure (cf. structures 1-1, 1-3, 1-5, 1-7, and 1-9 above). It is contemplated that the compounds recited below embody compounds in which R^{3a} projects toward the viewer while R^{3b} projects away from the viewer (cf. structures 1-2, 1-4, 1-6, 1-8, and 1-10). Moreover, it is contemplated that the compounds recited below also embody compounds in which R^{3a} projects away from the viewer while R^{3b} projects towards the viewer. Not meant to be limiting, however, it is contemplated that preferred compounds are those in which R^{3a} projects towards the viewer and R^{3b} projects away from the viewer such that the natural L-amino acid (S)-configuration is presented. Additionally, the inventors recognize that the phosphorus atom of the phosphoramidate moiety is another source of chirality. Although the structures below do not specifically depict chirality at phosphorus, the inventors recognize that stereochemical configurations are possible such that in a staggered (or zig-zag) line structure the oxo-substituent projects towards the viewer while the OR^1 substituent projects away from the viewer, and vice versa, i.e., where the Cahn-Ingold-Prelog stereochemical designation of phosphorous is either R or S. Therefore, the structures below include all possible stereochemical configurations possible for phosphorus."

- 19.4 This is then followed by the formula II



- 19.5 In the case of IX-25-2 each of R^2 - R^9 have been specified and should be read in combination with formula IX on page 246. The Patentee considered that the passage on page 99 referring to the first orientation of R^{3a} and R^{3b} is a preferred variation as is further shown e.g. by example 82, on pages 693-697, where all of the tested compounds have this configuration. There is also no

selection from lists involved because all substituents in formula II are set out in a single line. This should be combined with the statement of all possible stereochemical configurations possible for phosphorus and IX-25-2 which is considered to be directly related to it, thereby directly individualising the enantiomers of claims 2 and 3.

- 19.6 The Patentee also referred back to page 20, which reads "*It is contemplated that compounds of the formula I are racemic because the chirality at phosphorous. Applicants contemplate use of the racemate and/or the resolved enantiomers*".
- 19.7 Example 81 on pages 692-693, is said to make it clear that enantiomers of the patent have been separated and isolated. Claim 2 gives example 25 and its stereoisomer and this only gives the possibility of the two enantiomers. There is a direct disclosure of a single compound which includes the 2 stereoisomers thereof, the skilled person knows that these are at the P atom and knows how to extract them. The Patentee referred to T658/91, points 2.1 and 2.4, which was taken to show that documents not only disclose explicitly defined enantiomers, but also enantiomers when these are listed as being part of the invention. This is the same as the current case. With reference to T1046/97 this is said to only apply for the case where an optically active form can be a mixture, but not to the current case which has individualised enantiomers and is therefore less relevant. The Patentee considers that D13 makes specific reference to enantiomers and that these have effectively been individualised. When reading the table (i.e. for IX.25-2) both configurations for the phosphorous centre have to be read into the table, thereby providing a simple and direct disclosure. As there are only two possibilities, R and S, the compounds of claims 2 and 3 are also not just covered but also disclosed.
- 19.8 The following points were raised by the Opponents. O1 argued that the compounds of claims 2 and 3 show different configurations at the phosphorus atom. However, these structures are neither depicted nor described as such in D13. O1 considered the disclosure on p.20, l.8 to be only a general statement. Example 81 relates to the separation of a fast and a slow moving isomer. This example not only leaves undetermined the nature of the separated diastereoisomers, but also none of the separated examples in Example 81 relates to the compounds of claims 2 and 3. The claimed

compounds are seen as a selection from several lists, which require: a selection from the list of structures; the selection of one configuration as R and S; the selection of the configuration at the phosphorous atom, and the selection of a set of substituents within the Tables. O1 did not consider the claimed matter to be unambiguously and directly derivable from the application as originally filed. O2 considered that page 99 only says that P is a chiral atom, but does not individualise its isomers. O4 referred to the very large scope of D13 therefore necessitating that both a single compound and its individual isomers be disclosed. It was stressed that P chirality is not disclosed in example 25 and that while pages 99-100 specify some chiralities other chiralities are also given. With reference to the Case Law Book, 8th Edition, page 127 reference was made to T1048/92 which is taken to mean that a racemate does not disclose the enantiomers. Example 81 is irrelevant since it does not refer to example 25 and that this is in any case an Art. 83 EPC issue. O2 stressed that formula II does not specify the chirality of the carbon atom and that IX-25-2 is only the scope of the racemate of claim 1.

- 19.9 It is undisputed that the structures of claims 2 and 3 are not drawn in the application as originally filed, nor are the chemical names mentioned *expressis verbis*.
- 19.10 The OD notes that the decisions T1046/97 and T658/91 cited by the parties do not come to the same conclusions as to whether or not specific enantiomers are disclosed when compounds have been disclosed and there has only been a general reference to their enantiomers. The OD considers that the approach in T1046/97 is more relevant for the current situation. The OD notes that while p99-100 specify possible chiralities, phrases such as "not meant to be limiting" or "vice versa" mean that the disclosure is not limited thereto. Page 20 does not indicate a preferred chirality. Formula II and therefore IX-25-2 have two possible points of chirality leading to 4 possible enantiomers. Even when pages 20 and 99-100 are taken into account the enantiomers have not been individualised for IX-25-2.
- 19.11 In the case of example 25 on page 683 the chirality at the carbon atom has been defined so that there are two possibilities at the P atom. There is no indication on pages 682-683 that enantiomers are exemplified or comprised.

Pages 99-100 are taken to refer to IX-25-2, but it is not unambiguously clear that they apply to example 25 since page 99 refers to the "following tables", which would only be taken as a reference to tables which have been labelled as such i.e. Tables II-XXX-II. Example 25 is, however, only listed in the examples section. Even if p99-100 are combined with example 25 the OD notes that while p99-100 specify possible chiralities, phrases such as "not meant to be limiting" or "vice versa" mean that the disclosure is not limited thereto. It is not apparent that pages 99-100 should be read as a limitation to a fixed, simultaneous chirality at the P and C atoms.

- 19.12 The reference in claim 2 of the original application to stereochemistry reads "A compound its stereoisomer..."
- 19.13 Thereafter follows a list of individualised compounds including the compound of example 25. This means that claim 2 does not claim only example 25, but instead also claims a stereoisomer of example 25.
- 19.14 It is noted, that the passage of claim 2 mentioned above refers to the stereoisomer in singular form. In claim 2 this compound is in the (S) configuration i.e. where chirality has been defined at the carbon atom and not at the P atom. In the case of the compound of example 25 this means that a chirality has been defined for the carbon atom, but not for the phosphorus atom.
- 19.15 The OD considered that the stereoisomer of claim 2 mentioned in singular form can only relate to the corresponding (R) isomer, and not extend to more, other stereoisomers e.g. the specific enantiomers due to the two possibilities on the P atom.
- 19.16 Claim 2 of the original application does not therefore individualise the compounds of current claims 2 and 3.
- 19.17 The OD considers that the enantiomers of claims 2 and 3 as well as composition claims 5 and 6 containing them were not disclosed in D13 and that the main request fails to meet the requirements of Art 123 (2) EPC.

- 19.18 The OD notes that Aux Requests 2,4,6,8,10 were also not allowable under Art 123 (2) since they contain at least one of the enantiomers from the main request. This was not contested by any of the parties.

Auxiliary Request 1

- 20 This request has 2 claims and corresponds to claims 1 and 4 of the main request.

Art. 83 EPC sufficiency of disclosure

- 21 O2 referred to the statement in writing by the Patentee dated 03.08.2016, point 4.1 that the racemate also includes the enantiomers. O8 considered that the racemate does not include the pure enantiomers. O2 stated that since claim 1 includes both enantiomers it was necessary for the patent to disclose how they were obtained and how the configuration on the P atom should be assigned. There is no such information in the patent. Even if example 81 referring to slow and fast eluting can be applied to the racemate it would not be clear which isomer is in the R or S configuration. O2 disagreed with the written submissions of the Patentee that the use of X-ray can be considered to be a routine method for determining the configuration at the phosphorus atom. D59 and D60 were cited as evidence of potential problems associated with such a method. O2 considered that it is critical how to obtain a single crystal in a good quality. There is no information in the patent about how to obtain such a suitable crystal. With reference to D21, which was published two years after the filing date of the patent, O2 argued that at the time of filing the common general knowledge was insufficient to produce a suitable crystal of the compounds covered by claim 1. Furthermore, D15 shows that X-ray measurements were done on a specific crystal form II which proved to be unstable. To find the correct single crystal was difficult and would amount to an undue burden. Alternative methods for determining the phosphorus configuration are NMR methods. The patent does not disclose these methods. Common general knowledge, such as D7, suggested that this method is only successful for compounds which meet specific structural requirements (p.1844, Figure 2, compounds 34 or 35) which are not met by

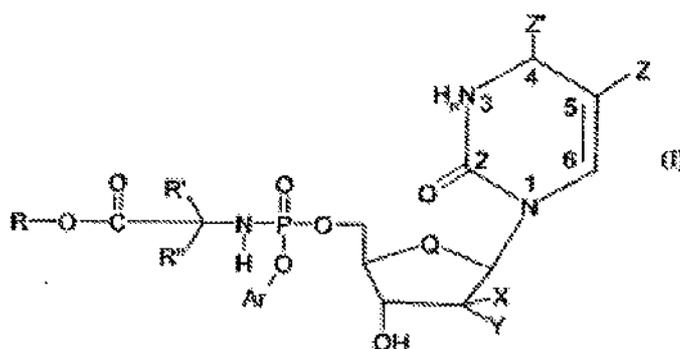
the claimed compound due to the absence of a comparable pi-pi interaction. O2 discussed D21, which mentions on p.7209 that this document is the first demonstration of a crystalline PPA. O2 insisted that the phosphorus configuration is a feature of the claim even when it is not depicted as a true 3D structure. Since the invention needed completeness in its disclosure, but this requirement is not met, an undue burden is placed on the public in searching for conditions which allow the crystallisation and analysis of the claimed product. O8 concluded from the fact that claim 2 of the granted patent was not drafted as a claim which is dependent on claim 1 that the latter was only directed to a racemate. In his understanding, claim 1 of the first auxiliary request does not embrace anything else but the racemate. Should however intermediate isomers also be covered by the claim then claim 1 is seen to infringe Art. 83 EPC for essentially the same reasons as presented by O2.

- 21.1 The Patentee stressed that claim 1 does not embrace individual pure stereoisomers. Art. 83 EPC requires that the skilled person is enabled to reproduce the invention. The Patentee noted that none of the opponents suggested that it would not be able to make the claimed product (example 25) or would see a problem with a chromatographic separation of isomers. It is therefore not at question that the claimed product can be made and purified. However, the structure of individual diastereoisomers is not a feature of claim 1, and therefore there is no legal justification for requiring that the skilled person must also be able to assign the individual diastereoisomers. In any case the skilled person would have been able to assign the absolute phosphorus configuration based on known and generally available techniques discussed in D59, D60, D61. D19 (p.6; p.32) and D21 (p.7214) were seen as further accounts of standard methods which were available before the filing date of the patent. The Patentee considered that methods were useful irrespective of whichever polymorph is analysed because each crystal form has the same configuration at the chiral centre. The growing of bigger crystals is within the prior art or common general knowledge. The Patentee considered that chromatographic separation methods were commonly applied techniques at the relevant time. One could therefore make and separate the individual isomers. They are within the scope of claim 1.

- 21.2 The OD considers that claim 1 is directed to a racemate i.e. a mixture of the enantiomers and not the pure enantiomers. As indicated by the Patentee it is not contested that the racemate of claim 1 can be produced. This interpretation of claim 1 means that it is not necessary to come to a decision as to whether or not the patent in suit sufficiently discloses how to isolate and characterise the enantiomers. This issue is not relevant to claim 1. The OD considers that the auxiliary request 1 meets the requirements of Art. 83 EPC.

Art. 54 EPC Novelty

- 22 The objections made by O1 and O10 are that the racemate of claim 1 is the racemate of a compound which falls fully within the Markush formula (I) of D4 shown below.



Each of the definitions for the substituents in formula (I) which correspond exactly to the racemate of the contested patent are included within the scope of D4 as is the correct chirality on the carbon atom.

- 22.1 The Patentee considers that D4 does not provide a direct and unambiguous disclosure of the racemate. Ten selections within (I) are required to obtain the racemate. In addition certain structural features in D4 (e.g. the uracil base or the 2 methyl, 2 fluoro deoxyribose ring) are not exemplified in D4.
- 22.2 The OD considers that the racemate falls within the scope of (I) in D4. In order to get from the general formula (I) in D4 to the racemate it is necessary to make selections from numerous lists and several of these selections are not preferred or exemplified embodiments. The racemate is not explicitly

disclosed in D4 and is not implicitly disclosed by a possible combination of preferred values. The racemate is therefore not clearly and unambiguously disclosed in D4 and is a novel selection within the scope of D4. The auxiliary request 1 is novel.

Late filed evidence and slide show request of O1:

- 23 Prior to the discussion on inventive step and the accompanying discussion of the entitlement to priority the Patentee objected to the admissibility of D87 and D90 and requested that if these documents were taken into account D89 should also be admitted. The Patentee also objected to a proposed slide show presentation by O1.
- 23.1 In response to a question from the OD about the subject of the presentation, O1 did not exclude the possibility that the presentation would contain new arguments or evidence. More precisely O1 stated that the slides were based on the written submissions with some modifications.
- 23.2 D87, D89 and D90 were filed to establish and prove the content of U.S. Law and are *prima facie* highly relevant for the question of formal priority entitlement. The OD thus decided to admit these documents under Article 114(2) EPC. On the other hand, the OD does not see that an oral presentation of his arguments only would affect the right to be heard of O1. For these reasons the OD exercised its discretion by not allowing O1 to support his submission with a slide show.

Formal entitlement to priority

- 24 Several opponents challenged the validity of the priority claims on the basis that D1 and D2 were not filed by the applicant of EP08732818.3 from which the patent-in-suit is derived.

- 24.1 In accordance with section A-III, 6.1 of the Guidelines for Examination in the EPO and the case law of the EPO Boards of Appeal as last summarised in and including decision T 205/14 of 18 June 2015 (see also decision T 517/14 of 19 June 2015), the succession in title requires that D1 and D2 (or the right to claim priority to these applications in the context of EP08732818.3) had been validly transferred to the applicant of EP08732818.3 when the latter was filed. The Patentee as the party claiming the right to priority carries the burden of proof and must establish the relevant national provisions, the requirements defined therein for a valid transfer and the compliance with these requirements.
- 24.2 Both the Patentee and the opponents challenging the priority rights argued based on U.S. Federal Law. The OD understands from the pertinent case law of the EPO Boards of Appeal that – depending on the specific circumstances of a given case - two legal regimes may be applied to assess the validity of the transfer of a priority application or priority right, namely the law of the country where the priority application was filed and/or the law governing the legal relationship between the parties to the transfer. The OD therefore agrees that the validity of the assignments must be assessed under U.S. (Federal) Law.
- 24.3 The content of any relevant national law is a question of fact which needs to be established on the basis of evidence brought forward by the parties (see, for example, decision J 19/87) and, to the extent available, the division's own knowledge of the relevant foreign law (Article 114(1) EPC).
- 24.4 The validity of the assignments A1 and A2 as such was not in dispute between the parties. The OD considers that indeed the assignment documents comply with the applicable requirements of U.S. Federal Law (35 U.S.C. § 261) in that A1 and A2 identify the relevant patent application and related invention, are signed by the assignors (a signature of the assignee not being required under U.S. Law) and contain the language required to bring about an immediate assignment of present and future rights as opposed to a promise to assign in the future (*"by these presents do sell, assign and transfer"*).

- 24.5 As is evident from D44, D87, D89 and D90 the parties disagreed, though, on the content of U.S. Law in so far as it concerns the requirement to explicitly mention the right to claim priority for it to be covered by an assignment of a patent application and/or invention as set out in A1 and A2. In this regard the OD cannot find any evidence in D44, D87, D89 or D90 that under U.S. Federal Law assignment of a priority right would require it to be explicitly mentioned in an assignment agreement and that the language used in A1 and A2 would not cover the right to claim priority from D1 and D2. D87 and D90 do not rely on codified or pertinent case law as evidence for the existence of such a requirement but rather in the first place on recommendations issued by law firms. In general, the OD does not consider such recommendations as relevant source for establishing specific requirements under a foreign national law. Moreover, it is rather doubtful whether the recommendations relate to the fulfilment of requirements under U.S. Federal Law. This applies in particular with regard to the title ("*...Pass Muster in Europe*") and the content of the publication mentioned in point 5.11 of D87 which *inter alia* mentions a signature of the assignee although not being a requirement under U.S. Federal Law. Such recommendations issued by law firms may thus well be written with a view to foreign jurisdictions (for example, France) in which the explicit mentioning of the priority right in an assignment agreement may be required. D87 and D90 do not, though, establish the existence of such a requirement under U.S. Federal Law. Rather, the OD accepts D44 and D89 as correctly representing U.S. Federal Law in that the language used in paragraph 3 of A1 and A2 ("*...the full and exclusive right to the said invention in the United States....and in all foreign countries and the entire right, title and interest in and to any and all Letters Patent which may be granted therefor in any and all foreign countries...*") included the right to claim priority from D1 and D2 without the need for the priority rights to be explicitly mentioned.
- 24.6 The OD does not agree either with the suggestion brought forward by O7 that Articles 87-89 EPC as implementation of Article 4 of the Paris Convention require the explicit mentioning of the priority right. Articles 87 to 89 EPC as complete, self-contained code of rules on claiming priority for the purpose of filing a European patent application do not set forth any such (or other) formal requirement for a valid succession-in-title (T 517/14, at 2.4 and 2.7.1). Nothing else can be derived from decision T 204/15 relied on by several opponents. On the contrary, in point 3.3 of the reasons of the decision immediately following the passage cited by O1 and O5 in support of their

position, it is stated: *"The board does not share the respondent's view that the characterisation of the right of priority as a right independent of the right to the priority application implies that the valid transfer of a priority right inevitably requires a separate and express assignment declaration. The requirements for a valid transfer of rights of priority is a matter distinct from the characterisation as an independent legal right."* The OD understands this as a clear rejection of the suggestion that the requirement to explicitly mention the priority right in an assignment could be derived from the EPC.

- 25 For these reasons the OD considers it as sufficiently proven that before the filing date of EP08732818.3 D1 and D2 were validly assigned to Pharmasset Inc. The Patentee is thus formally entitled to claim priority to these applications under Article 87(1) EPC as successor-in-title.

Entitlement to priority of D1 on substantive grounds

- 25.1 The argumentation concerning the entitlement to the priority of D1 on substantive grounds is similar to the discussion on Art 123 (2) for the main request with the following differences: example 25 is not present in the first priority D1; IX-25-2 is on page 195 of D1; formula IX is on page 187 of D1; formula II is on page 64 of D1; the passage corresponding to pages 99-100 in D13 is on pages 63-64 of D1; the passage corresponding to page 20 of D13 about the P atom is not found in D1. The remaining difference with the previous discussion is that disclosure is required for the racemate, not for the enantiomers.
- 25.2 The compound of claim 1 is a racemate with two chiral centres whereby the chirality is defined at the carbon atom and not at the phosphorus atom. The specific indication is IX-25-2 where neither chiral centre is defined. In order to arrive at the racemate form IX-25-2 it is necessary to make at least two choices: firstly to define chirality at one, but not both, of the two possible sites and secondly to specify the chirality at that particular site. This represents a selection from within two lists. On account of this and the general teaching of T1046/97 which is taken to represent the standard way of understanding the scope of an application which discloses compounds and has a reference to the (non-exemplified) enantiomers, the OD considers that the auxiliary request 1 is not entitled to the priority of D1.

Entitlement to priority of D2 on substantive grounds

- 26 D2 specifically discloses example 25 (see e.g. page 1) which is the racemate. None of the parties contested the entitlement of the patent to D2. The effective priority date of the auxiliary request 1 is therefore that of D2, namely 24.10.2007

Public Availability of D5

- 27 The Patentee objected to the admissibility of D86A and D86B as not being relevant and because from the dates it was clear that O1 could have filed these documents earlier. If D86A and D86B were taken into account D48C should also be admitted. O1 explained that he had taken the submission date of the thesis as publication date and only later obtained evidence D86A and D86B and filed these documents when the Patentee challenged the publication date.
- 27.1 Since D86A, D86B and D48C are *prima facie* highly relevant for the question of when D5 was made available to the public, the OD decides to admit these documents under Article 114(2) EPC.
- 27.2 A university thesis such as D5 can normally be considered to be made available to the public if it is shelved, catalogued or otherwise prepared in a public library so that a member of the public can acquire knowledge of the document and access, reproduce, distribute, transmit or otherwise exploit the information content without restriction (see, for example, decisions T 381/87; T 314/99; T 834/09).
- 27.3 The burden of proof lies with O1 as the party claiming that the information in question was made available to the public on a certain date. The standard of proof to be applied is the "balance of probabilities" (see, for example, decision

T 729/91) and not the stricter standard of “beyond reasonable doubt” or “up to the hilt” since both O1 and the Patentee had access to relevant evidence. The division must thus decide what was more likely than not to have happened.

- 27.4 From D86A, D86B and D48C the OD is satisfied – and this does not seem to be at dispute between the parties – that D5 was catalogued in a library in March 2007. The decisive questions are, though, whether it was subject to a bar on access, what the effect of such bar on access was and during which period it applied.
- 27.5 Page 3 of D5 suggests that the thesis was subject to a bar on access “*approved by the Graduate Development Committee*”. O1 did not clearly state whether it challenged the existence of a bar on access *per se* or only that such bar would have prevented public availability (see below). The only evidence for the existence of an *approved* bar on access is the e-mail dated 12 November 2015 (15:21) in D48B. While the e-mail is addressed to Amanda Simons, the addressee in the text seems to be the author of the thesis (“*Hi Plinio*”). During oral proceedings the Patentee confirmed that the text of the e-mail had been copied into D48B from a different source. In general the OD does not attribute significant evidential weight to a statement copy-pasted from a different unknown source and where in addition the original request to which this information was sent in reply is missing. On the other hand, the OD was not presented with any evidence that approval of a bar on access was subject to any special restrictive conditions and would only exceptionally be granted. On this basis the OD considers it more likely than not that Dr. Perrone’s request for a bar on access had been approved and that D5 was therefore subject to a bar on access.
- 27.6 Regarding the effect of the bar on access, O1 argued supported by D86B that it would not have prevented at least “*consultation within the library*”. Page 3 of D5 mentions that “*photocopying and inter-library loans*” would not be allowed before expiry of the bar on access but does not explicitly mention consultation within the library. This is challenged by the Patentee on the basis of D48B and D48C according to which the thesis would have been held back (according to D48B “*by the school*”; according to D48C “*by the cataloguing office*”) and would not have been added to the library stock until expiry of the bar on access. The OD is thus presented with conflicting evidence and has to

decide what was more likely to have been the case. In this regard the OD notes that neither the enquiries nor the replies in D86B mention the bar on access. It is thus not clear whether in their reply the library staff was aware of the bar on access or not. The information was provided by the cataloguing department ("*This query was passed to our cataloguing department...*"; "*Our cataloguing department have confirmed...*") but the evidence clearly shows that cataloguing on the one hand and availability for consultation on the other have to be distinguished. This is confirmed by D86A concerning the cataloguing code and date which does not mention the bar on access either. The statements made on the basis of the cataloguing date of the thesis are thus of limited evidential weight for the question of actual availability for consultation. The OD furthermore considers that, although there may be different reasons for a bar on access, the main purpose will be to delay the public availability of commercially sensitive or confidential information. This purpose could not be achieved if the bar would just prevent photocopying and inter-library loans but allow university students and staff or even any member of the public to consult the thesis within the library and freely transmit or otherwise exploit the information. On the balance of probabilities the OD thus concludes that the bar on access prevented that a member of the public had access to anything more than the thesis title before expiry of the bar.

- 27.7 Concerning the precise time period during which the bar on access was in place, O1 argued that according to the Patentee's own evidence D48B and D48C the bar on access only took effect on 26 March 2007 so that at least between the cataloguing on 6 March 2007 and the start of the bar the thesis was publicly available. The OD does not agree. It is much more likely that 26 March 2007 is the date on which the Graduate Development Committee had taken its decision to approve the bar and informed Dr. Perrone. 26 March 2007 therefore rather marks the start of the *period* for which the bar was approved than the first day on which public accessibility was restricted. It would go against common experience and the purpose of a bar on access if a thesis were freely available while the request is examined by the competent body. Regarding the exact duration of the bar, the OD was only presented with D48B to which, for the above-mentioned reasons, it does not attribute significant evidential weight. O1 did not provide any evidence concerning the date of expiry of the bar on access. Neither was the OD presented with any information on how long a bar on access would last normally, how long in exceptional cases and whether there was a maximum period for a bar on access. In view of the purpose of a bar on access as mentioned above it

appears more likely that a bar would have lasted a longer period of some months or even one to two years rather than only a short period of some days or a few weeks. Since there was a bar on access, because it is likely that it lasted at least for a couple of months and since O1 as the party carrying the burden of proof did not provide any evidence that it lasted less than 7 months and expired before 24 October 2007, the OD considers on the balance of probabilities that the bar on access was still effective on the earliest valid priority date of the patent-in-suit.

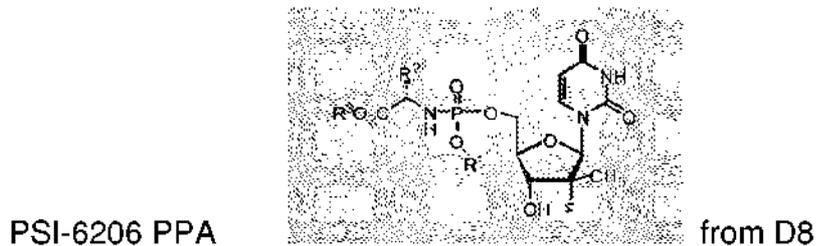
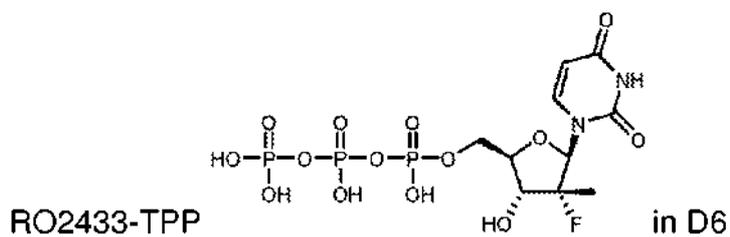
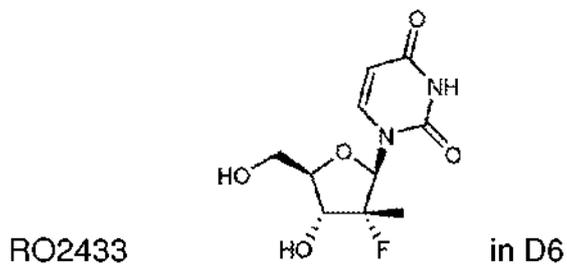
- 28 For the above-mentioned reasons D5 does not form part of the state of the art under Article 54(2) EPC for the present proceedings.

Article 56 EPC inventive step

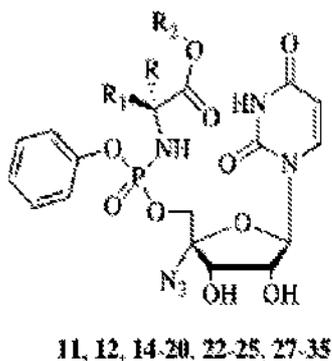
Expert oral intervention

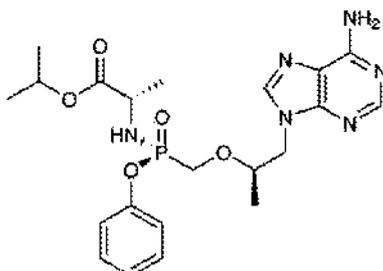
- 29 In the context of the inventive step submissions, the Patentee requested an intervention of the expert Dr. Ray which was objected to by several opponents. Dr. Ray was announced as an accompanying person and not as an expert in the sense of Article 117(1)(e) EPC. The principles set out in decision G 4/95 (OJ 1996, 412) are controlling. In the Patentee's submission of 3 August 2016 the subject-matter of the proposed oral submissions was specified as concerning the declaration D45 / D45A. However, the Patentee's request for Dr. Ray's intervention during oral proceedings did not relate to his declaration and the OD, having read it, also sees no need for oral clarifications in this regard. Rather, the Patentee's request concerned an intervention on the feasibility of explaining SARs in a different technical context. The submission of 3 August 2016 did not specify the proposed intervention in this regard, but referred in very general terms to "*the technical teaching of any of the cited documents and a skilled person's knowledge at the relevant date*". The OD did not consider a submission by Dr. Ray on the proposed question to be *prima facie* relevant and the Patentee was not restricted in its right to be heard as it could present arguments in this regard itself. For these reasons the OD exercised its discretion by not allowing an oral intervention by Dr. Ray.

30 The following formulae are relevant for considering inventive step:

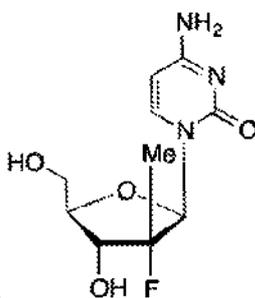


D7 compound 15 in the formula below R = H, R₁ = methyl and R₂ = isopropyl



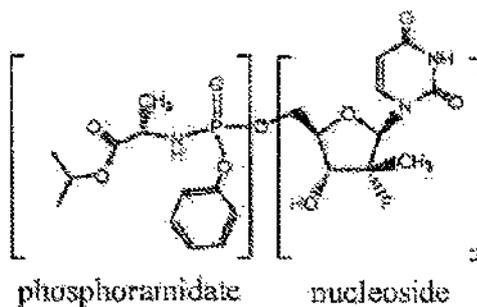


GS 7340 in D18



PSI-6130 in D6 and D8

30.1 The claimed compound has two components, a phosphoramidate (PPA) and a nucleoside as shown in this formula.



The nucleoside is made up of a sugar moiety (2' deoxyribose) attached to a uracil base.

Closest Prior Art

- 31 The Opponents argued that the 1st auxiliary request lacked an inventive step and gave their arguments for starting from D6 (O6/O10), D7 (O1, O3, O7) or D8 (O2, O4/O5, O8, O9) as the closest prior art.
- 31.1 It is established practice of the Boards of Appeal that the closest prior art for assessing inventive step is normally a prior art document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common, i.e. requiring the minimum of structural modifications.
- 31.2 Claim 1 of Auxiliary request 1 is a compound claim, the objective or purpose as HCV replication inhibitors is disclosed in the description.

D8 as the closest prior art

- 32 The OD considers that D8 is the closest prior art as it aims at the same purpose and has structurally the most features in common with the compound of auxiliary request 1. More precisely, D8 teaches that selected PPAs are potent HCV inhibitors, show no cytotoxicity and have good stability under stimulated gastrointestinal conditions.
- 32.1 D6 and D7 are prior art documents which also refer to HCV inhibition, but are structurally more remote. In the case of the formula PSI-6206 PPA in D8 the nucleoside is the same as the nucleoside in the patent in suit and the PPA component only differs from the PPA in the patent in suit in that the R¹-R³ groups have not been defined. As can be seen in the formulae in point 30, D6 discloses compounds without a PPA while D7, see the formula in point 30, discloses compounds with a significant difference in the nucleosides on account of the substitution on the ribose ring.

Technical problem

- 32.2 Starting from D8 as the closest prior art means starting from PSI-6206 PPA. This formula differs from the racemate in that it has substituents R¹-R³ which are not defined. There is literally no possibility in D8 of determining what these substituents could be. In the case of the patent in suit these groups are

alkyl (methyl or isopropyl) or phenyl. The skilled person is obliged to determine the most likely possibilities of the undefined R^1 - R^3 substituents. While D8 (see the SAR results in Tables 1-4) indicates that structural variation occurs for R^1 - R^3 there is no information at all as to which possibilities are encompassed and whether or not this includes alkyl or phenyl. PSI-6206 PPA in D8 must be seen as an incompletely defined and open-ended Markush formula at 3 positions of the PPA part of the molecule.

- 32.3 In view of this prior art the objective technical problem is the provision of alternative HCV inhibitors.

Obviousness

- 33 The Opponents have argued that the level of evidence in the tables 1 to 6 of D8 is such that the skilled person would have tried different suitable groups R^1 - R^3 with a reasonable expectation of success. The OD considers that for a defined group of substituents this argumentation might apply, but that in D8 there is no limitation at all in terms of R^1 - R^3 so that there is no larger group from within which a skilled person could make a considered choice with a reasonable expectation of successfully choosing suitable substituents.
- 33.1 D8 on its own is an incomplete teaching since it is impossible to determine what R^1 - R^3 could be. The OD decided that D8 on its own does not therefore provide sufficient information to lead the skilled person to the racemate in an obvious way.
- 33.2 The Opponents considered that the question of which substituents should be used was already answered in D7 where example 15 has the same PPA as the patent in suit and is one of the most active compounds. In addition it was argued that there is a clear teaching in D8 about the need to bypass the first phosphorylation step and the aspects linked to drug potency and safety. These points are said to lead to the conclusion that the skilled person would seek to optimise R^1 - R^3 and that this could be achieved without inventive effort. The Opponents consider that D7 provides a clear indication as to what the undefined substituents in D8 should be and consider that a simple combination of the best values from D7 would be combined with D8.

- 33.3 The Patentee has argued that every known variation of a group corresponding to R¹-R³ in D8 would lead the skilled person to believe that different moieties are the best candidates and stated that the prior art, particularly D7, suggested that there at least 10 different known residues for R¹, at least 25 for R² and at least 17 for R³. Even if each of those choices would have been considered the skilled person would still not have been able to predict the best combination thereof, because D8 made clear that every time one of R¹-R³ is modified, significant SAR differences were obtained for the activity and toxicity of the compounds. The OD considers that compounds with more than one difference in terms of R¹-R³ will have even more unpredictable levels of activity and toxicity. D7, page 1843 "*...matrix based optimization of amino acid and ester functions may be preferred over stepwise approaches*" reinforces this viewpoint. D8 shows that structural variation leads to pronounced differences in activity. Compounds indicated in Table 3 of D8 as being more active may be more toxic according to Table 5. Each of the moieties R¹-R³ affects the potency and toxicity to a different extent. The skilled person could not simply select a best possible combination of substituents as the tables in D8 refer to compounds by number. There is no explanation of which number corresponds to which compounds. It is not therefore possible to deduce from D8 which specific values of R¹-R³ have which properties.
- 33.4 Independently of the question of whether or not the D7 provides an indication to the exact substituents for R¹-R³ the first question to be resolved is whether or not the skilled person would indeed have combined D8 and D7. The compounds of D7 differ from D8 and the claimed compounds in that the deoxyribose sugar ring is substituted by a 2'-Me, 2'-F, 4-H in the racemate of D8 and is 2-OH, 2'-H, 4-N₃ in D7. This is a significant structural difference. It is considered that D43 gives a strong indication (see especially points 5-5.3) that the skilled person would not have considered modifying the known nucleosides of D7 by adding the 2'-Me, 2'-F substituents. The skilled person seeking to modify D7 could have considered making changes to the basic structure of compound 15 of D7 in terms of substituents on the oxygen atoms in the PPA, but would not have been led to make major modifications to the deoxyribose substituents. There does not appear to be any evidence to unambiguously show the equivalence of these two deoxyribose systems. The major difference in the substitution pattern on the ribose ring must be

considered to lead to unpredictable quantitative and/or qualitative effects. In addition D7 refers to D38 (see reference 6 on page 1840). The skilled person would be taught by D38, discussion section on page 3797, to keep the 4'-substituted nucleoside derivative as other nucleosides were said to have limited selectivity, which would be seen as a warning about toxicity. D38 provides an additional indication why the skilled person would not consider methyl/fluoro-substituted nucleosides as alternative nucleosides. The OD considers that the pronounced difference in the ribose substituents and the lack of an indication in D7 that variation in terms of these substituents is possible, means that the skilled person would not have combined D7 with D8.

- 33.5 As well as D8 other documents were cited as providing pointers to the undefined R¹-R³ substituents in D8 and these include D16: pp372-374, D18: p.1899, Tables 1 and 5 for GS7340; D37: p.3580; D20: p.7219, Table 2, see 4ab. These are in particular said to provide a particular indication that the isopropyl and phenyl substituents are the most suitable. The OD considers that the prior art indicates that one cannot simply extrapolate the SAR of PPAs (D7: p.1843, "This reinforces our earlier conclusion that a separate ProTide motif optimization is need for each nucleoside analogue versus a given target" "...quite distinct SARs emerge from this family"; D37: first page "unique in vivo properties"; D20: p.7219, "very significant differences...", p7220 "complex metabolic SAR"). GS7340 in D18 has the same PPA as the claimed compound in the patent in suit, but this moiety is attached to a structurally unrelated parent compound. Additionally D18 refers in its title to HIV and is therefore not readily apparent that it is relevant for the field of HCV inhibition. The said documents are less structurally close than D7, since they are more structurally remote from the patent in suit in terms of the nucleoside or the specific substitution on the nucleoside. The OD considers that the skilled person would not have combined them with D8.
- 33.6 The OD does not consider that it can be said that starting from D8 the skilled person would have been led to combine it with D7 or any of D18, D20 or D37. The racemate is seen as a non-obvious solution to the problem of providing further potent HCV inhibitors.
- 34 D7 as closest prior art

- 34.1 O1 considers D7 as the closest prior art and that the skilled person would have combined compound 15, the structurally closest compound in D7, with the nucleoside of D8.
- 34.2 It is noted that in D7 in terms of activity the best phenyl-substituted compound, for instance as measured by the lowest EC50 of the compound, was a PPA prodrug 17 which had a benzyl ester and not the isopropyl ester group of the compound claimed in the patent. Moreover D7 teaches, that the most active compound was in fact a naphthyl-substituted PPA. Selecting compound 15 of D7 for its PPA substituents is therefore based on hindsight if obviousness of substituents is based on the activity of the compounds.
- 34.3 D7 on its own also does not render the auxiliary request 1 obvious because there was no indication to use the specific nucleoside component of the patent in suit. The OD's opinion that the PPA of D7 would not be combined with the nucleoside of D8 is given above. The reasoning that the skilled person starting from the closest prior art D7 would not have combined it with D8 applies for the same reasons that the skilled person starting from the closest prior art D8 would not have combined it with D7. Given the pronounced difference in the riboside the OD does not consider that it can be said that starting from D7 the skilled person would have been led to change it by incorporating the riboside from D8. The racemate is seen as a non-obvious solution to the problem of providing further HCV inhibitors.
- 35 D6 as closest prior art
- 35.1 O6/10 considered that in the closest prior art, D6, the strategy of aiming at the problem of getting high amounts of the active substance into the cell is fully disclosed. The penultimate page of D6 pointed to the previously existing problem to transfer the inhibiting compound into the cell and to the solution. A clear pointer to a prodrug as the solution was said to be found in the reference in D6 to D7 (i.e. reference (21) on page 29819), which described in detail the nature of the prodrug, and also describes as exceptional those compounds 14, 15 and 17 having the 2-butyl, isopropyl and benzyl residues shown at the places in the claimed compound of the patent. Any additional effect achieved was considered as a bonus effect which would not establish an inventive activity. The very good results achieved in D7 (p.1843, Table 1)

for compounds having methyl, isopropyl and phenyl groups in the PPA moiety would have generated a reasonable expectation of success to consider any of these prodrugs as an obvious modification of the compounds of D6. In the light of the data in D55, O6/10 considered the claimed compounds no better than a skilled person would have expected on the basis of D7 (p.1843).

- 35.2 The Patentee referred to the metabolism of cytidine nucleosides (D6, Figure 7). The cytidine derivatives were disclosed as highly potent drugs which efficiently enter the cell. The data did not suggest a particular problem and therefore the difference between D6 and invention was in the specific way in which the compound was delivered to cells. The Patentee referred to the data in D55 (Table 1; Figure 2) to show that a huge difference existed in the generation of triphosphates when comparing the PPA of the opposed patent with the compounds of D6. D6 (Table 1) did not provide a hint to the solution since the cytidine derivative was presented as being about 2x more active than the uridine derivative. Also two different active species were obtained as uridine and cytidine would bind at different areas. Starting from D6, the skilled person would thus have looked at cytidine but would not be led to uridine derivatives. There was no reason for the skilled person to combine D6 and D7 because D6 already teaches compounds which were sufficiently good. Even if he did so, he would still have to work out the specific methyl, isopropyl and phenyl groups in the PPA moiety for which no pointer existed.
- 35.3 D6 indicates that the cytidine derivative PSI-6130 appears to be more active than the uridine compound R02433 (see Table 1). The final paragraph on page 29819 of D6 shows that R02433-TP is more stable than PSI-6130-TP. It is not therefore unambiguously clear that the skilled person would take D6 as giving a clear teaching towards using uridine derivatives rather than cytidine derivatives. The OD is therefore of the opinion that the auxiliary request 1 is not obvious in the light of D6 alone because D6 does not provide any indication as to the correct PPA moiety. In order to arrive at the claimed racemate from D6 it is necessary to take R02433 which has the correct nucleoside, but no PPA and modify it with the PPA known from D7, compound 15. This requires taking half a compound from D6 and combining it with half a compound from D7- there is no overlapping structural component.

- 35.4 The OD further notes that compound 15 is not the most active compound in Table 1 of D7 since this is compound 17 which has a benzyl group instead of an isopropyl group so that D7, compound 17 would have been a more obvious starting point. The compounds of D7 differ from the compounds of D6 and the patent in suit in that the deoxyribose sugar ring is substituted by a 2'-Me, 2'-F, 4-H in the racemate or D6 and is 2-OH, 2'-H, 4-N₃ in D7. This is a significant structural difference. It is considered that D43 gives a strong indication (see especially points 5-5.3) that the skilled person would not have considered modifying the known nucleosides of D7 by adding the 2'-Me, 2'-F substituents. The skilled person seeking to modify D6 could have considered making changes to the basic structure of PSI-6130 or RO2433 in terms of substituents on the oxygen atoms (see e.g. PPAs), but would not have been led to make major modifications to the deoxyribose substituents. There does not appear to be any evidence to unambiguously show the equivalence of these two deoxyribose systems. The major difference in the substitution pattern on the ribose ring must be considered to lead to unpredictable quantitative and/or qualitative effects. It is therefore not unambiguously evident that the skilled person seeking to provide further HCV inhibitors would have combined the teachings of D6 and D7. The skilled person could have considered taking the nucleoside known from D6 and combining it with one of the preferred PPAs from D7, but there was no clear and convincing expectation that this would lead to compounds which solved the given problem. If D6 is taken as the closest prior art the racemate is seen as a non-obvious solution to the problem of providing further HCV inhibitors.

Inventive Step conclusion

- 36 Since the auxiliary request 1 is inventive when starting from any of D6-D8 the said request is considered to meet the requirements of Art. 56 EPC.
- 37 The first auxiliary request 1 meets the requirements of the EPC.
- 38 The Patentee submitted an amended description page 9 where the only changes were the deletion of clauses 2,3, 5 and 6 corresponding to the subject matter of claims 2, 3, 5 and 6 of the granted patent and the renumbering of previous clause 4 as clause 2. No objections were made by

any parties under Art. 84 or 123 (2), (3) EPC. The amended description is therefore allowed and is considered to harmonise the description with the scope of the claims.

- 39 In view of the foregoing, the OD concludes that, taking into consideration the amendments made by the Proprietor of the European patent during the opposition proceedings, the patent and the invention to which it relates according to the auxiliary request 1 meet the requirements of the EPC.

Design, Synthesis, and Antiviral Activity of 2'-Deoxy-2'-fluoro-2'-C-methylcytidine, a Potent Inhibitor of Hepatitis C Virus Replication

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The pyrimidine nucleoside beta-D-2'-deoxy-2'-fluoro-2'-C-methylcytidine (**1**) was designed as a hepatitis C virus RNA-dependent RNA polymerase (HCV RdRp) inhibitor. The title compound was obtained by a DAST fluorination of *N*⁴-benzoyl-1-(2-methyl-3,5-di-*O*-benzoyl-β-D-arabino-furanosyl)cytosine (**6**) to provide *N*⁴-benzoyl-1-[2-fluoro-2-methyl-3,5-di-*O*-benzoyl-β-D-ribofuranosyl]cytosine (**7a**). The protected 2'-*C*-methylcytidine (**7c**) was obtained as a byproduct from the DAST fluorination and allowed for the preparation of two biologically active compounds from a common precursor. Compound **1** and 2'-*C*-methylcytidine were assayed in a subgenomic HCV replicon assay system and found to be potent and selective inhibitors of HCV replication. Compound **1** shows increased inhibitory activity in the HCV replicon assay compared to 2'-*C*-methylcytidine and low cellular toxicity.

Introduction

Hepatitis C virus infection is a major health problem that leads to chronic liver disease, such as cirrhosis and hepatocellular carcinoma, in a substantial number of infected individuals. Once infected, about 20% of people clear the virus, but the rest can harbor HCV the rest of their lives. Ten to 20% of chronically infected individuals eventually develop liver-destroying cirrhosis or cancer. The current standard of care for chronic hepatitis C is combination therapy with an interferon-α and ribavirin. Studies have shown that more patients with hepatitis C respond to pegylated interferon-α/ribavirin combination therapy than to combination therapy with unpegylated interferon-α. The overall response rate to treatment, defined as loss of HCV from serum 6 months after completion of treatment, is 40%. Because of the low response rates as well as toxic side effects and unsustainable viral load reductions, these therapies are inadequate. Moreover, there is no established vaccine for HCV, and there is an urgent need for improved therapeutic agents that effectively combat chronic HCV infection.¹

The nonstructural protein NS5B has been characterized as an RNA-dependent RNA polymerase (RdRp) that is required for viral replication. This polymerase is considered to be an essential component in the HCV

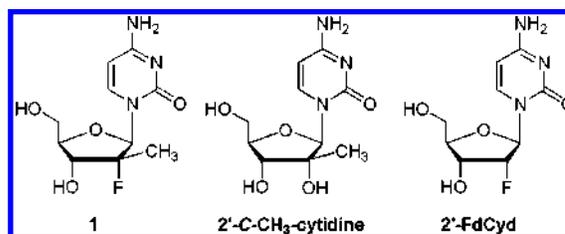


Figure 1. Structures of 2'-deoxy-2'-fluoro-2'-*C*-methylcytidine (**1**), 2'-*C*-methylcytidine, and 2'-deoxy-2'-fluorocytidine (2'-FdCyd).

replication complex and therefore is an ideal target for drug discovery. Recently, several 2'-modified nucleoside analogues with potent inhibitory activity against the HCV NS5B polymerase have been identified. Among the most potent compounds in this class are 2'-deoxy-2'-fluorocytidine (2'-FdCyd)² and 2'-*C*-methyl nucleosides (Figure 1).^{3–5} Despite the potent HCV inhibition of 2'-FdCyd, its therapeutic potential as an antiviral agent is diminished due to a lack of selectivity between host cells and the viral target. 2'-FdCyd triphosphate has been demonstrated to be a substrate for both RNA and DNA polymerases.^{6,7} Here we describe the synthesis and biological activity of 2'-deoxy-2'-fluoro-2'-*C*-methyl cytidine (**1**) as a potent anti-HCV agent.

Chemistry

For the synthesis of 2'-deoxy-2'-fluoro-2'-*C*-methylcytidine (**1**), *N*⁴-benzoyl-1-(2-methyl-3,5-di-*O*-benzoyl-β-D-arabino-furanosyl)cytosine (**6**) was chosen as the key intermediate and was prepared in approximately 20% yield in six steps from cytidine (Scheme 1).⁸ Briefly, selective benzylation of cytidine with benzoic anhydride in DMF,⁹ followed by treatment with TIDPSCl₂ in pyridine, afforded *N*⁴-benzoyl-3',5'-*O*-(tetraisopropyl-disiloxane-1,3-diyl)cytidine (**2**).¹⁰ Oxidation of the 2'-alcohol to the 2'-ketone derivative (**3**) was achieved with

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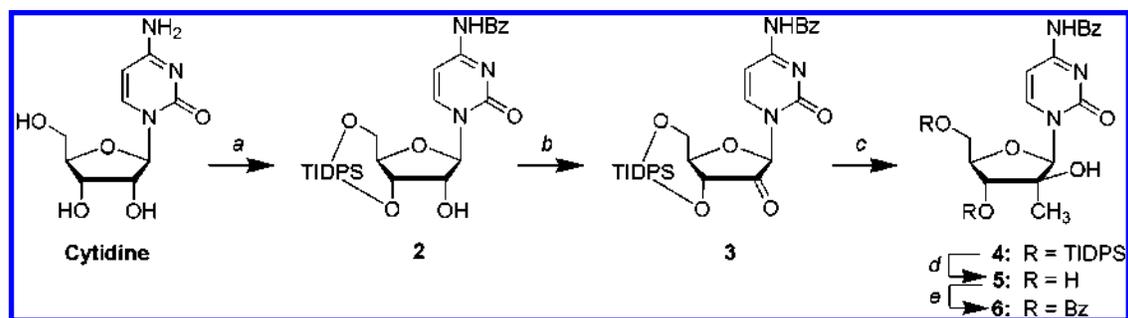
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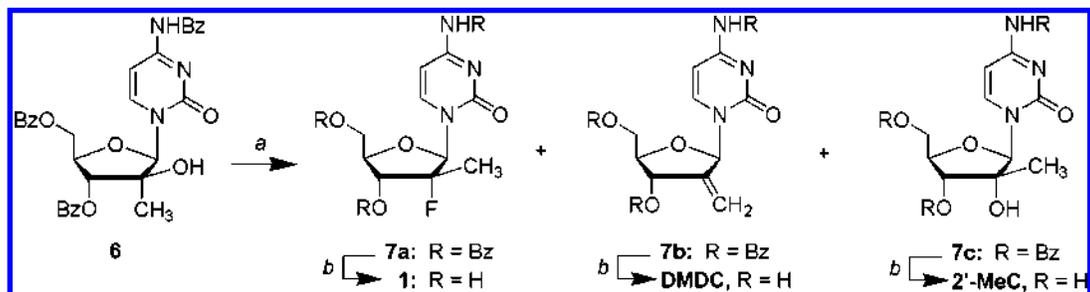
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Scheme 1^a

^a Reagents and conditions: (a) (i) Bz₂O, DMF, room temp, (ii) TIDPSCl₂, DMF; (b) DMSO, TFAA, TEA, -15 °C; (c) MeLi, -78 °C; (d) 1 M TBAF, concd HOAc, rt; (e) BzCl, pyridine, rt.

Scheme 2^a

^a Reagents and conditions: (a) DAST, toluene, -20 °C to rt; (b) MeOH/NH₃, rt.

Table 1. Fluorine-Coupled ¹H and ¹³C NMR Chemical Shifts, Multiplicities, and *J* Values for Compounds **1**, **7a**, **8**, and **9**^a

compd	2CH ₃	H-1/C-1	C-2	H-3/C-3
1	δ 1.17, d, ³ J _{H-F} = 22.3 δ 16.6, d, ² J _{C-F} = 25.9	δ 6.07, d, ³ J _{H-F} = 18.9 δ 88.6, d, ² J _{C-F} = 37.4	δ 101.2, d, ¹ J _{C-F} = 180.1	overlapping mult. δ 70.5, d, ² J _{C-F} = 18.3
7a	δ 1.49, d, ³ J _{H-F} = 22.4 δ 17.4, d, ² J _{C-F} = 25.2	δ 6.52, d, ³ J _{H-F} = 18.0 δ 91.2, d, ² J _{C-F} = 42.0	δ 100.2, d, ¹ J _{C-F} = 187.7	δ 5.56, dd, ³ J _{H-F} = 20.7 δ 72.4, d, ² J _{C-F} = 16.0
8	δ 1.39, d, ³ J _{H-F} = 22.3 δ 17.3, d, ² J _{C-F} = 25.1	δ 6.17, d, ³ J _{H-F} = 19.3 δ 90.7, d, ² J _{C-F} = 44.2	δ 99.9, d, ¹ J _{C-F} = 186.2	δ 5.49, d, ³ J _{H-F} = 21.2 δ 72.7, d, ² J _{C-F} = 16.1
9	δ 1.35, d, ³ J _{H-F} = 22.3 δ 16.9, d, ² J _{C-F} = 25.2	δ 6.13, d, ³ J _{H-F} = 18.9 δ 90.6, d, ² J _{C-F} = 44.0	δ 102.1, d, ¹ J _{C-F} = 180.1	overlapping mult. δ 72.5, d, ² J _{C-F} = 17.6

^a NMR spectra were recorded at 30 °C (400 MHz) in DMSO-*d*₆ for compound **1**, CDCl₃ for compound **7a**, CD₃OD + CDCl₃ for **8**, and CD₃OD for **9** with concentrations of ~40 mg/0.75 mL. *J* values are in Hz.

trifluoroacetic anhydride/DMSO under Swern oxidation conditions.¹¹ Purification of compound **3** by silica gel chromatography followed by crystallization from petroleum ether-CH₂Cl₂ provided a white solid that was stable when stored at room temperature with minimal atmospheric exposure.¹² Treatment of the 2'-ketone (**3**) with methyllithium at -78 °C in diethyl ether gave exclusively the protected 1-[2-C-methyl-3,5-O-(tetra-isopropylidisiloxane-1,3-diyl)-β-D-arabinofuranosyl]cytosine (**4**).¹³ The 3',5'-silyl protecting group was removed with TBAF/acetic acid and replaced with benzoyl protecting groups to provide compound **6**.

The fluorination of tertiary alcohols using DAST has been reported, but the stereochemistry of such transformations is substrate-specific and often unpredictable. For instance, Yang et al. reported that the DAST fluorination of a tertiary alcohol in 2-bromomethyl-DL-*myo*-inositol proceeds with retention of configuration.¹⁴ Wachtmeister et al. obtained a 4-fluoro-1-cyclopentanol containing a tertiary fluorine in 25% yield using DAST as a fluorinating reagent, and this transformation proceeded with inversion of configuration.¹⁵ Furthermore, dehydrations or eliminations, rearrangements, and ring contractions are often pervading problems in the DAST fluorination of highly functionalized molecules.¹⁶

Upon treating **6** with DAST in toluene or dichloromethane, a clean mixture of three products (**7a**–**7c**) in 15–20% yield for each compound was obtained (Scheme 2). The desired transformation of **6** to *N*⁴-benzoyl-1-[2-fluoro-2-methyl-3,5-di-*O*-benzoyl]cytosine (**7a**) proceeded with inversion of configuration and was stereospecific; no diastereomeric *N*⁴-benzoyl-1-[2-fluoro-2-methyl-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl]cytosine was detected in the crude reaction mixture. The presence of the tertiary fluorine at the 2' position in **7a** was confirmed by the ¹H and ¹³C NMR multiplicities and coupling constants (Table 1), whereas the stereochemistry of the fluorination was determined by nuclear Overhauser enhancement ¹H NMR difference spectroscopy (Figure 2).

Analysis of the ¹H NMR spectrum of compound **7a** revealed three distinct multiplicities due to H–F coupling: a doublet at δ 1.49 (2'-CH₃), a doublet of doublets at δ 5.56 (H-3'), and a doublet at δ 6.52 (H-1'). Irradiation of the H-3' resonance resulted in a relatively large enhancement of both the H-5' signal (4.8%) and the 2'-methyl signal (5.9%), while irradiation of the 2'-methyl signal resulted in an enhancement of both H-3' (3.4%) and, to a lesser extent, H-1' (1.7%). Deprotection of **7a** using methanolic ammonia provided the title compound,

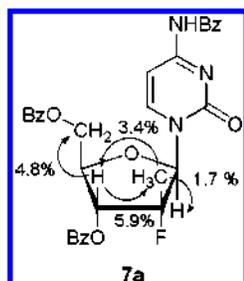


Figure 2. ^1H NMR NOE correlations of compound **7a**.

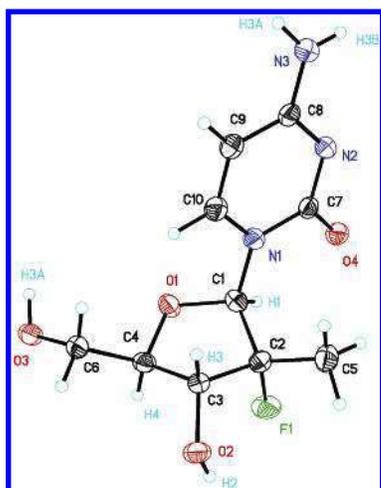
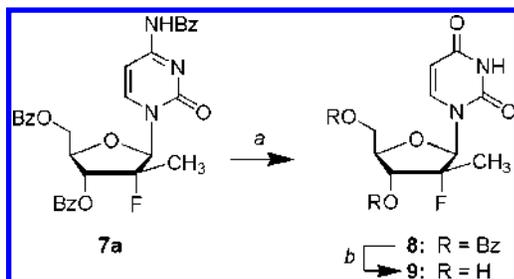


Figure 3. ORTEP drawing of 2'-deoxy-2'-fluoro-2'-C-methylcytidine (**1**).

Scheme 3^a



^a Reagents and conditions: (a) 80% HOAc, reflux; (b) MeOH/ NH_3 , rt.

1, whose structure was unambiguously confirmed by X-ray crystallography and revealed the expected 3'-endo conformation (Figure 3).

The degradation enzymes cytidine deaminase (CDA) and deoxycytidine monophosphate deaminase (dCMP-DA) are responsible for the *in vivo* metabolic conversion of cytidine or cytidine monophosphate to uridine. To facilitate future *in vivo* studies of compound **1**, 2'-deoxy-2'-fluoro-2'-C-methyluridine (**9**) was prepared from **7a** by deamination in refluxing 80% acetic acid followed by debenzoylation using methanolic ammonia (Scheme 3).

Results and Discussion

The novel pyrimidine nucleoside analogues 2'-deoxy-2'-fluoro-2'-C-methylcytidine (**1**) and 2'-deoxy-2'-fluoro-2'-C-methyluridine (**9**) were tested for anti-HCV activity in both a cell-based quantitative real-time RT-PCR assay and surrogate bovine viral diarrhea virus (BVDV) assays as previously described (Table 2).¹⁷ The activity and cytotoxicity profiles of 2'-C-methylcytidine and 2'-deoxy-2'-fluorocytidine (2'-FdCyd) are included for com-

Table 2. Anti-HCV Activity and Cellular Toxicity of Compounds **1**, **9**, 2'-C-Methylcytidine (2'-C-MeCyd), and 2'-Deoxy-2'-fluorocytidine (2'-FdCyd)

compound	cpBVDV ^a (MDBK cells)		HCV replicon ^b	
	EC ₉₀ (μM) ^b	CC ₅₀ (μM)	EC ₉₀ (μM)	CC ₅₀ ^c (μM)
1	>100	>100	5.40 \pm 2.6	>100
9	>100	>100	>100	>100
2-C-MeCyd	2.30 \pm 0.1	>100	19.0 \pm 5.7	>100
2-FdCyd	>100	>100	6.50 \pm 1.6	>100

^a cpBVDV = cytopathic BVDV. ^b 96 h, average of at least four experiments. ^c MTS CC₅₀ was determined in a 4-day assay using the Celltiter 96 nonradioactive cell proliferation assay from Promega (Madison, WI).

parison and indicate that compound **1** demonstrated a similar potency as 2'-FdCyd in the HCV replicon assay. Dynamic profiling of the cell growth in this replicon assay revealed no cytostasis for compound **1** at the HCV replicon EC₉₀ value. As previously reported, 2'-FdCyd, although not cytotoxic, induced cytostasis at the EC₉₀ value.² Additionally, much like 2'-FdCyd, but unlike 2'-C-MeCyd, compound **1** was inactive in the BVDV assays. Compound **9** demonstrated no activity or cytotoxicity in any assay.

Experimental Section

All reagents and anhydrous solvents were purchased from Aldrich or Acros and were used as received. ^1H , ^{19}F , and ^{13}C NMR spectra were obtained with a Varian Unity Plus 400 spectrometer at 400, 376, and 100 MHz, respectively. ^1H and ^{13}C NMR chemical shifts are reported as δ (ppm) downfield with respect to an internal standard of tetramethylsilane, while ^{19}F chemical shifts are reported downfield from an external standard of hexafluorobenzene. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter at the sodium D line (589 nm) in a 1-dm cell. Melting points were determined using an electrothermal digital melting point apparatus and are uncorrected. Atlantic Microlab, Inc. of Norcross, GA provided the elemental analysis.

N⁴-Benzoyl-1-[2-C-methyl-3,5-O-(tetraisopropylidisiloxane-1,3-diyl)- β -D-arabinofuranosyl]cytosine (4**).** Compound **3** (37.6 g, 64 mmol) was dissolved in anhydrous Et_2O (800 mL) under argon and cooled to -78°C with stirring. To this solution was added MeLi (103 mL, 1.6 M in Et_2O) dropwise over 1 h. After stirring for an additional 2 h, the reaction mixture was quenched by dropwise addition of 1 M NH_4Cl (165 mL). Upon warming to room temperature, the mixture was diluted with EtOAc (600 mL) and H_2O (130 mL). The organic phase was separated, washed with H_2O (1×130 mL), dried (Na_2SO_4), and the concentrated to dryness to give a brown foam (42.5 g, >100%) that was used without further purification. An analytical sample was obtained by silica gel chromatography eluting with 2:1 Et_2O –petroleum ether: $[\alpha]_{\text{D}}^{25} +52.2^\circ$ (*c* 1, CHCl_3); ^1H NMR (CDCl_3) δ 0.96–1.15 (m, 24H), 1.60 (s, 3H), 3.81 (dt, 1H, $J = 1.9, 9.2$ Hz), 4.02 (dd, 1H, $J = 2.5, 13.7$ Hz), 4.17–4.23 (m, 2H), 5.85 (s, 1H), 7.50–7.54 (m, 2H), 7.60–7.64 (m, 2H), 7.91 (d, 2H, $J = 7.3$ Hz), 8.38 (d, 1H, $J = 7.3$ Hz), 8.89 (bs, 1H); ^{13}C NMR (CDCl_3) δ 12.5, 13.0, 13.1, 13.6, 16.9, 17.1, 17.2, 17.4, 17.5, 17.6, 17.7, 20.9, 60.4, 72.9, 81.7, 91.2, 96.5, 127.8, 129.0, 133.2, 145.2, 156.6, 162.5, 166.7. Anal. Calcd ($\text{C}_{29}\text{H}_{45}\text{N}_3\text{O}_7\text{Si}_2$): C, 57.68; H, 7.51; N, 6.96. Found: C, 57.63; H, 7.55; N, 6.82.

N⁴-Benzoyl-2'-C-methyl- β -D-arabinofuranosylcytidine (5**).** Crude **4** (128.0 g, 0.212 mol) was dissolved in THF (1.28 L) and treated with glacial HOAc (23.0 mL, 0.401 mol). To this solution was added tetrabutylammonium fluoride (384 mL, 1 M in THF) at room temperature and stirred for 0.75 h. The mixture was treated with silica gel (750 g) and concentrated to dryness *in vacuo*, and the tan-colored residue was placed onto a silica gel column. Eluting with 1:7 EtOH – CH_2Cl_2 afforded a waxy solid that was preadsorbed onto silica gel

(300 g) and chromatographed as before to give an off-white solid (46.4 g, 61%). Crystallization from aqueous acetone afforded an analytical sample: mp 197–200 °C; $[\alpha]_D^{22} + 132.0^\circ$ (*c* 1, MeOH); $^1\text{H NMR}$ (DMSO-*d*₆) δ 1.20 (s, 3H), 3.62–3.69 (m, 2H), 3.73–3.78 (m, 2H), 5.19 (t, 1H, *J* = 5.4 Hz), 5.25 (s, 1H), 5.52 (d, 1H, *J* = 5.0 Hz), 5.99 (s, 1H), 7.32 (d, 1H, *J* = 5.8 Hz), 7.50 (Ψ t, 2H, *J* = 7.7 Hz), 7.62 (Ψ t, 1H, *J* = 7.3 Hz), 8.00 (d, 2H, *J* = 7.3 Hz), 8.14 (d, 1H, *J* = 6.9 Hz), 11.22 (s, 1H); $^{13}\text{C NMR}$ (DMSO-*d*₆) δ 19.6, 61.3, 77.5, 78.5, 85.2, 88.9, 95.2, 128.5, 132.8, 133.3, 147.3, 154.9, 162.9, 167.4. Anal. Calcd (C₁₇H₁₉N₃O₆·0.5H₂O): C, 55.14; H, 5.41; N, 11.35. Found: C, 55.21; H, 5.47; N, 11.33.

N⁴-Benzoyl-1-(2-C-methyl-3,5-di-O-benzoyl-β-D-arabino-furanosyl)cytosine (6). Compound **5** (46.0 g, 0.127 mol) was dissolved in anhydrous pyridine (200 mL) and the solvent was removed in vacuo. The resulting syrup was dissolved in anhydrous pyridine, cooled to 0 °C under argon with stirring, and treated with BzCl (30.0 mL, 0.250 mol) dropwise over 10 min. After the addition was complete, the ice bath was removed and stirring was continued for 1.5 h. Water (5 mL) was added and the mixture was concentrated to dryness in vacuo. The residue was dissolved in CH₂Cl₂ and washed with saturated NaHCO₃ (1 × 500 mL) and water (1 × 500 mL). The organic layer was dried (Na₂SO₄), filtered, and concentrated to dryness to afford a syrup that was purified by silica gel chromatography eluting with 1:1 EtOAc–hexanes. Compound **6** was isolated as an off-white solid (48.5 g, 67%): $[\alpha]_D^{22} + 9.6^\circ$ (*c* 1, CHCl₃); $^1\text{H NMR}$ (CDCl₃) δ 1.64 (s, 3H), 4.50 (m, 1H), 4.78–4.85 (m, 2H), 5.50 (d, 1H, *J* = 3.4 Hz), 6.42 (s, 1H), 7.44–7.54 (m, 7H), 7.57–7.66 (m, 3H), 7.94 (d, 2H, *J* = 7.8 Hz), 8.05–8.09 (m, 4H), 8.21 (d, 1H, *J* = 7.3 Hz); $^{13}\text{C NMR}$ (CDCl₃) δ 19.9, 64.4, 79.2, 80.6, 81.6, 90.3, 96.7, 128.1, 128.6, 128.8, 128.9, 129.5, 129.9, 130.0, 133.2, 133.5, 134.0, 147.5, 156.0, 162.7, 165.9, 166.6. Anal. Calcd (C₃₁H₂₇N₃O₈): C, 65.37; H, 4.78; N, 7.38. Found: C, 65.59; H, 4.79; N, 7.16.

Reaction of 6 with DAST. To a stirred solution of **6** (2.96 g, 5.20 mmol) in anhydrous toluene (50 mL) was added DAST (1.0 mL, 7.8 mmol) at –20 °C under argon. After the addition was complete, the cooling bath was removed and stirring was continued for 1 h. The reaction mixture was poured into saturated NaHCO₃ (50 mL) and washed until gas evolution ceased. The organic phase was dried (Na₂SO₄), concentrated to dryness, and purified by silica gel chromatography eluting with 1:1:1 EtOAc–CHCl₃–hexanes to afford compound **7a** (0.55 g, 19%) as a white solid, followed by compound **7b** (0.39 g, 14%) as an off-white solid. Elution was continued with 1:1:1 EtOH–EtOAc–CHCl₃–hexanes to afford compound **7c** (0.451 g, 15%) as an off-white solid. Analytical samples were obtained by recrystallization from the indicated solvents.

N⁴-Benzoyl-3',5'-di-O-benzoyl-2'-fluoro-2'-C-methylcytidine (7a): mp 241 °C (CH₂Cl₂–hexanes); $[\alpha]_D^{22} + 82.0^\circ$ (*c* 1, CHCl₃); $^1\text{H NMR}$ (CDCl₃) δ 1.49 (d, 3H, *J* = 22.4 Hz), 4.64 (dd, 1H, *J* = 3.44, 12.9 Hz), 4.73 (d, 1H, *J* = 9.5 Hz), 4.90 (dd, 1H, *J* = 2.4, 12.7 Hz), 5.56 (dd, 1H, *J* = 8.6, 20.7 Hz), 6.52 (d, 1H, *J* = 18.0 Hz), 7.47–7.57 (m, 7H), 7.62–7.71 (m, 3H), 7.89 (d, 2H, *J* = 6.9 Hz), 8.07–8.11 (m, 5H), 8.67 (bs, 1H); $^{13}\text{C NMR}$ (CDCl₃) δ 17.4 (d, *J* = 25.2 Hz), 62.1, 72.4 (d, *J* = 16.0 Hz), 77.7, 91.2 (d, *J* = 42.0 Hz), 97.5, 100.2 (d, *J* = 187.7 Hz), 127.8, 128.6, 128.8, 128.9, 129.2, 129.6, 129.7, 130.3, 133.2, 133.4, 133.8, 134.1, 143.8, 154.6, 162.6, 165.6, 166.1; $^{19}\text{F NMR}$ (CDCl₃) δ 3.9 (m). Anal. Calcd (C₃₁H₂₅FN₃O₇·0.7H₂O): C, 63.74; H, 4.73; N, 7.19. Found: C, 63.71; H, 4.54; N, 7.20.

N⁴-Benzoyl-1-[2-deoxy-2-methylidene-3,5-di-O-benzoyl-β-D-glycero-pentofuranosyl]cytosine (7b): mp 173.4–174.4 (EtOH); $[\alpha]_D^{22} - 40.4^\circ$ (*c* 1, CHCl₃); $^1\text{H NMR}$ (CDCl₃) δ 4.58 (dd, 1H, *J* = 3.7, 5.0 Hz), 4.70–4.81 (m, 2H), 5.55 (s, 1H), 6.09–6.11 (m, 1H), 7.03 (d, 1H, *J* = 1.3 Hz), 7.40–7.66 (m, 10H), 7.85 (d, 1H, *J* = 7.3 Hz), 7.91 (d, 2H, *J* = 7.7 Hz), 8.03 (dd, 2H, *J* = 0.9, 8.3 Hz), 8.03 (dd, 2H), 8.86 (bs, 1H); $^{13}\text{C NMR}$ (CDCl₃) δ 63.9, 73.3, 80.7, 85.9, 97.8, 117.3, 127.8, 128.0, 128.7, 128.9, 129.1, 129.4, 129.6, 129.9, 130.2, 133.0, 133.6, 133.8, 144.1, 144.9, 155.1, 162.5, 165.9, 166.1. Anal. Calcd (C₃₁H₂₅N₃O₇): C, 67.51; H, 4.57; N, 7.62. Found: C, 67.21; H, 4.51; N, 7.66

N⁴-Benzoyl-2'-C-methyl-3',5'-di-O-benzoylcytidine (7c): mp 176.7–179.1 °C (EtOH); $[\alpha]_D^{22} + 46.2^\circ$ (*c* 1, CHCl₃); $^1\text{H NMR}$ (CDCl₃) δ 1.29 (s, 3H), 4.67–4.80 (m, 3H), 4.82–4.87 (m, 1H), 5.30 (d, 1H, *J* = 5.8 Hz), 6.09 (s, 1H), 7.47–7.56 (m, 4H), 7.89 (d, 2H, *J* = 7.3 Hz), 8.07–8.14 (m, 4H), 8.68 (s, 1H); $^{13}\text{C NMR}$ (CDCl₃) δ 21.4, 62.7, 75.8, 78.5, 79.0, 93.4, 97.3, 127.8, 128.5, 128.7, 128.9, 129.0, 129.4, 129.6, 130.0, 132.9, 133.2, 133.6, 144.3, 156.0, 162.8, 165.8, 166.2, 166.8. Anal. Calcd (C₃₁H₂₇N₃O₈·0.4H₂O): C, 64.56; H, 4.86; N, 7.29. Found: C, 64.54; H, 4.81; N, 7.32.

3',5'-Di-O-benzoyl-2'-deoxy-2'-fluoro-2'-methyluridine (8). Compound **7a** (0.225 g, 0.394 mmol) was suspended in 80% aqueous HOAc (15 mL) and heated under reflux with stirring for 12 h. The clear solution was cooled, concentrated to dryness in vacuo, and coevaporated with 50% MeOH–water (3 × 5 mL) to remove the residual HOAc. Purification by silica gel chromatography, eluting with 2% EtOH–CH₂Cl₂, gave 0.160 g of **8** (87%) as a white solid. Crystallization from 2-propanol afforded an analytical sample: mp 256.4–257.6 °C; $[\alpha]_D^{22} + 71.7^\circ$ (*c* 1, CHCl₃); $^1\text{H NMR}$ (CDCl₃ + CD₃OD) δ 1.39 (d, 3H, *J* = 22.3 Hz), 4.49 (dd, 1H, *J* = 3.9, 12.7 Hz), 4.57 (m, 1H), 4.79 (dd, 1H, *J* = 2.7, 12.5 Hz), 5.42 (d, 1H, *J* = 8.1 Hz), 5.49 (dd, 1H, *J* = 9.20, 21.2 Hz), 6.17 (d, 1H, *J* = 19.3), 7.37–7.50 (m, 4H), 7.51–7.57 (m, 3H, H-6) 7.93–8.01 (m, 4H); $^{13}\text{C NMR}$ (CDCl₃ + CD₃OD) δ 17.3 (d, *J* = 25.1 Hz), 62.1, 72.7 (d, *J* = 16.1 Hz), 90.7 (d, *J* = 44.2 Hz), 99.9 (d, *J* = 186.2 Hz), 103.1, 128.5, 128.6, 128.7, 129.4, 129.5, 130.1, 133.6, 134.0, 139.3, 150.4, 163.2, 165.7, 166.1; $^{19}\text{F NMR}$ (CDCl₃ + CD₃OD) δ 6.02 (m). Anal. Calcd (C₂₄H₂₁FN₂O₇): C, 61.54; H, 4.52; N, 5.98. Found: C, 61.42; H, 4.51; N, 5.96.

General Procedure for Deprotection. The free nucleosides were prepared by treating compounds **7a–c** and **8** with NH₃/MeOH (ca. 7 N, ~12 mL/mmol) followed by stirring at room temperature overnight (8–12 h). The solvent was removed in vacuo, and the compounds were isolated as indicated.

2'-Deoxy-2'-fluoro-2'-C-methylcytidine (1). Compound **7a** (6.30 g, 0.011 mol) was deprotected to give **1** (2.18 g, 76%) as a white powder after column chromatography eluting with 9% EtOH in CHCl₃ and then 17% EtOH and finally 25% EtOH in CHCl₃: mp 216.4–218.0 °C (EtOH); $[\alpha]_D^{22} + 125.6^\circ$ (*c* 1, H₂O); $^1\text{H NMR}$ (DMSO-*d*₆) δ 1.17 (d, 3H, *J* = 22.3 Hz), 3.63 (dd, 1H, *J* = 2.7, 13.7 Hz), 3.70–3.84 (m, 3H), 5.24 (app s, 1H), 5.60 (d, 1H, *J* = 5.4 Hz), 5.74 (d, 1H, *J* = 7.71 Hz), 6.07 (d, 1H, *J* = 18.9 Hz), 7.31 (s, 1H, NH₂), 7.42 (s, 1H, NH₂), 7.90 (d, 1H, *J* = 7.3 Hz); $^{13}\text{C NMR}$ (DMSO-*d*₆) δ 16.6 (d, *J* = 25.9 Hz), 58.5, 70.5 (d, *J* = 18.3 Hz), 81.4, 88.6 (d, *J* = 37.4 Hz), 94.4, 101.2 (d, *J* = 180.1 Hz), 140.5, 154.8, 165.2; $^{19}\text{F NMR}$ (DMSO-*d*₆) δ 2.60 (m). Anal. Calcd (C₁₀H₁₄FN₃O₄·1.5H₂O): C, 41.96; H, 5.94; N, 14.69. Found: C, 42.24; H, 5.63; N, 14.54.

Compound **1** was converted to the HCl salt and crystallized from aqueous ethanol: mp 243 °C (dec); $[\alpha]_D^{22} + 108.4^\circ$ (*c* 1, H₂O); $^1\text{H NMR}$ (DMSO-*d*₆) δ 1.29 (d, 3H, *J* = 22.6 Hz), 3.65 (dd, 1H, *J* = 2.3, 12.7 Hz), 3.76–3.90 (m, 3H), 5.96 (d, 1H, *J* = 17.3 Hz), 6.15 (d, 1H, *J* = 7.9 Hz), 8.33 (d, 1H, *J* = 7.9 Hz), 8.69 (s, 1.5H), 9.78 (s, 1.5H); $^{13}\text{C NMR}$ (DMSO-*d*₆) δ 16.2 (d, *J* = 24.4 Hz), 58.2, 69.9 (d, *J* = 16.8 Hz), 82.1, 88.8 (d, *J* = 32.0 Hz), 94.6, 101.1 (d, *J* = 181.5 Hz), 143.2, 147.6, 159.6; $^{19}\text{F NMR}$ (DMSO-*d*₆) δ 1.69 (m). Anal. Calcd (C₁₀H₁₅ClFN₃O₄): C, 40.62; H, 5.11; N, 14.21. Found: C, 40.80; H, 5.09; N, 14.23.

2'-Deoxy-2'-fluoro-2'-C-methyluridine (9). Deprotection of **8** (0.120 g, 0.209 mmol) followed by column chromatography eluting with 5–10% acetone in diethyl ether provided **9** (0.054 g, 100%) as a white solid: mp 237.3–238.0 °C; $[\alpha]_D^{25} + 83.2^\circ$ (*c* 1, MeOH); $^1\text{H NMR}$ (CD₃OD) δ 1.35 (d, 3H, *J* = 22.3 Hz), 3.79 (dd, 1H, *J* = 2.1, 12.5 Hz), 3.94–4.02 (m, 3H), 5.70 (d, 1H, *J* = 8.1 Hz), 6.13 (d, 1H, *J* = 18.9 Hz), 8.09 (d, 1H); $^{13}\text{C NMR}$ (CD₃OD) δ 16.9 (d, *J* = 25.2 Hz), 60.1, 72.5 (d, *J* = 17.6 Hz), 83.5, 90.6 (d, *J* = 44.0 Hz), 102.1 (d, *J* = 180.1 Hz), 103.0, 142.0, 152.4, 166.0; $^{19}\text{F NMR}$ (CD₃OD) δ 4.07 (bs). Anal. Calcd (C₁₀H₁₃FN₂O₅): C, 46.16; H, 5.04; N, 10.77. Found: C, 45.96; H, 4.93; N, 10.49.

Isolation of 2'-C-methylcytidine. Compound **7c** (0.1 g, 0.176 mmol) upon deprotection and crystallization from MeOH

gave a white solid (0.032 g, 71%): mp 244.2–245.8 °C (lit.¹³ mp 239.5–242 °C, lit.¹⁸ mp 243–245 °C); $[\alpha]_D^{23} +135.7^\circ$ (c 1, H₂O) {lit.¹⁸ $[\alpha]_D +132^\circ$ (c 0.5, H₂O), lit.¹⁹ $[\alpha]_D^{20} +128^\circ$ (c 1, H₂O)}; ¹H NMR (DMSO-*d*₆) δ 0.92 (s, 1H), 3.58–3.62 (m, 2H), 3.70–3.77 (m, 2H), 4.98 (s, 1H), 5.06 (d, 1H, *J* = 7.3 Hz), 5.11 (t, 1H, *J* = 5.0 Hz), 5.67 (d, 1H, *J* = 7.7 Hz), 5.87 (s, 1H), 7.11 (s, 1H), 7.17 (s, 1H), 7.94 (d, 1H, *J* = 7.2 Hz); ¹³C NMR (DMSO-*d*₆) δ 20.0, 59.0, 71.8, 78.3, 81.8, 91.3, 93.6, 141.1, 155.5, 165.4.

Isolation of 2'-Deoxy-2'-methylidenecytidine (DMDC).

Compound **7b** was deprotected and crystallized from H₂O: mp 190–194 °C (dec) (transition at 90–92 °C) (lit.²⁰ mp 89–90 °C); $[\alpha]_D^{22} -39.8^\circ$ (c 1, H₂O); ¹H NMR (DMSO-*d*₆) δ 3.50–3.70 (m, 3H), 4.43 (broad t, 1H), 4.93 (t, 1H, *J* = 5.2 Hz), 5.13 (s, 1H), 5.29 (s, 1H), 5.61 (d, 1H, *J* = 6.4 Hz), 5.69 (d, 1H, *J* = 7.6 Hz), 6.51 (s, 1H), 7.20 (s, 1H), 7.23 (s, 1H), 7.47 (d, 1H, *J* = 7.6 Hz); ¹³C NMR (DMSO-*d*₆) δ 60.5, 69.8, 84.1, 84.2, 94.6, 110.8, 142.0, 151.1, 155.3, 165.5.

Biological Methods. Antiviral assays with bovine viral diarrhea virus and the HCV replicon were performed as described previously.¹⁷

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(54) Title: MODIFIED FLUORINATED NUCLEOSIDE ANALOGUES

(57) Abstract: The disclosed invention provides compositions and methods of treating a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection in a host, including animals, and especially humans, using a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleosides, or a pharmaceutically acceptable salt or prodrug thereof.

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MODIFIED FLUORINATED NUCLEOSIDE ANALOGUES

This application is being filed on 21 April 2004 as a PCT International Patent application in the name of PHARMASSET LTD. a US resident, applicants for all designations except the US.

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FIELD OF THE INVENTION

The present invention includes (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleosides having the natural β -D configuration and methods for the treatment of *Flaviviridae* infections, especially hepatitis C virus (HCV).

BACKGROUND OF THE INVENTION

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Hepatitis C virus (HCV) infection is a major health problem that leads to chronic liver disease, such as cirrhosis and hepatocellular carcinoma, in a substantial number of infected individuals, estimated to be 2-15% of the world's population. There are an estimated 4.5 million infected people in the United States alone, according to the U.S. Center for Disease Control. According to the World Health Organization, there are more than 200 million infected individuals worldwide, with at least 3 to 4 million people being infected each year. Once infected, about 20% of people clear the virus, but the rest can harbor HCV the rest of their lives. Ten to twenty percent of chronically infected individuals eventually develop liver-destroying cirrhosis or cancer. The viral disease is transmitted parenterally by contaminated blood and blood products, contaminated needles, or sexually and vertically from infected mothers or carrier mothers to their offspring. Current treatments for HCV infection, which are restricted to immunotherapy with recombinant interferon- α alone or in combination with the nucleoside analog ribavirin, are of limited clinical benefit as resistance develops rapidly. Moreover, there is no established vaccine for HCV. Consequently, there is an urgent need for improved therapeutic agents that effectively combat chronic HCV infection.

The HCV virion is an enveloped positive-strand RNA virus with a single oligoribonucleotide genomic sequence of about 9600 bases which encodes a

polyprotein of about 3,010 amino acids. The protein products of the HCV gene consist of the structural proteins C, E1, and E2, and the non-structural proteins NS2, NS3, NS4A and NS4B, and NS5A and NS5B. The nonstructural (NS) proteins are believed to provide the catalytic machinery for viral replication. The NS3 protease releases NS5B, the RNA-dependent RNA polymerase from the polyprotein chain. HCV NS5B polymerase is required for the synthesis of a double-stranded RNA from a single-stranded viral RNA that serves as a template in the replication cycle of HCV. Therefore, NS5B polymerase is considered to be an essential component in the HCV replication complex (K. Ishi, et al., "Expression of Hepatitis C Virus NS5B Protein: Characterization of Its RNA Polymerase Activity and RNA Binding," *Heptology*, 29: 1227-1235 (1999); V. Lohmann, et al., "Biochemical and Kinetic Analysis of NS5B RNA-Dependent RNA Polymerase of the Hepatitis C Virus," *Virology*, 249: 108-118 (1998)). Inhibition of HCV NS5B polymerase prevents formation of the double-stranded HCV RNA and therefore constitutes an attractive approach to the development of HCV-specific antiviral therapies.

HCV belongs to a much larger family of viruses that share many common features.

Flaviviridae Viruses

The Flaviviridae family of viruses comprises at least three distinct genera: *pestiviruses*, which cause disease in cattle and pigs; *flaviviruses*, which are the primary cause of diseases such as dengue fever and yellow fever; and *hepaciviruses*, whose sole member is HCV. The flavivirus genus includes more than 68 members separated into groups on the basis of serological relatedness (Calisher et al., *J. Gen. Virol.*, 1993,70,37-43). Clinical symptoms vary and include fever, encephalitis and hemorrhagic fever (*Fields Virology*, Editors: Fields, B. N., Knipe, D. M., and Howley, P. M., Lippincott-Raven Publishers, Philadelphia, PA, 1996, Chapter 31, 931-959). Flaviviruses of global concern that are associated with human disease include the Dengue Hemorrhagic Fever viruses (DHF), yellow fever virus, shock syndrome and Japanese encephalitis virus (Halstead, S. B., *Rev. Infect. Dis.*, 1984, 6, 251-264; Halstead, S. B., *Science*, 239:476-481, 1988; Monath, T. P., *New Eng. J. Med.*, 1988, 319, 641-643).

The pestivirus genus includes bovine viral diarrhea virus (BVDV), classical swine fever virus (CSFV, also called hog cholera virus) and border disease virus (BDV) of sheep (Moennig, V. et al. *Adv. Vir. Res.* 1992, 41, 53-98). Pestivirus infections of domesticated livestock (cattle, pigs and sheep) cause significant economic losses worldwide. BVDV causes mucosal disease in cattle and is of significant economic importance to the livestock industry (Meyers, G. and Thiel, H.J., *Advances in Virus Research*, 1996, 47, 53-118; Moennig V., et al, *Adv. Vir. Res.* 1992, 41, 53-98). Human pestiviruses have not been as extensively characterized as the animal pestiviruses. However, serological surveys indicate considerable pestivirus exposure in humans.

Pestiviruses and hepaciviruses are closely related virus groups within the Flaviviridae family. Other closely related viruses in this family include the GB virus A, GB virus A-like agents, GB virus-B and GB virus-C (also called hepatitis G virus, HGV). The hepacivirus group (hepatitis C virus; HCV) consists of a number of closely related but genotypically distinguishable viruses that infect humans. There are at least 6 HCV genotypes and more than 50 subtypes. Due to the similarities between pestiviruses and hepaciviruses, combined with the poor ability of hepaciviruses to grow efficiently in cell culture, bovine viral diarrhea virus (BVDV) is often used as a surrogate to study the HCV virus.

The genetic organization of pestiviruses and hepaciviruses is very similar. These positive stranded RNA viruses possess a single large open reading frame (ORF) encoding all the viral proteins necessary for virus replication. These proteins are expressed as a polyprotein that is co- and post-translationally processed by both cellular and virus-encoded proteinases to yield the mature viral proteins. The viral proteins responsible for the replication of the viral genome RNA are located within approximately the carboxy-terminal. Two-thirds of the ORF are termed nonstructural (NS) proteins. The genetic organization and polyprotein processing of the nonstructural protein portion of the ORF for pestiviruses and hepaciviruses is very similar. For both the pestiviruses and hepaciviruses, the mature nonstructural (NS) proteins, in sequential order from the amino-terminus of the nonstructural protein coding region to the carboxy-terminus of the ORF, consist of p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

The NS proteins of pestiviruses and hepaciviruses share sequence domains that are characteristic of specific protein functions. For example, the NS3 proteins of viruses in both groups possess amino acid sequence motifs characteristic of serine proteinases and of helicases (Gorbalenya et al. (1988) *Nature* 333:22; Bazan and Fletterick (1989) *Virology* 171:637-639; Gorbalenya et al. (1989) *Nucleic Acid Res.* 17:3889-3897). Similarly, the NS5B proteins of pestiviruses and hepaciviruses have the motifs characteristic of RNA-directed RNA polymerases (Koonin, E.V. and Dolja, V.V. (1993) *Crit. Rev. Biochem. Molec. Biol.* 28:375-430).

The actual roles and functions of the NS proteins of pestiviruses and hepaciviruses in the lifecycle of the viruses are directly analogous. In both cases, the NS3 serine proteinase is responsible for all proteolytic processing of polyprotein precursors downstream of its position in the ORF (Wiskerchen and Collett (1991) *Virology* 184:341-350; Bartenschlager et al. (1993) *J. Virol.* 67:3835-3844; Eckart et al. (1993) *Biochem. Biophys. Res. Comm.* 192:399-406; Grakoui et al. (1993) *J. Virol.* 67:2832-2843; Grakoui et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:10583-10587; Hijikata et al. (1993) *J. Virol.* 67:4665-4675; Tome et al. (1993) *J. Virol.* 67:4017-4026). The NS4A protein, in both cases, acts as a cofactor with the NS3 serine protease (Bartenschlager et al. (1994) *J. Virol.* 68:5045-5055; Failla et al. (1994) *J. Virol.* 68: 3753-3760; Xu et al. (1997) *J. Virol.* 71:53 12-5322). The NS3 protein of both viruses also functions as a helicase (Kim et al. (1995) *Biochem. Biophys. Res. Comm.* 215: 160-166; Jin and Peterson (1995) *Arch. Biochem. Biophys.*, 323:47-53; Warrenner and Collett (1995) *J. Virol.* 69:1720-1726). Finally, the NS5B proteins of pestiviruses and hepaciviruses have the predicted RNA-directed RNA polymerases activity (Behrens et al. (1996) *EMBO.* 15:12-22; Lechmann et al. (1997) *J. Virol.* 71:8416-8428; Yuan et al. (1997) *Biochem. Biophys. Res. Comm.* 232:231-235; Hagedorn, PCT WO 97/12033; Zhong et al. (1998) *J. Virol.* 72:9365-9369).

Treatment of HCV Infection with Interferon

Interferons (IFNs) have been commercially available for the treatment of chronic hepatitis for nearly a decade. IFNs are glycoproteins produced by immune cells in response to viral infection. IFNs inhibit replication of a number of viruses,

including HCV, and when used as the sole treatment for hepatitis C infection, IFN can in certain cases suppress serum HCV-RNA to undetectable levels. Additionally, IFN can normalize serum amino transferase levels. Unfortunately, the effect of IFN is temporary and a sustained response occurs in only 8%-9% of patients chronically
5 infected with HCV (Gary L. Davis. Gastroenterology 18:S104-S114, 2000). Most patients, however, have difficulty tolerating interferon treatment, which causes severe flu-like symptoms, weight loss, and lack of energy and stamina.

A number of patents disclose Flaviviridae, including HCV, and treatments using interferon-based therapies. For example, U.S. Patent No. 5,980,884 to Blatt et
10 al. discloses methods for retreatment of patients afflicted with HCV using consensus interferon. U.S. Patent No. 5,942,223 to Bazer et al. discloses an anti-HCV therapy using ovine or bovine interferon-tau. U.S. Patent No. 5,928,636 to Alber et al. discloses the combination therapy of interleukin-12 and interferon alpha for the treatment of infectious diseases including HCV. U.S. Patent No. 5,849,696 to
15 Chretien et al. discloses the use of thymosins, alone or in combination with interferon, for treating HCV. U.S. Patent No. 5,830,455 to Valtuena et al. discloses a combination HCV therapy employing interferon and a free radical scavenger. U.S. Patent No. 5,738,845 to Imakawa discloses the use of human interferon tau proteins for treating HCV. Other interferon-based treatments for HCV are disclosed in U.S.
20 Patent No. 5,676,942 to Testa et al., U.S. Patent No. 5,372,808 to Blatt et al., and U.S. Patent No. 5,849,696. A number of patents also disclose pegylated forms of interferon, such as U.S. Patent Nos. 5,747,646, 5,792,834 and 5,834,594 to Hoffmann-La Roche; PCT Publication No. WO 99/32139 and WO 99/32140 to Enzon; WO 95/13090 and U.S. Patent Nos. 5,738,846 and 5,711,944 to Schering;
25 and U.S. Patent No. 5,908,621 to Glue et al.

Interferon alpha-2a and interferon alpha-2b are currently approved as monotherapy for the treatment of HCV. ROFERON®-A (Roche) is the recombinant form of interferon alpha-2a. PEGASYS® (Roche) is the pegylated (i.e. polyethylene glycol modified) form of interferon alpha-2a. INTRON®A (Schering Corporation)
30 is the recombinant form of Interferon alpha-2b, and PEG-INTRON® (Schering Corporation) is the pegylated form of interferon alpha-2b.

Other forms of interferon alpha, as well as interferon beta, gamma, tau and omega are currently in clinical development for the treatment of HCV. For example, INFERGEN (interferon alphacon-1) by InterMune, OMNIFERON (natural interferon) by Viragen, ALBUFERON by Human Genome Sciences, REBIF
5 (interferon beta-1a) by Ares-Serono, Omega Interferon by BioMedicine, Oral Interferon Alpha by Amarillo Biosciences, and interferon gamma, interferon tau, and interferon gamma-1b by InterMune are in development.

Ribavirin

Ribavirin (1- β -D-ribofuranosyl-1-1,2,4-triazole-3-carboxamide) is a
10 synthetic, non-interferon-inducing, broad spectrum antiviral nucleoside analog sold under the trade name, Virazole (The Merck Index, 11th edition, Editor: Budavari, S., Merck & Co., Inc., Rahway, NJ, p1304, 1989). United States Patent No. 3,798,209 and RE29,835 disclose and claim ribavirin. Ribavirin is structurally similar to guanosine, and has in vitro activity against several DNA and RNA viruses including
15 *Flaviviridae* (Gary L. Davis. *Gastroenterology* 118: 5104-51 14, 2000).

Ribavirin reduces serum amino transferase levels to normal in 40% of patients, but it does not lower serum levels of HCV-RNA (Gary L. Davis, 2000). Thus, ribavirin alone is not effective in reducing viral RNA levels. Additionally, ribavirin has significant toxicity and is known to induce anemia. Ribavirin is not
20 approved for monotherapy against HCV. It has been approved in combination with interferon alpha-2a or interferon alpha-2b for the treatment of HCV.

Ribavirin is a known inosine monophosphate dehydrogenase inhibitor that does not have specific anti-HCV activity in the HCV replicon system (Stuyver et al. *Journal of Virology*, 2003, 77, 10689-10694).

25 *Combination of Interferon and Ribavirin*

The current standard of care for chronic hepatitis C is combination therapy with an alpha interferon and ribavirin. The combination of interferon and ribavirin for the treatment of HCV infection has been reported to be effective in the treatment of interferon naïve patients (Battaglia, A.M. et al., *Ann. Pharmacother.* 34:487-494,
30 2000), as well as for treatment of patients when histological disease is present

(Berenguer, M. et al. *Antivir. Ther.* 3(Suppl. 3):125-136, 1998). Studies have shown that more patients with hepatitis C respond to pegylated interferon-alpha/ribavirin combination therapy than to combination therapy with unpegylated interferon alpha. However, as with monotherapy, significant side effects develop during combination
5 therapy, including hemolysis, flu-like symptoms, anemia, and fatigue. (Gary L. Davis, 2000). Combination therapy with PEG-INTRON® (peginterferon alpha-2b) and REBETOL® (Ribavirin, USP) capsules are available from Schering Corporation. REBETOL® (Schering Corporation) has also been approved in combination with INTRON® A (Interferon alpha-2b, recombinant, Schering
10 Corporation). Roche's PEGASYS® (pegylated interferon alpha-2a) and COPEGUS® (ribavirin), as well as Three River Pharmaceutical's Ribosphere® are also approved for the treatment of HCV.

PCT Publication Nos. WO 99/59621, WO 00/37110, WO 01/81359, WO 02/32414 and WO 03/02446 1 by Schering Corporation disclose the use of
15 pegylated interferon alpha and ribavirin combination therapy for the treatment of HCV. PCT Publication Nos. WO 99/15 194, WO 99/64016, and WO 00/24355 by Hoffmann-La Roche Inc. also disclose the use of pegylated interferon alpha and ribavirin combination therapy for the treatment of HCV.

Additional Methods to Treat Flaviviridae Infections

20 The development of new antiviral agents for *Flaviviridae* infections, especially hepatitis C, is currently underway. Specific inhibitors of HCV-derived enzymes such as protease, helicase, and polymerase inhibitors are being developed. Drugs that inhibit other steps in HCV replication are also in development, for example, drugs that block production of HCV antigens from the RNA (IRES
25 inhibitors), drugs that prevent the normal processing of HCV proteins (inhibitors of glycosylation), drugs that block entry of HCV into cells (by blocking its receptor) and nonspecific cytoprotective agents that block cell injury caused by the virus infection. Further, molecular approaches are also being developed to treat hepatitis C, for example, ribozymes, which are enzymes that break down specific viral RNA
30 molecules, antisense oligonucleotides, which are small complementary segments of DNA that bind to viral RNA and inhibit viral replication, and RNA interference

techniques are under investigation (Bymoock et al. *Antiviral Chemistry & Chemotherapy*, 11:2; 79-95 (2000); De Francesco et al. in *Antiviral Research*, 58: 1-16 (2003); and Kronke et al., *J. Virol.*, 78:3436-3446 (2004).

5 Bovine viral diarrhea virus (BVDV) is a pestivirus belonging to the family *Flaviviridae* and has been used as a surrogate for *in vitro* testing of potential antiviral agents. While activity against BVDV may suggest activity against other flaviviruses, often a compound can be inactive against BVDV and active against another flavivirus. Sommadossi and La Colla have revealed ("Methods and compositions for treating flaviviruses and pestiviruses", PCT WO 01/92282) that
10 ribonucleosides containing a methyl group at the 2' "up" position have activity against BVDV. However, it is unclear whether these compounds can inhibit other flaviviruses, including HCV in cell culture or at the HCV NS5B level. Interestingly while this publication discloses a large number of compounds that are 2'-methyl-2'-X-ribonucleosides, where X is a halogen, fluorine is not considered. Furthermore, a
15 synthetic pathway leading to nucleosides halogenated at the 2' "down" position is not shown by these inventors.

Dengue virus (DENV) is the causative agent of Dengue hemorrhagic fever (DHF). According to the world Health Organization (WHO), two fifths of the world
20 population are now at risk for infection with this virus. An estimated 500,000 cases of DHF require hospitalization each year with a mortality rate of 5% in children.

West Nile virus (WNV), a flavivirus previously known to exist only in
intertropical regions, has emerged in recent years in temperate areas of Europe and North America, presenting a threat to public health. The most serious manifestation of WNV infection is fatal encephalitis in humans. Outbreaks in New York City and
25 sporadic occurrences in the Southern United States have been reported since 1999.

There is currently no preventive treatment of HCV, Dengue virus (DENV) or West Nile virus infection. Currently approved therapies, which exist only against HCV, are limited. Examples of antiviral agents that have been identified as active against the hepatitis C flavivirus include:

30 1) Protease inhibitors:

Substrate-based NS3 protease inhibitors (Attwood et al., PCT WO 98/22496, 1998; Attwood et al., *Antiviral Chemistry and Chemotherapy* 1999, 10, 259-273; Attwood et al., Preparation and use of amino acid derivatives as anti-viral agents, German Patent Pub. DE 19914474; Tung et al. Inhibitors of serine proteases, particularly hepatitis C virus NS3 protease, PCT WO 98/17679), including
5 alphaketoamides and hydrazinoureas, and inhibitors that terminate in an electrophile such as a boronic acid or phosphonate (Llinas-Brunet et al, Hepatitis C inhibitor peptide analogues, PCT WO 99/07734) are being investigated.

Non-substrate-based NS3 protease inhibitors such as 2,4,6-trihydroxy-3-nitro-benzamide derivatives (Sudo K. et al., *Biochemical and Biophysical Research Communications*, 1997, 238, 643-647; Sudo K. et al. *Antiviral Chemistry and Chemotherapy*, 1998, 9, 186), including RD3-4082 and RD3-4078, the former substituted on the amide with a 14 carbon chain and the latter processing a para-phenoxyphenyl group are also being investigated.

SCH 68631, a phenanthrenequinone, is an HCV protease inhibitor (Chu M. et al., *Tetrahedron Letters* 37:7229-7232, 1996). In another example by the same authors, SCH 351633, isolated from the fungus *Penicillium griseofulvum*, was identified as a protease inhibitor (Chu M. et al., *Bioorganic and Medicinal Chemistry Letters* 9:1949-1952). Nanomolar potency against the HCV NS3
15 protease enzyme has been achieved by the design of selective inhibitors based on the macromolecule eglin c. Eglin c, isolated from leech, is a potent inhibitor of several serine proteases such as *S. griseus* proteases A and B, α -chymotrypsin, chymase and subtilisin (Qasim M.A. et al., *Biochemistry* 36:1598-1607, 1997).

Several U.S. patents disclose protease inhibitors for the treatment of HCV. For example, U.S. Patent No. 6,004,933 to Spruce et al. discloses a class of cysteine protease inhibitors for inhibiting HCV endopeptidase 2. U.S. Patent No. 5,990,276 to Zhang et al. discloses synthetic inhibitors of hepatitis C virus NS3 protease. The inhibitor is a subsequence of a substrate of the NS3 protease or a substrate of the NS4A cofactor. The use of restriction enzymes to treat HCV is disclosed in U.S.
25 Patent No. 5,538,865 to Reyes et al. Peptides as NS3 serine protease inhibitors of HCV are disclosed in WO 02/008251 to Corvas International, Inc. and WO
30

02/08187 and WO 02/008256 to Schering Corporation. HCV inhibitor tripeptides are disclosed in U.S. Patent Nos. 6,534,523, 6,410,531, and 6,420,380 to Boehringer Ingelheim and WO 02/060926 to Bristol Myers Squibb. Diaryl peptides as NS3 serine protease inhibitors of HCV are disclosed in WO 02/48172 to Schering Corporation. Imidazoleidinones as NS3 serine protease inhibitors of HCV are disclosed in WO 02/08198 to Schering Corporation and WO 02/48157 to Bristol Myers Squibb. WO 98/17679 to Vertex Pharmaceuticals and WO 02/48116 to Bristol Myers Squibb also disclose HCV protease inhibitors.

2) Thiazolidine derivatives which show relevant inhibition in a reverse-phase HPLC assay with an NS3/4A fusion protein and NS5A/5B substrate (Sudo K. et al., *Antiviral Research*, 1996, 32, 9-18), especially compound RD-1-6250, possessing a fused cinnamoyl moiety substituted with a long alkyl chain, RD4 6205 and RD4 6193;

3) Thiazolidines and benzanilides identified in Kakiuchi N. et al. *J. EBS Letters* 421, 217-220; Takeshita N. et al. *Analytical Biochemistry*, 1997, 247,242-246;

4) A phenanthrenequinone possessing activity against protease in a SDS-PAGE and autoradiography assay isolated from the fermentation culture broth of *Streptomyces* sp., Sch 68631 (Chu M. et al., *Tetrahedron Letters*, 1996, 37, 7229-7232), and Sch 351633, isolated from the fungus *Penicillium griseofulvum*, which demonstrates activity in a scintillation proximity assay (Chu M. et al., *Bioorganic and Medicinal Chemistry Letters* 9, 1949-1952);

5) Helicase inhibitors (Diana G.D. et al., Compounds, compositions and methods for treatment of hepatitis C, U.S. Pat. No. 5,633,358; Diana G.D. et al., Piperidine derivatives, pharmaceutical compositions thereof and their use in the treatment of hepatitis C, PCT WO 97/36554);

6) Nucleotide polymerase inhibitors and gliotoxin (Ferrari R. et al. *Journal of Virology*, 1999, 73, 1649-1654), and the natural product cerulenin (Lohmann V. et al, *Virology*, 1998, 249, 108-118);

7) Antisense phosphorothioate oligodeoxynucleotides (S-ODN) complementary to sequence stretches in the 5' non-coding region (NCR) of the virus (Alt M. et al.,

Hepatology, 1995, 22, 707-717), or nucleotides 326-348 comprising the 3' end of the NCR and nucleotides 371-388 located in the core coding region of the HCV RNA (Alt M. et al., *Archives of Virology*, 1997, 142, 589-599; Galderisi U. et al., *Journal of Cellular Physiology*, 1999, 181, 251-257);

5 8) Inhibitors of IRES-dependent translation (Ikeda N. et al., Agent for the prevention and treatment of hepatitis C, Japanese Patent Pub. JP-8268890; Kai Y. et al. Prevention and treatment of viral diseases, Japanese Patent Pub. JP-101 01591);

9) Ribozymes, such as nuclease-resistant ribozymes (Maccjak, D. J. et al., *Hepatology* 1999, 30, abstract 995) and those disclosed in U.S. Patent No. 6,043,077
10 to Barber et al., and U.S. Patent Nos. 5,869,253 and 5,610,054 to Draper et al.;

10) Nucleoside analogs have also been developed for the treatment of Flaviviridae infections.

Idenix Pharmaceuticals discloses the use of certain branched nucleosides in the treatment of flaviviruses (including HCV) and pestiviruses in International
15 Publication Nos. WO 01/90121 and WO 01/92282. Specifically, a method for the treatment of hepatitis C virus infection (and flaviviruses and pestiviruses) in humans and other host animals is disclosed in the Idenix publications that includes administering an effective amount of a biologically active 1', 2', 3' or 4'-branched β -
D or β -L nucleosides or a pharmaceutically acceptable salt or derivative thereof,
20 administered either alone or in combination with another antiviral agent, optionally in a pharmaceutically acceptable carrier.

WO 2004/002422 to Idenix published January 8, 2004 discloses a family of 2'-methyl nucleosides for the treatment of flavivirus infections. WO 2004/002999 to
Idenix, published January 8, 2004 discloses a series of 2' or 3' prodrugs of 1', 2', 3',
25 or 4' branch nucleosides for the treatment of flavivirus infections including HCV infections.

Other patent applications disclosing the use of certain nucleoside analogs to treat hepatitis C virus infection include: PCT/CAOO/01316 (WO 01/32153; filed
November 3, 2000) and PCT/CAOI/00197 (WO 01/60315; filed February 19, 2001)
30 filed by BioChem Pharma, Inc. (now Shire Biochem, Inc.); PCT/USO2/01531 (WO

02/057425; filed January 18, 2002) and PCT/U502/03086 (WO 02/057287; filed
January 18, 2002) filed by Merck & Co., Inc., PCT/EPOT/09633 (WO 02/18404;
published August 21, 2001) filed by Roche, and PCT Publication Nos. WO
01/79246 (filed April 13, 2001), WO 02/32920 (filed October 18, 2001) and WO
5 02/48 165 by Pharmasset, Ltd.

WO 2004/007512 to Merck & Co. discloses a number of nucleoside
compounds disclosed as inhibitors of RNA-dependent RNA viral polymerase. The
nucleosides disclosed in this publication are primarily 2'-methyl-2'-hydroxy
substituted nucleosides. WO 02/057287 to Merck et al. published July 25, 2002,
10 discloses a large genus of pyrimidine derivative nucleosides of the 2'-methyl-2'-
hydroxy substitutions. WO 2004/009020 to Merck et al. discloses a series of
thionucleoside derivatives as inhibitors of RNA dependent RNA viral polymerase.
WO 03/105770 to Merck et al. discloses a series of carbocyclic nucleoside
derivatives that are useful for the treatment of HCV infections.

15 PCT Publication No. WO 99/43691 to Emory University, entitled "2'-
Fluoronucleosides" discloses the use of certain 2'-fluoronucleosides to treat HCV.
U.S. Patent No. 6,348,587 to Emory University entitled "2'-fluoronucleosides"
discloses a family of 2'-fluoronucleosides useful for the treatment of hepatitis B,
HCV, HIV and abnormal cellular proliferation. The 2' substituent is disclosed to be
20 in either the "up" or "down" position.

Eldrup et al. (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th
International Conference on Antiviral Research (April 27, 2003, Savannah, Ga.))
described the structure activity relationship of 2'-modified nucleosides for inhibition
of HCV.

25 Bhat et al. (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International
Conference on Antiviral Research (April 27, 2003, Savannah, Ga.); p A75) describe
the synthesis and pharmacokinetic properties of nucleoside analogues as possible
inhibitors of HCV RNA replication. The authors report that 2'-modified nucleosides
demonstrate potent inhibitory activity in cell-based replicon assays.

Olsen et al. (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International Conference on Antiviral Research (April 27, 2003, Savannah, Ga.) p A76) also described the effects of the 2'-modified nucleosides on HCV RNA replication.

5 11) Other miscellaneous compounds including 1-amino-alkylcyclohexanes (U.S. Patent No. 6,034,134 to Gold et al.), alkyl lipids (U.S. Pat. No. 5,922,757 to Chojkier et al.), vitamin E and other antioxidants (U.S. Pat. No. 5,922,757 to Chojkier et al.), squalene, amantadine, bile acids (U.S. Pat. No. 5,846,964 to Ozeki et al.), N-(phosphonoacetyl)-L-aspartic acid, (U.S. Pat. No. 5,830,905 to Diana et al.), benzenedicarboxamides (U.S. Pat. No. 5,633,388 to Diana et al.), polyadenylic acid derivatives (U.S. Pat. No. 5,496,546 to Wang et al.), 2,3-dideoxyinosine (U.S. Pat. No. 5,026,687 to Yarchoan et al.), benzimidazoles (U.S. Pat. No. 5,891,874 to Colacino et al.), plant extracts (U.S. Patent No. 5,837,257 to Tsai et al., U.S. Patent No. 5,725,859 to Omer et al., and U.S. Patent No. 6,056,961), and piperidines (U.S. Patent No. 5,830,905 to Diana et al.).

12) Other compounds currently in preclinical or clinical development for treatment of hepatitis C virus infection include: Interleukin-10 by Schering-Plough, IP-SOI by Intemuron, Merimebodib (VX-497) by Vertex, AMANTADINE® (Symmetrel) by Endo Labs Solvay, HEPTAZYME® by RPI, IDN-6556 by Idun Pharma., XTL-002 by XTL., HCV/MFS9 by Chiron, CIVACIR® (hepatitis C Immune Globulin) by NABI, LEVOVIRIN® by ICN/Ribapharm, VIRAMIDINE® by ICN/Ribapharm, ZADAXIN® (thymosin alpha-1) by SciClone, thymosin plus pegylated interferon by Sci Clone, CEPLENE® (histamine dihydrochloride) by Maxim, VX 950 / LY 570310 by Vertex/Eli Lilly, ISIS 14803 by Isis Pharmaceutical/Elan, IDN-6556 by Idun Pharmaceuticals, Inc., JTK 003 by AKROS Pharma, BILN-2061 by Boehringer Ingelheim, CellCept (mycophenolate mofetil) by Roche, T67, a β -tubulin inhibitor, by Tularik, a therapeutic vaccine directed to E2 by Innogenetics, FK788 by Fujisawa Healthcare, Inc., 1dB 1016 (Siliphos, oral silybin-phosphatidylcholine phytosome), RNA replication inhibitors (VP50406) by ViroPharma/Wyeth, therapeutic vaccine by Intercell, therapeutic vaccine by Epimmune/Genencor, IRES inhibitor by Anadys, ANA 245 and ANA 246 by Anadys, immunotherapy (Therapore) by Avant, protease inhibitor by Corvas/SChering, helicase inhibitor by Vertex, fusion inhibitor

by Trimeris, T cell therapy by CellExSys, polymerase inhibitor by Biocryst, targeted RNA chemistry by PTC Therapeutics, Dication by Immtech, Int., protease inhibitor by Agouron, protease inhibitor by Chiron/Medivir, antisense therapy by AVI BioPharma, antisense therapy by Hybridon, hemopurifier by Aethlon Medical, therapeutic vaccine by Merix, protease inhibitor by Bristol-Myers Squibb/Axys, Chron-VacC, a therapeutic vaccine, by Tripep, UT 231 B by United Therapeutics, protease, helicase and polymerase inhibitors by Genelabs Technologies, IRES inhibitors by Immusol, R803 by Rigel Pharmaceuticals, INFERGEN® (interferon alphacon-1) by InterMune, OMNIFERON® (natural interferon) by Viragen, ALBUFERON® by Human Genome Sciences, REBIF® (interferon beta-1a) by Ares-Serono, Omega Interferon by BioMedicine, Oral Interferon Alpha by Amarillo Biosciences, interferon gamma, interferon tau, and Interferon gamma- 1b by InterMune. Rigel Pharmaceuticals is developing a non-nucleoside HCV polymerase inhibitor, R803, that shows promise as being synergistic with IFN and ribavirin.

13) A summary of several investigational drugs, including several discussed above, that are currently in various phases of development for the treatment of HCV, are summarized below:

Drug	Mechanism / Target	Company	U.S. Status
BILN-2061	NS3 Serine-protease inhibitor	Boehringer Ingelheim	Phase II
ISIS 14803	Antisense / Prevent Translation of RNA	ISIS / Elan	Phase II
Viramidine	Prodrug of Ribavirin	Ribapharm	Phase II
NM 283	Inhibitor of HCV RNA Polymerase	Idenix	Phase II / III
VX-497	IMPDH Inhibitor	Vertex	Phase I / II
JKT-003	Inhibitor of HCV RNA Polymerase	Japan Tobacco / Akros	Phase I / II
Levovirin	L-Ribavirin analog	Ribapharm / Roche	Phase I / II
Isatoribine; ANA245	Nucleoside analog Interact with TLR7 receptor	Anadys	Phase I
Albuferon	Immune modulator	Human Genome Sciences	Phase I

Peg-Infergen	Immune modulator	Intermune	Phase I
VX-950	Inhibitor of HCV NS3-4A protease	Vertex	Preclinical
SCH 6	Inhibitor of HCV NS3-4A protease	Schering Plough	Preclinical
R803	Inhibitor of HCV RNA polymerase	Rigel	Phase I
HCV-086	--	ViroPharma/Wyeth	Phase I
R1479	Inhibitor of HCV RNA polymerase	Roche	Phase I

Nucleoside prodrugs have been previously described for the treatment of other forms of hepatitis. WO 00/09531 and WO 01/96353 to Idenix Pharmaceuticals, discloses 2'-deoxy- β -L-nucleosides and their 3'-prodrugs for the treatment of HBV. U.S. Patent No. 4,957,924 to Beauchamp discloses various therapeutic esters of acyclovir.

In light of the fact that HCV infection has reached epidemic levels worldwide, and has tragic effects on the infected patient, there remains a strong need to provide new effective pharmaceutical agents to treat hepatitis C that have low toxicity to the host.

Further, given the rising threat of other flaviviridae infections, there remains a strong need to provide new effective pharmaceutical agents that have low toxicity to the host.

SUMMARY OF THE INVENTION

There is currently no preventive treatment of Hepatitis C virus (HCV), Dengue virus (DENV) or West Nile virus (WNV) infection, and currently approved therapies, which exist only against HCV, are limited. Design and development of pharmaceutical compounds is essential, especially those that are synergistic with other approved and investigational *Flaviviridae*, and in particular HCV, therapeutics

for the evolution of treatment standards, including more effective combination therapies.

The present invention provides a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L), or its pharmaceutically acceptable salt or prodrug thereof, and the use of such compounds for the treatment of a host infected with a virus belonging to the *Flaviviridae* family, including hepatitis C, West Nile Virus and yellow fever virus. In addition, the nucleosides of the present invention show actively against rhinovirus. Rhinoviruses (RVs) are small (30 nm), nonenveloped viruses that contain a single-strand ribonucleic acid (RNA) genome within an icosahedral (20-sided) capsid. RVs belong to the *Picornaviridae* family, which includes the genera Enterovirus (polioviruses, coxsackieviruses groups A and B, echoviruses, numbered enteroviruses) and Hepatovirus (hepatitis A virus). Approximately 101 serotypes are identified currently. Rhinoviruses are most frequently associated with the common cold, nasopharyngitis, croup, pneumonia, otitis media and asthma exacerbations.

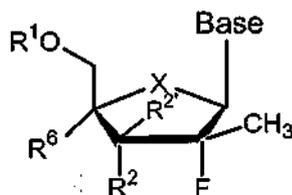
The inventor has made the unexpected discovery that the 2' substitutions on the β -D or β -L nucleosides of the present invention impart greater specificity for hepatitis C virus as well as exhibiting lower toxicity following administration to a host. The invention also includes a method for treating a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection, that includes the administration of an anti-virally effective amount of a β -D or β -L nucleoside disclosed herein, or its pharmaceutically acceptable salt or prodrug, optionally in a pharmaceutically acceptable carrier or diluent, optionally in combination or alternation with another effective antiviral agent.

The nucleosides of the present invention, possess the unique properties of having greater specificity for the hepatitis C virus and lower toxicity in culture or when administered into an animal. One potential, but non-limiting reason for this is the presence of the 2'-fluoro substitution on the ribose ring. For example, U.S. Patent No. 6,348,587 to Schinazi et al., discloses a family of 2'-fluoro nucleoside compounds that are useful in the treatment of hepatitis C virus infection. In contrast, are 2'-methyl substitutions such as found in 2'-*C*-methylcytidine as shown in WO

2004/02999 to Idenix wherein the 2'-methyl substitution on the nucleoside ring at the 2' position is not specific to hepatitis C.

Thus, in one aspect, the antivirally effective nucleoside is a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) or its pharmaceutically acceptable salt or prodrug thereof of the general formula:

5



wherein

(a) Base is a naturally occurring or modified purine or pyrimidine base;

(b) X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R,S*, or racemic), C(W)₂,
 10 wherein W is F, Cl, Br, or I;

(c) R¹ and R⁷ are independently H, phosphate, including 5'-monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including
 15 lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable
 20 leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is OH or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group; and

(d) R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkenyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl),
 25

SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, R² and R^{2'} can be together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂; OR⁷ and

(e) R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro.

In various aspects of the invention, the Base can be selected from



20

wherein

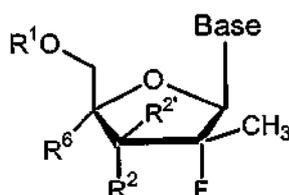
- (a) Y is N or CH.
- (b) R³, R⁴ and R⁵ are independently H, halogen (including F, Cl, Br, I), OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂,

25

5 halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

10 wherein R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

In still another aspect, the (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof can be of the formula:



15 wherein

(a) Base, Y, R¹, R², R²', R³, R⁴, R⁵, R⁶, R⁷ and R' are as described above.

20 Various aspects of the present invention also include pharmaceutical compositions comprising any of the (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) described herein or their pharmaceutically acceptable salts or prodrugs thereof and a pharmaceutically acceptable carrier.

25 The present invention also provides in various aspects, methods for the treatment or prophylaxis of hepatitis C virus infection, West Nile virus infection, a yellow fever viral infection or a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside disclosed herein. The invention also includes methods for treating or preventing *Flaviviridae* infection, including all members of the Hepacivirus genus

(HCV), Pestivirus genus (BVDV, CSFV, BDV), or Flavivirus genus (Dengue virus, Japanese encephalitis virus group (including West Nile Virus), and Yellow Fever virus).

5 In various aspects, the (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl β -D-nucleoside has an EC₅₀ (effective concentration to achieve 50% inhibition) when tested in an appropriate cell-based assay, of less than 15 micromolar, and more particularly, less than 10 or 5 micromolar. In other aspects, the nucleoside is enantiomerically enriched.

10 The present invention also provides methods for the treatment or prophylaxis of a hepatitis C virus infection, West Nile virus infection, a yellow fever viral infection or a rhinovirus infection in a host comprising administering an effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleosides (β -D or β -L) disclosed herein, or its pharmaceutically acceptable salt or prodrug thereof, in combination or alternation with one or more other effective antiviral agent(s), optionally in a pharmaceutically acceptable carrier or diluent thereof, as described herein. Nonlimiting examples of the types of antiviral agents or their prodrugs that can be used in combination with the compounds disclosed herein include, but are not limited to: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; 15 an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon in combination with ribavirin; a protease inhibitor including NS3 inhibitor; a helicase inhibitor; a polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadneylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; 20 silybin-phosphatidylcholine phytosome; and mycophenolate.

25 The following non-limiting aspects illustrate some general methodology to obtain the nucleosides of the present invention. Specifically, the synthesis of the present nucleosides can be achieved by either of two general means:

1) alkylating the appropriately modified carbohydrate building block, subsequent fluoroination, followed by coupling to form the nucleosides of the present invention (Scheme 1) or

2) glycosylation to form the nucleoside followed by alkylation and fluorination of the pre-formed nucleosides of the present invention (Scheme 2).

In addition, the L-enantiomers corresponding to the compounds of the invention can be prepared following the same general methods (Schemes 1 or 2), beginning with the corresponding L-carbohydrate building block or nucleoside L-enantiomer as the starting material.

Thus, the present invention includes at least the following general features:

- (a) β -D and β -L nucleosides of the general formulas disclosed, or their pharmaceutically acceptable salts or prodrugs thereof, as described herein;
- (b) processes for the preparation of the β -D and β -L nucleosides of the general formula disclosed, or their pharmaceutically acceptable salts or prodrugs thereof, as described herein;
- (c) pharmaceutical compositions comprising a β -D or β -L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier or diluent thereof, as described herein, for the treatment or prophylaxis of a viral infection in a host;
- (d) pharmaceutical compositions comprising a β -D or β -L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, in combination with one or more other effective antiviral agent(s), optionally in a pharmaceutically acceptable carrier or diluent thereof, as described herein, for the treatment or prophylaxis of a viral infection in a host;
- (e) methods for the treatment or prophylaxis of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host comprising administering an effective

amount of a β -D or β -L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier or diluent thereof, as described herein;

- 5 (f) methods for the treatment or prophylaxis of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host comprising administering an effective amount of a β -D or β -L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, in combination or
10 alternation with one or more other effective antiviral agent(s), optionally in a pharmaceutically acceptable carrier or diluent thereof, as described herein;
- (g) use of a β -D or β -L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, optionally in a
15 pharmaceutically acceptable carrier, as described herein, for the treatment or prophylaxis of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host;
- (h) use of a β -D or β -L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, in combination or
20 alternation with one or more other effective antiviral agent(s), optionally in a pharmaceutically acceptable carrier, as described herein, for the treatment or prophylaxis of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host;
- (i) use of a β -D or β -L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, optionally in a
25 pharmaceutically acceptable carrier, as described herein, in the manufacture of a medicament for the treatment or prophylaxis of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and
30 yellow fever virus and rhinovirus infection in a host;

- 5 (j) use of a β -D or β -L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, in combination or alternation with one or more other effective antiviral agent(s), optionally in a pharmaceutically acceptable carrier, as described herein, in the manufacture of a medicament for the treatment or prophylaxis of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host;
- 10 (k) use of a β -D or β -L nucleoside of the general formulas disclosed, or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier or diluent, as described herein, in a medical therapy, i.e. as antiviral for example for the treatment or prophylaxis of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection;
- 15 (l) use of a β -D or β -L nucleoside of the general formulas disclosed, as described herein, or its pharmaceutically acceptable salt or prodrug thereof, i.e. as antiviral agent, in combination or alternation with one or more other effective therapeutic agent(s), i.e. another antiviral agent, optionally in a pharmaceutically acceptable carrier or diluent, as described herein, in a medical therapy, for example for the treatment or prophylaxis
- 20 of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection in a host.

BRIEF DESCRIPTION OF THE FIGURES

25 **Figure 1** is a graphical depiction of the dose-dependant reduction of the replicon HCV RNA based on the treatment with β -D-(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine. **(A)**: The viral reduction was compared to the reduction of cellular RNA levels (ribosomal RNA) to obtain therapeutic index values. EC_{90} which represents the effective concentration 90% at 96 hours following the dose dependant administration of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine was determined to be 5 μ M. **(B)**: HCV RNA was significantly reduced in a dose-dependent manner for 7

30 days following treatment with 25 μ M.

Figure 2 depicts the average weight change (%) of female Swiss mice in the toxicity study of β -D-(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine at various doses. Intraperitoneal injections were given on days 0 to day 5 of the 0, 3.3, 10, 33, 100 mg/kg. Each dosing group contained 5 mice and no mice died during the 30-day study.

Figure 3 depicts the pharmacokinetics of β -D-(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine in Rhesus monkeys given a single dose (33.3 mg/kg) oral or intravenous dose of β -D-(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine.

DETAILED DESCRIPTION OF THE INVENTION

Various embodiments of the invention are now described in detail. As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein and throughout the claims that follow, the meaning of "in" includes "in" and "on" unless the context clearly dictates otherwise.

The terms used in this specification generally have their ordinary meanings in the art, within the context of the invention, and in the specific context where each term is used. Certain terms that are used to describe the invention are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them. For convenience, certain terms may be highlighted, for example using italics and/or quotation marks. The use of highlighting has no influence on the scope and meaning of a term; the scope and meaning of a term is the same, in the same context, whether or not it is highlighted. It will be appreciated that the same thing can be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein, nor is any special significance to be placed upon whether or not a term is elaborated or discussed herein. Synonyms for certain terms are provided. A recital of one or more synonyms does not exclude the use of other synonyms. The use of examples anywhere in this specification, including examples of any terms discussed herein, is illustrative only, and in no way limits the scope and meaning of the

invention or of any exemplified term. Likewise, the invention is not limited to various embodiments given in this specification.

As used herein, "about" or "approximately" shall generally mean within 20 percent, preferably within 10 percent, and more preferably within 5 percent of a given value or range. Numerical quantities given herein are approximate, meaning
5 that the term "about" or "approximately" can be inferred if not expressly stated.

The present invention provides (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleosides and their pharmaceutically acceptable salts and prodrugs for the treatment of hepatitis C virus infection, West Nile virus infection, a yellow fever
10 viral infection or a rhinovirus infection in a host.

The disclosed compounds or their pharmaceutically acceptable derivatives or salts or pharmaceutically acceptable formulations containing these compounds are useful in the prevention and treatment of HCV infections. In addition, these compounds or formulations can be used prophylactically to prevent or retard the
15 progression of clinical illness in individuals who are anti-HCV antigen positive or who have been exposed to HCV.

The compounds disclosed herein can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agent, for example, an acid halide or anhydride. The compound or its pharmaceutically acceptable
20 derivative can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment with an appropriate base. The ester or salt of the compound can be converted into the parent compound, for example, by hydrolysis.

Definitions

The term "independently" is used herein to indicate that the variable, which is independently applied, varies independently from application to application. Thus, in a compound such as R^aXYR^a , wherein R^a is "independently carbon or nitrogen", both R^a can be carbon, both R^a can be nitrogen, or one R^a can be carbon and the other R^a nitrogen.

As used herein, the terms "enantiomerically pure" or "enantiomerically enriched" refers to a nucleoside composition that comprises at least approximately 95%, and preferably approximately 97%, 98%, 99% or 100% of a single enantiomer of that nucleoside.

As used herein, the term "substantially free of" or "substantially in the absence of" refers to a nucleoside composition that includes at least 85 or 90% by weight, preferably 95% to 98% by weight, and even more preferably 99% to 100% by weight, of the designated enantiomer of that nucleoside. In a preferred embodiment, in the methods and compounds of this invention, the compounds are substantially free of enantiomers.

Similarly, the term "isolated" refers to a nucleoside composition that includes at least 85 or 90% by weight, preferably 95% to 98% by weight, and even more preferably 99% to 100% by weight, of the nucleoside, the remainder comprising other chemical species or enantiomers.

The term "alkyl," as used herein, unless otherwise specified, refers to a saturated straight, branched, or cyclic, primary, secondary, or tertiary hydrocarbon of typically C_1 to C_{10} , and specifically includes methyl, trifluoromethyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, *t*-butyl, pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, isohexyl, cyclohexyl, cyclohexylmethyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. The term includes both substituted and unsubstituted alkyl groups. Alkyl groups can be optionally substituted with one or more moieties selected from the group consisting of hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, or any other viable

functional group that does not inhibit the pharmacological activity of this compound, either unprotected, or protected, as necessary, as known to those skilled in the art, for example, as taught in T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999, hereby incorporated by
5 reference.

The term "lower alkyl," as used herein, and unless otherwise specified, refers to a C₁ to C₄ saturated straight, branched, or if appropriate, a cyclic (for example, cyclopropyl) alkyl group, including both substituted and unsubstituted forms. Unless otherwise specifically stated in this application, when alkyl is a suitable
10 moiety, lower alkyl is preferred. Similarly, when alkyl or lower alkyl is a suitable moiety, unsubstituted alkyl or lower alkyl is preferred.

The terms "alkylamino" or "arylamino" refer to an amino group that has one or two alkyl or aryl substituents, respectively.

The term "protected," as used herein and unless otherwise defined, refers to a
15 group that is added to an oxygen, nitrogen, or phosphorus atom to prevent its further reaction or for other purposes. A wide variety of oxygen and nitrogen protecting groups are known to those skilled in the art of organic synthesis. Non-limiting examples include: C(O)-alkyl, C(O)Ph, C(O)aryl, CH₃, CH₂-alkyl, CH₂-alkenyl, CH₂Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-alkyl, SO₂-aryl, *tert*-
20 butyldimethylsilyl, *tert*-butyldiphenylsilyl, and 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene).

The term "aryl," as used herein, and unless otherwise specified, refers to phenyl, biphenyl, or naphthyl, and preferably phenyl. The term includes both substituted and unsubstituted moieties. The aryl group can be substituted with one
25 or more moieties selected from the group consisting of hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999.

The terms "alkaryl" or "alkylaryl" refer to an alkyl group with an aryl substituent. The terms "aralkyl" or "arylalkyl" refer to an aryl group with an alkyl substituent.

The term "halo," as used herein, includes chloro, bromo, iodo and fluoro.

5 The term "acyl" refers to a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic alkyl or lower alkyl, alkoxyalkyl including methoxymethyl, aralkyl including benzyl, aryloxyalkyl such as phenoxymethyl, aryl including phenyl optionally substituted with halogen (F, Cl, Br, I), C₁ to C₄ alkyl or C₁ to C₄ alkoxy, sulfonate esters such as alkyl or
10 aralkyl sulphonyl including methanesulfonyl, the mono, di or triphosphate ester, trityl or monomethoxytrityl, substituted benzyl, trialkylsilyl (e.g. dimethyl-*t*-butylsilyl) or diphenylmethylsilyl. Aryl groups in the esters optimally comprise a phenyl group.

 The term "lower acyl" refers to an acyl group in which the non-carbonyl
15 moiety is lower alkyl.

 The term "purine" or "pyrimidine" base includes, but is not limited to, adenine, N⁶-alkylpurines, N⁶-acylpurines (wherein acyl is C(O)(alkyl, aryl, alkylaryl, or arylalkyl), N⁶-benzylpurine, N⁶-halopurine, N⁶-vinylpurine, N⁶-acetylenic purine, N⁶-acyl purine, N⁶-hydroxyalkyl purine, N⁶-allylaminopurine,
20 N⁶-thioallyl purine, N²-alkylpurines, N²-alkyl-6-thiopurines, thymine, cytosine, 5-fluorocytosine, 5-methylcytosine, 6-azapyrimidine, including 6-azacytosine, 2-and/or 4-mercaptopyrimidine, uracil, 5-halouracil, including 5-fluorouracil, C⁵-alkylpyrimidines, C⁵-benzylpyrimidines, C⁵-halopyrimidines, C⁵-vinylpyrimidine, C⁵-acetylenic pyrimidine, C⁵-acyl pyrimidine, C⁵-hydroxyalkyl purine, C⁵-
25 amidopyrimidine, C⁵-cyanopyrimidine, C⁵-iodopyrimidine, C⁶-iodo-pyrimidine, C⁵-Br-vinyl pyrimidine, C⁶-Br-vinyl pyrimidine, C⁵-nitro-pyrimidine, C⁵-amino-pyrimidine, N²-alkylpurines, N²-alkyl-6-thiopurines, 5-azacytidinyl, 5-azauracilyl, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, and pyrazolopyrimidinyl. Purine bases include, but are not limited to, guanine, adenine, hypoxanthine, 2,6-
30 diaminopurine, and 6-chloropurine. Functional oxygen and nitrogen groups on the base can be protected as necessary or desired. Suitable protecting groups are well

known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, *t*-butyldimethylsilyl, and *t*-butyldiphenylsilyl, trityl, alkyl groups, and acyl groups such as acetyl and propionyl, methanesulfonyl, and *p*-toluenesulfonyl.

The term "acyl" or "O-linked ester" refers to a group of the formula C(O)R',
5 wherein R' is an straight, branched, or cyclic alkyl (including lower alkyl), amino acid, aryl including phenyl, alkaryl, aralkyl including benzyl, alkoxyalkyl including methoxymethyl, aryloxyalkyl such as phenoxymethyl; or substituted alkyl (including lower alkyl), aryl including phenyl optionally substituted with chloro, bromo, fluoro, iodo, C₁ to C₄ alkyl or C₁ to C₄ alkoxy, sulfonate esters such as alkyl
10 or aralkyl sulphonyl including methanesulfonyl, the mono, di or triphosphate ester, trityl or monomethoxy-trityl, substituted benzyl, alkaryl, aralkyl including benzyl, alkoxyalkyl including methoxymethyl, aryloxyalkyl such as phenoxymethyl. Aryl groups in the esters optimally comprise a phenyl group. In particular, acyl groups include acetyl, trifluoroacetyl, methylacetyl, cyclopropylacetyl, cyclopropyl
15 carboxy, propionyl, butyryl, hexanoyl, heptanoyl, octanoyl, neo-heptanoyl, phenylacetyl, 2-acetoxy-2-phenylacetyl, diphenylacetyl, α -methoxy- α -trifluoromethyl-phenylacetyl, bromoacetyl, 2-nitro-benzeneacetyl, 4-chloro-benzeneacetyl, 2-chloro-2,2-diphenylacetyl, 2-chloro-2-phenylacetyl, trimethylacetyl, chlorodifluoroacetyl, perfluoroacetyl, fluoroacetyl, bromodifluoroacetyl, methoxyacetyl, 2-thiopheneacetyl, chlorosulfonylacetyl, 3-methoxyphenylacetyl, phenoxyacetyl, tert-butylacetyl, trichloroacetyl, monochloro-
20 acetyl, dichloroacetyl, 7H-dodecafluoro-heptanoyl, perfluoro-heptanoyl, 7H-dodecafluoroheptanoyl, 7-chlorododecafluoro-heptanoyl, 7-chloro-dodecafluoro-heptanoyl, 7H-dodecafluoroheptanoyl, 7H-dodeca-fluoroheptanoyl, nona-fluoro-3,6-dioxaheptanoyl, nonafluoro-3,6-dioxaheptanoyl, perfluoroheptanoyl, methoxybenzoyl, methyl 3-amino-5-phenylthiophene-2-carboxyl, 3,6-dichloro-2-methoxy-benzoyl, 4-(1,1,2,2-tetrafluoro-ethoxy)-benzoyl, 2-bromo-propionyl, omega-aminocapryl, decanoyl, n-pentadecanoyl, stearyl, 3-cyclopentyl-propionyl, 1 -benzene-carboxyl, O-acetylimandelyl, pivaloyl acetyl, 1-adamantane-carboxyl, cyclohexane-carboxyl,
25 2,6- pyridinedicarboxyl, cyclopropane-carboxyl, cyclobutane-carboxyl, perfluorocyclohexyl carboxyl, 4-methylbenzoyl, chloromethyl isoxazolyl carbonyl, perfluorocyclohexyl carboxyl, crotonyl, 1-methyl-1H-indazole-3-carbonyl, 2-

propenyl, isovaleryl, 1-pyrrolidinecarbonyl, 4-phenylbenzoyl. When the term acyl is used, it is meant to be a specific and independent disclosure of acetyl, trifluoroacetyl, methylacetyl, cyclopropylacetyl, propionyl, butyryl, hexanoyl, heptanoyl, octanoyl, neo-heptanoyl, phenylacetyl, diphenylacetyl, *o*-trifluoromethyl-phenylacetyl, bromoacetyl, 4-chloro-benzeneacetyl, 2-chloro-2,2-diphenylacetyl, 2-chloro-2-phenylacetyl, trimethylacetyl, chlorodifluoroacetyl, perfluoroacetyl, fluoroacetyl, bromodifluoroacetyl, 2-thiopheneacetyl, tert-butylacetyl, trichloroacetyl, monochloro-acetyl, dichloroacetyl, methoxybenzoyl, 2-bromo-propionyl, decanoyl, n-pentadecanoyl, stearyl, 3-cyclopentyl-propionyl, 1-benzene-carboxyl, pivaloyl acetyl, 1-adamantane-carboxyl, cyclohexane-carboxyl, 2,6-pyridinedicarboxyl, cyclopropane-carboxyl, cyclobutane-carboxyl, 4-methylbenzoyl, crotonyl, 1-methyl-1H-indazole-3-carbonyl, 2-propenyl, isovaleryl, 4-phenylbenzoyl.

The term "amino acid" includes naturally occurring and synthetic α , β , γ or δ amino acids, and includes but is not limited to, amino acids found in proteins, i.e. glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartate, glutamate, lysine, arginine and histidine. In a preferred embodiment, the amino acid is in the L-configuration. Alternatively, the amino acid can be a derivative of alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaroyl, lysinyl, argininyl, histidinyl, β -alanyl, β -valinyl, β -leucinyl, β -isoleucinyl, β -prolinyl, β -phenylalaninyl, β -tryptophanyl, β -methioninyl, β -glycyl, β -serinyl, β -threoninyl, β -cysteinyl, β -tyrosinyl, β -asparaginyl, β -glutaminyl, β -aspartoyl, β -glutaroyl, β -lysinyl, β -argininyl or β -histidinyl. When the term amino acid is used, it is considered to be a specific and independent disclosure of each of the esters of α , β , γ or δ glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartate, glutamate, lysine, arginine and histidine in the D and L-configurations.

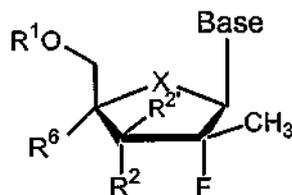
The term "host," as used herein, refers to a unicellular or multicellular organism in which the virus can replicate, including cell lines and animals, and preferably a human. Alternatively, the host can be carrying a part of the viral

genome, whose replication or functions can be altered by the compounds of the present invention. The term host specifically refers to infected cells, cells transfected with all or part of the viral genome, and animals, in particular, primates and humans. In most animal applications of the present invention, the host is a human patient. Veterinary applications, in certain indications, however, are clearly anticipated by the present invention.

The term "pharmaceutically acceptable salt or prodrug" is used throughout the specification to describe any pharmaceutically acceptable form (such as an ester, phosphate ester, salt of an ester or a related group) of a compound which, upon administration to a patient, provides the active compound. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic or organic bases and acids. Suitable salts include those derived from alkali metals such as potassium and sodium, alkaline earth metals such as calcium and magnesium, among numerous other acids well known in the pharmaceutical art. Pharmaceutically acceptable prodrugs refer to a compound that is metabolized, for example hydrolyzed or oxidized, in the host to form the compound of the present invention. Typical examples of prodrugs include compounds that have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated to produce the active compound.

I. Active Compound, and Physiologically Acceptable Derivatives and Salts Thereof

A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:



wherein Base refers to a naturally occurring or modified purine or pyrimidine base; X is O, S, CH₂, Se, NH, N-alkyl, CHW, C(W)₂, wherein W is F, Cl, Br, or I;

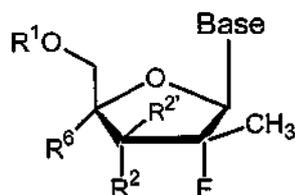
5 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is OH or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group; and

20 R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄

alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl),
 SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄
 4 acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄
 alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl),
 N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷, R² and R^{2'} can be linked
 5 together to form a vinyl optionally substituted by one or two
 of N₃, CN, Cl, Br, F, I, NO₂; and

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano
 (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH),
 10 fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂,
 CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or
 fluoro.

In a second embodiment, a (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside
 or its pharmaceutically acceptable salt or prodrug thereof is provided of the
 15 structure:



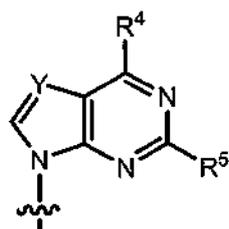
wherein Base, R¹, R², R^{2'}, R⁶ and R⁷ are as defined above.

A third embodiment provides a (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl
 nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:

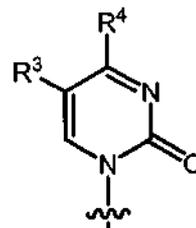


wherein X, R¹, R², R^{2'}, R⁶ and R⁷ are as defined above, and

Base is selected from



(a)



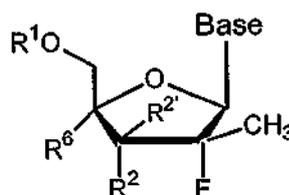
(b)

Y is N or CH;

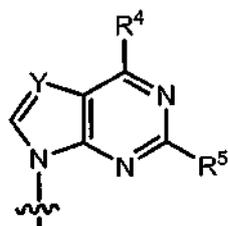
5 R^3 , R^4 and R^5 are independently H, halogen (including F, Cl, Br, I), OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

15 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

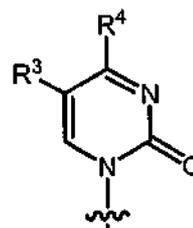
20 In a fourth embodiment, a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:



wherein Base is selected from



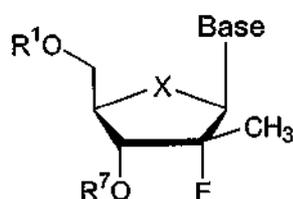
(a)



(b)

and, wherein R^1 , R^2 , R^3 , R^4 , R^5 , R^6 and Y are as defined above.

5 A fifth embodiment provides a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:

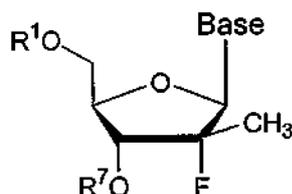


wherein Base refers to a naturally occurring or modified purine or pyrimidine base;

10 R^7 is independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound
15
20 wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7 can also be linked with cyclic phosphate group; and

wherein X and R^1 are as defined above.

In a sixth embodiment, a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:

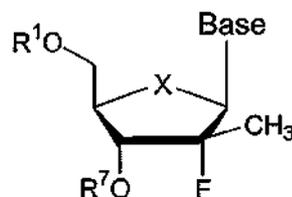


5

wherein Base refers to a naturally occurring or modified purine or pyrimidine base; and

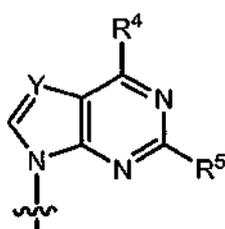
wherein R¹ and R⁷ are as defined above.

A seventh embodiment provides a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:

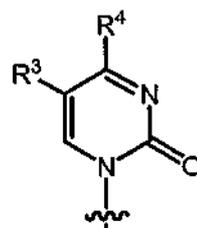


10

wherein Base is selected from



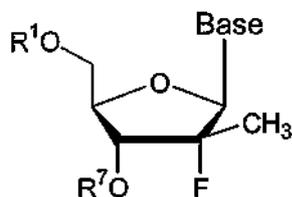
(a)



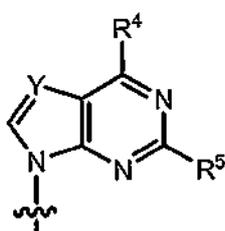
(b)

and wherein X, Y, R¹, R³, R⁴, R⁵, R⁷ and R' are as defined above.

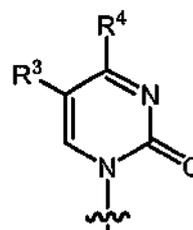
In an eighth embodiment, a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:



5 wherein Base is selected from



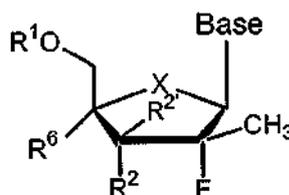
(a)



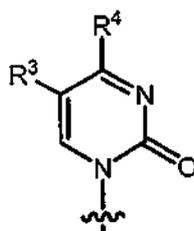
(b)

and, wherein Y, R¹, R³, R⁴, R⁵, R⁷ and R' are as defined above.

10 A ninth embodiment provides a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:



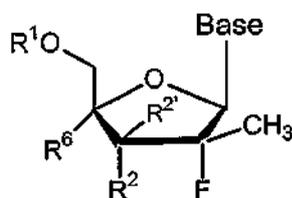
wherein Base is:



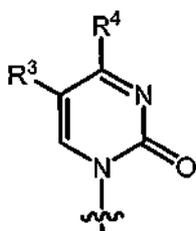
and wherein X is defined as above, R¹ is H, R² is OH, R^{2'} is H, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

In a tenth embodiment, a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:

5



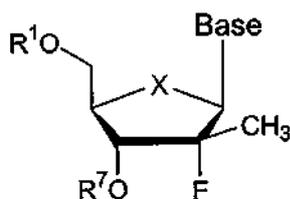
wherein Base is:



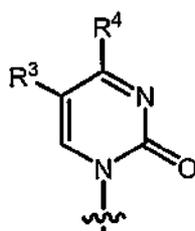
and wherein R¹ is H, R² is OH, R^{2'} is H, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

10

An eleventh embodiment provides a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:



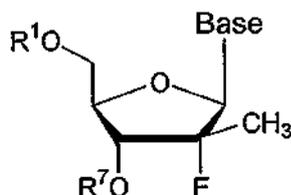
wherein Base is:



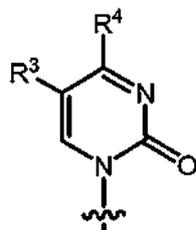
15

and wherein X is defined as above, R¹ is H, R³ is H, R⁴ is NH₂ or OH, R⁶ is H, and R⁷ is H.

5 In a twelfth embodiment, a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof is provided of the structure:

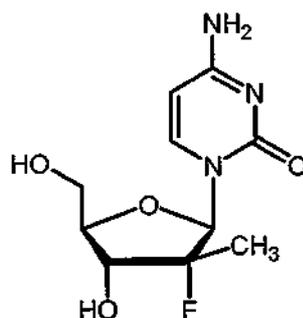


wherein Base is:

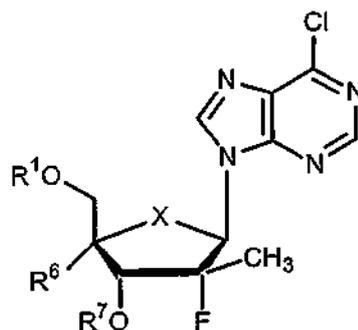


and wherein R¹ is H, R³ is H, R⁴ is NH₂ or OH, and R⁷ is H.

10 A thirteenth embodiment provides a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside or its pharmaceutically acceptable salt or prodrug thereof of the structure:

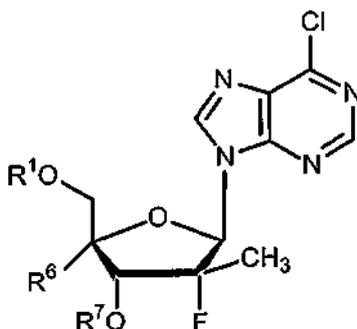


15 In a fourteenth embodiment, a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:



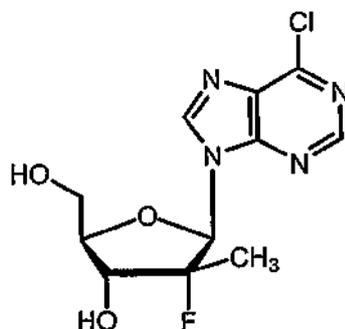
wherein X, R¹, R⁶ and R⁷ are as defined above.

In a fifteenth embodiment, a (2*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:



wherein R¹, R⁶ and R⁷ are as defined above.

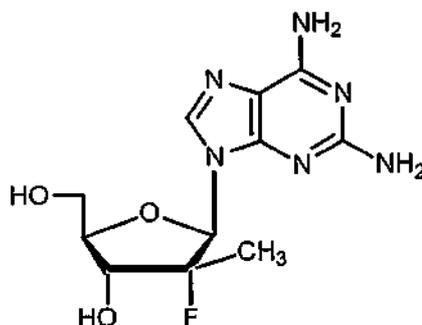
In a sixteenth embodiment, a (2*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:



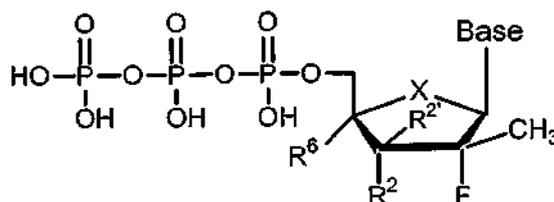
wherein X and R¹ are as defined above.

In a twentieth embodiment, a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside, its pharmaceutically acceptable salt or product thereof is provided by the structure:

5



The present invention also contemplates 5'-triphosphate triphosphoric acid ester derivatives of the 5'-hydroxyl group of a nucleoside compound of the present invention having the following general structural formula:

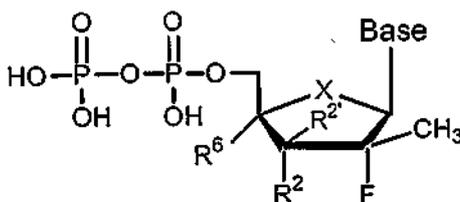


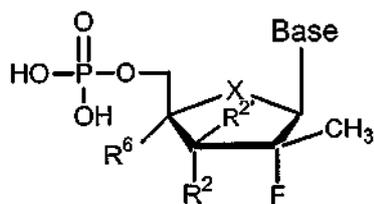
10

wherein Base, X, R², R^{2'}, and R⁶ are as defined as above.

The compounds of the present invention are also intended to include pharmaceutically acceptable salts of the triphosphate ester as well as pharmaceutically acceptable salts of 5'-diphosphate and 5'-monophosphate ester derivatives of the following structural formulas, respectively.

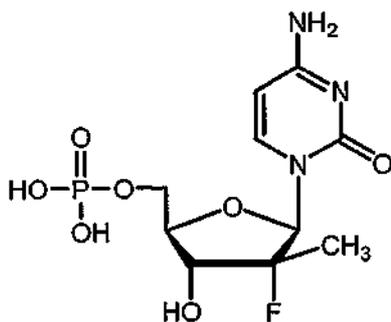
15



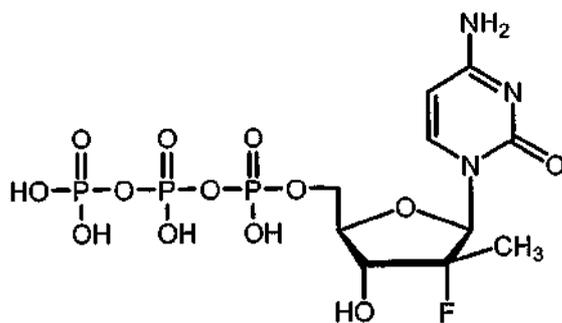
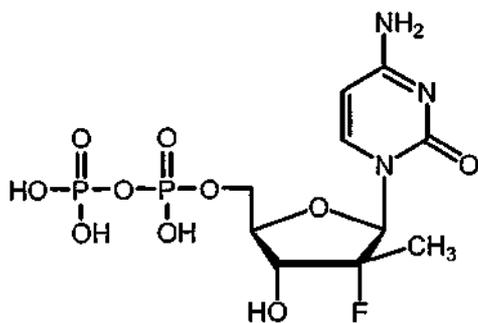


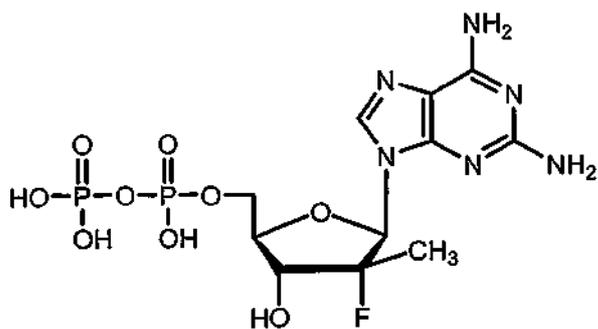
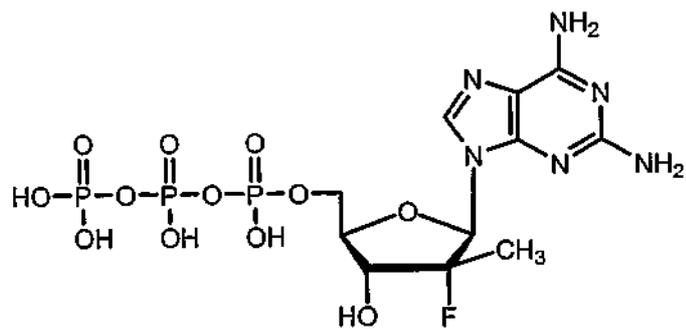
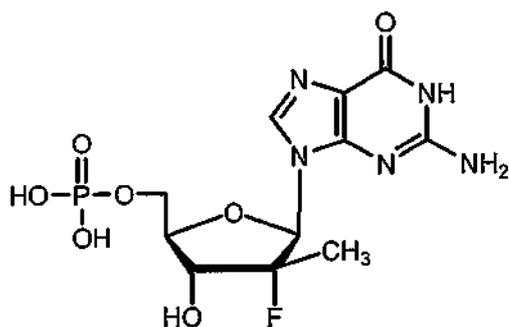
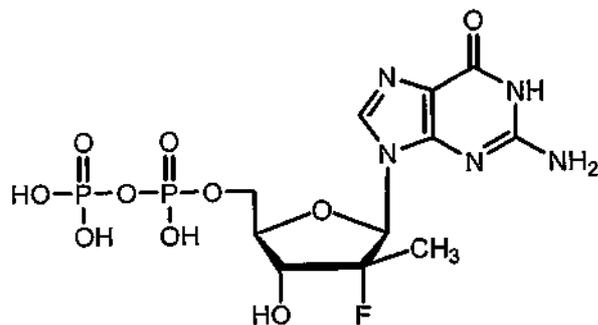
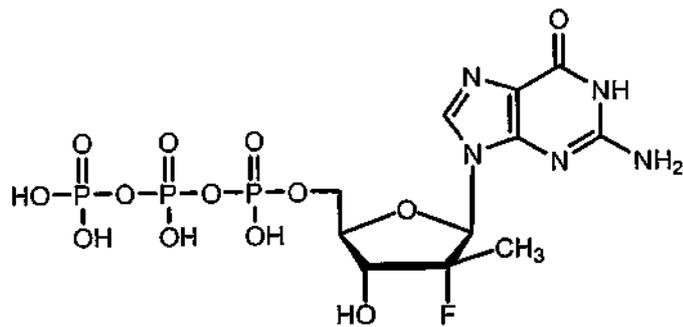
wherein Base, X, R², R^{2'} and R⁶ are as defined above.

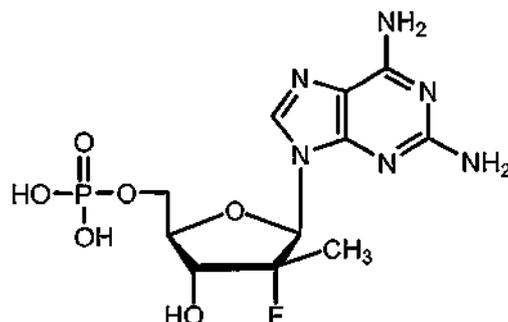
Further non-limiting examples of phosphoric acid derivatives are the nucleosides of the present invention are shown below:



5







The present invention also contemplates that any phosphate nucleoside derivative can include a 5'-(*S*-acyl-2-thioethyl)phosphate or "SATE" mono or diester derivative of the 5'-monophosphates.

5 Alternative embodiments are also contemplated wherein the N-4 amino group on a phosphate nucleoside derivative can be replaced with H, F, Cl, Br or I.

Additional embodiments include 3' and/or 5' prodrugs as described in more detail herein.

In the various embodiments, the fluorinated derivatives are preferred. Fluorine is viewed as "isosteric" with hydrogen because of its size (Van der Waals radii for H is 1.20A and for F 1.35A). However, the atomic weight (18.998) and electronegativity of fluorine (4.0 [Pauling's scale], 4.000 [Sanderson's scale]) are more similar to oxygen (3.5 [Pauling], 3.654 [Sanderson]) than hydrogen (2.1 [Pauling], 2.592 [Sanderson]) (March, J., "Advances in Organic Chemistry: Reactions, Mechanisms, and Structure" Third edition, 1985, p. 14., Wiley Interscience, New York). Fluorine is known to be capable of forming a hydrogen bond, but unlike a hydroxyl group (which can act both as proton acceptor and proton donor) fluorine acts only as a proton acceptor. On the other hand, 2'-fluoro-ribonucleosides can be viewed as analogues of both ribonucleosides and deoxynucleosides. They may be better recognized by viral RNA polymerase at the triphosphate level than by the host RNA polymerase thus selectively inhibiting the viral enzyme.

II. Pharmaceutically Acceptable Salts and Prodrugs

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compound as a pharmaceutically

acceptable salt may be appropriate. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic or organic bases and acids. Suitable salts include those derived from alkali metals such as potassium and sodium, alkaline earth metals such as calcium and magnesium, among numerous other acids well known in the pharmaceutical art. In particular, examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids, which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α -ketoglutarate, and α -glycerophosphate. Suitable inorganic salts may also be formed, including, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

Any of the nucleosides described herein can be administered as a nucleotide prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A number of nucleotide prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the mono, di or triphosphate of the nucleoside will increase the stability of the nucleotide. Examples of substituent groups that can replace one or more hydrogens on the phosphate moiety are alkyl, aryl, steroids, carbohydrates, including sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones and N. Bischofberger, *Antiviral Research*, 27 (1995) 1-17. Any of these can be used in combination with the disclosed nucleosides to achieve a desired effect.

The active nucleoside can also be provided as a 5'-phosphoether lipid or a 5'-ether lipid, as disclosed in the following references, which are incorporated by reference herein: Kucera, L.S., N. Iyer, E. Leake, A. Raben, Modest E.K., D.L.W., and C. Piantadosi. 1990. "Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation." *AIDS Res. Hum. Retro Viruses*. 6:491-501; Piantadosi, C., J. Marasco C.J., S.L. Morris-

Natschke, K.L. Meyer, F. Gumus, J.R. Surlis, K.S. Ishaq, L.S. Kucera, N. Iyer, C.A. Wallen, S. Piantadosi, and E.J. Modest. 1991. "Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity." *J. Med. Chem.* 34:1408.1414; Hosteller, K.Y., D.D. Richman, D.A. Carson, L.M. Stuhmiller, G.M. T. van Wijk, and H. van den Bosch. 1992. "Greatly enhanced inhibition of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 3'-deoxythymidine diphosphate dimyristoylglycerol, a lipid prodrug of 3'-deoxythymidine." *Antimicrob. Agents Chemother.* 36:2025.2029; Hosetler, K.Y., L.M. Stuhmiller, H.B. Lenting, H. van den Bosch, and D.D. Richman, 1990. "Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides." *J. Biol. Chem.* 265:61127.

Nonlimiting examples of U.S. patents that disclose suitable lipophilic substituents that can be covalently incorporated into the nucleoside, preferably at the 5'-OH position of the nucleoside or lipophilic preparations, include U.S. Patent Nos. 5,149,794; 5,194,654; 5,223,263; 5,256,641; 5,411,947; 5,463,092; 5,543,389; 5,543,390; 5,543,391; and 5,554,728, all of which are incorporated herein by reference. Foreign patent applications that disclose lipophilic substituents that can be attached to the nucleosides of the present invention, or lipophilic preparations, include WO 89/02733, WO 90/00555, WO 91/16920, WO 91/18914, WO 93/00910, WO 94/26273, WO 96/15132, EP 0 350 287, EP 93917054.4, and WO 91/19721.

III. Pharmaceutical Compositions

Pharmaceutical compositions based upon a β -D or β -L compound disclosed herein or its pharmaceutically acceptable salt or prodrug can be prepared in a therapeutically effective amount for treating a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection, optionally in combination with a pharmaceutically acceptable additive, carrier or excipient. The therapeutically effective amount may vary with the infection or condition to be treated, its severity, the treatment regimen to be employed, the pharmacokinetics of the agent used, as well as the patient treated.

In one aspect according to the present invention, the compound according to the present invention is formulated preferably in a mixture with a pharmaceutically

acceptable carrier. In general, it is preferable to administer the pharmaceutical composition in orally administrable form, but formulations may be administered via parenteral, intravenous, intramuscular, transdermal, buccal, subcutaneous, suppository or other route. Intravenous and intramuscular formulations are preferably administered in sterile saline. One of ordinary skill in the art may modify the formulation within the teachings of the specification to provide numerous formulations for a particular route of administration without rendering the compositions of the present invention unstable or compromising its therapeutic activity. In particular, a modification of a desired compound to render it more soluble in water or other vehicle, for example, may be easily accomplished by routine modification (salt formulation, esterification, etc.).

In certain pharmaceutical dosage forms, the prodrug form of the compound, especially including acylated (acetylated or other) and ether derivatives, phosphate esters and various salt forms of the present compounds, is preferred. One of ordinary skill in the art will recognize how to readily modify the present compound to a prodrug form to facilitate delivery of active compound to a targeted site within the host organism or patient. The artisan also will take advantage of favorable pharmacokinetic parameters of the prodrug form, where applicable, in delivering the desired compound to a targeted site within the host organism or patient to maximize the intended effect of the compound in the treatment of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection.

The amount of compound included within therapeutically active formulations, according to the present invention, is an effective amount for treating the infection or condition, in preferred embodiments, a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection. In general, a therapeutically effective amount of the present compound in pharmaceutical dosage form usually ranges from about 50 mg to about 2,000 mg or more, depending upon the compound used, the condition or infection treated and the route of administration. For purposes of the present invention, a prophylactically or preventively effective amount of the compositions, according to the present invention, falls within the same concentration range as set forth above for

therapeutically effective amount and is usually the same as a therapeutically effective amount.

Administration of the active compound may range from continuous (intravenous drip) to several oral administrations per day (for example, Q.I.D., B.I.D., etc.) and may include oral, topical, parenteral, intramuscular, intravenous, subcutaneous, transdermal (which may include a penetration enhancement agent), buccal and suppository administration, among other routes of administration. Enteric-coated oral tablets may also be used to enhance bioavailability and stability of the compounds from an oral route of administration. The most effective dosage form will depend upon the pharmacokinetics of the particular agent chosen, as well as the severity of disease in the patient. Oral dosage forms are particularly preferred, because of ease of administration and prospective favorable patient compliance.

To prepare the pharmaceutical compositions according to the present invention, a therapeutically effective amount of one or more of the compounds according to the present invention is preferably mixed with a pharmaceutically acceptable carrier according to conventional pharmaceutical compounding techniques to produce a dose. A carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral. In preparing pharmaceutical compositions in oral dosage form, any of the usual pharmaceutical media may be used. Thus, for liquid oral preparations such as suspensions, elixirs and solutions, suitable carriers and additives including water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like may be used. For solid oral preparations such as powders, tablets, capsules, and for solid preparations such as suppositories, suitable carriers and additives including starches, sugar carriers, such as dextrose, mannitol, lactose and related carriers, diluents, granulating agents, lubricants, binders, disintegrating agents and the like may be used. If desired, the tablets or capsules may be enteric-coated for sustained release by standard techniques. The use of these dosage forms may significantly impact the bioavailability of the compounds in the patient.

For parenteral formulations, the carrier will usually comprise sterile water or aqueous sodium chloride solution, though other ingredients, including those that aid dispersion, also may be included. Where sterile water is to be used and maintained as sterile, the compositions and carriers must also be sterilized. Injectable
5 suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed.

Liposomal suspensions (including liposomes targeted to viral antigens) may also be prepared by conventional methods to produce pharmaceutically acceptable carriers. This may be appropriate for the delivery of free nucleosides, acyl
10 nucleosides or phosphate ester prodrug forms of the nucleoside compounds according to the present invention.

In particularly preferred embodiments according to the present invention, the compounds and compositions are used to treat, prevent or delay the onset of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus, yellow fever
15 virus, and a rhinovirus infection. The present compounds are preferably administered orally, but may be administered parenterally, topically or in suppository form.

The compounds according to the present invention, because of their low toxicity to host cells in certain instances, may be advantageously employed
20 prophylactically to prevent a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection or to prevent the occurrence of clinical symptoms associated with the viral infection or condition. Thus, the present invention also encompasses methods for the prophylactic treatment of viral infection, and in particular a *Flaviviridae* infection, including
25 hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection. In this aspect, according to the present invention, the present compositions are used to prevent or delay the onset of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection. This prophylactic method comprises administration to a patient in need of such treatment, or who is at
30 risk for the development of the virus or condition, an amount of a compound according to the present invention effective for alleviating, preventing or delaying

the onset of the viral infection or condition. In the prophylactic treatment according to the present invention, it is preferred that the antiviral compound utilized should be low in toxicity and preferably non-toxic to the patient. It is particularly preferred in this aspect of the present invention that the compound that is used should be maximally effective against the virus or condition and should exhibit a minimum of toxicity to the patient. In the case of a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus, yellow fever virus, and a rhinovirus infection, compounds according to the present invention, which may be used to treat these disease states, may be administered within the same dosage range for therapeutic treatment (i.e., about 250 micrograms up to 1 gram or more from one to four times per day for an oral dosage form) as a prophylactic agent to prevent the proliferation of the viral infection, or alternatively, to prolong the onset of the viral infection, which manifests itself in clinical symptoms.

In addition, compounds according to the present invention can be administered in combination or alternation with one or more antiviral agents, including other compounds of the present invention. Certain compounds according to the present invention may be effective for enhancing the biological activity of certain agents according to the present invention by reducing the metabolism, catabolism or inactivation of other compounds and as such, are co-administered for this intended effect.

IV. Stereoisomerism and Polymorphism

It is appreciated that nucleosides of the present invention have several chiral centers and may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically active, diastereomeric, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein. It being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase).

5 Carbons of the nucleoside are chiral, their nonhydrogen substituents (the base and the CHOR groups, respectively) can be either *cis* (on the same side) or *trans* (on opposite sides) with respect to the sugar ring system. The four optical isomers therefore are represented by the following configurations (when orienting the sugar moiety in a horizontal plane such that the oxygen atom is in the back): *cis* (with both groups "up", which corresponds to the configuration of naturally occurring β -D nucleosides), *cis* (with both groups "down", which is a nonnaturally occurring β -L configuration), *trans* (with the C2' substituent "up" and the C4' substituent "down"), and *trans* (with the C2' substituent "down" and the C4' substituent "up"). The "D-nucleosides" are *cis* nucleosides in a natural configuration and the "L-nucleosides" are *cis* nucleosides in the nonnaturally occurring configuration.

15 Likewise, most amino acids are chiral (designated as L or D, wherein the L enantiomer is the naturally occurring configuration) and can exist as separate enantiomers.

Examples of methods to obtain optically active materials are known in the art, and include at least the following.

- 20 i) physical separation of crystals - a technique whereby macroscopic crystals of the individual enantiomers are manually separated. This technique can be used if crystals of the separate enantiomers exist, i.e., the material is a conglomerate, and the crystals are visually distinct;
- ii) simultaneous crystallization - a technique whereby the individual enantiomers are separately crystallized from a solution of the racemate, possible only if the latter is a conglomerate in the solid state;
- 25 iii) enzymatic resolutions - a technique whereby partial or complete separation of a racemate by virtue of differing rates of reaction for the enantiomers with an enzyme;
- iv) enzymatic asymmetric synthesis - a synthetic technique whereby at least one step of the synthesis uses an enzymatic reaction to obtain an

enantiomerically pure or enriched synthetic precursor of the desired enantiomer;

- 5 v) chemical asymmetric synthesis - a synthetic technique whereby the desired enantiomer is synthesized from an achiral precursor under conditions that produce asymmetry (i.e., chirality) in the product, which may be achieved using chiral catalysts or chiral auxiliaries;
- 10 vi) diastereomer separations - a technique whereby a racemic compound is reacted with an enantiomerically pure reagent (the chiral auxiliary) that converts the individual enantiomers to diastereomers. The resulting diastereomers are then separated by chromatography or crystallization by virtue of their now more distinct structural differences and the chiral auxiliary later removed to obtain the desired enantiomer;
- 15 vii) first- and second-order asymmetric transformations - a technique whereby diastereomers from the racemate equilibrate to yield a preponderance in solution of the diastereomer from the desired enantiomer or where preferential crystallization of the diastereomer from the desired enantiomer perturbs the equilibrium such that eventually in principle all the material is converted to the crystalline diastereomer from the desired enantiomer. The desired enantiomer is then released from the
- 20 diastereomer;
- viii) kinetic resolutions - this technique refers to the achievement of partial or complete resolution of a racemate (or of a further resolution of a partially resolved compound) by virtue of unequal reaction rates of the enantiomers with a chiral, non-racemic reagent or catalyst under kinetic
- 25 conditions;
- ix) enantiospecific synthesis from non-racemic precursors - a synthetic technique whereby the desired enantiomer is obtained from non-chiral starting materials and where the stereochemical integrity is not or is only minimally compromised over the course of the synthesis;

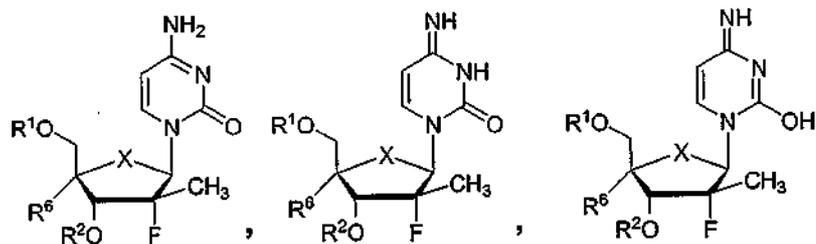
- 5 x) chiral liquid chromatography - a technique whereby the enantiomers of a racemate are separated in a liquid mobile phase by virtue of their differing interactions with a stationary phase. The stationary phase can be made of chiral material or the mobile phase can contain an additional chiral material to provoke the differing interactions;
- 10 xi) chiral gas chromatography - a technique whereby the racemate is volatilized and enantiomers are separated by virtue of their differing interactions in the gaseous mobile phase with a column containing a fixed non-racemic chiral adsorbent phase;
- 15 xii) extraction with chiral solvents - a technique whereby the enantiomers are separated by virtue of preferential dissolution of one enantiomer into a particular chiral solvent;
- xiii) transport across chiral membranes - a technique whereby a racemate is placed in contact with a thin membrane barrier. The barrier typically separates two miscible fluids, one containing the racemate, and a driving force such as concentration or pressure differential causes preferential transport across the membrane barrier. Separation occurs as a result of the non-racemic chiral nature of the membrane which allows only one enantiomer of the racemate to pass through.

20 Chiral chromatography, including simulated moving bed chromatography, is used in one embodiment. A wide variety of chiral stationary phases are commercially available.

Some of the compounds described herein contain olefinic double bonds and unless otherwise specified, are meant to include both E and Z geometric isomers.

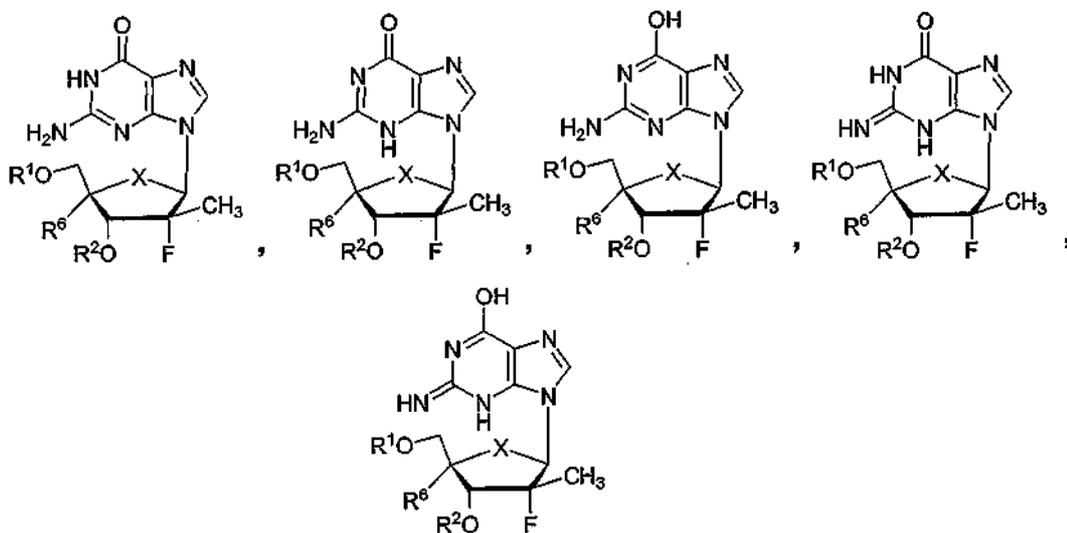
25 In addition, some of the nucleosides described herein, may exist as tautomers, such as, keto-enol tautomers. The individual tautomers as well as mixtures thereof are intended to be encompassed within the compounds of the present invention as illustrated below.

A (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine:

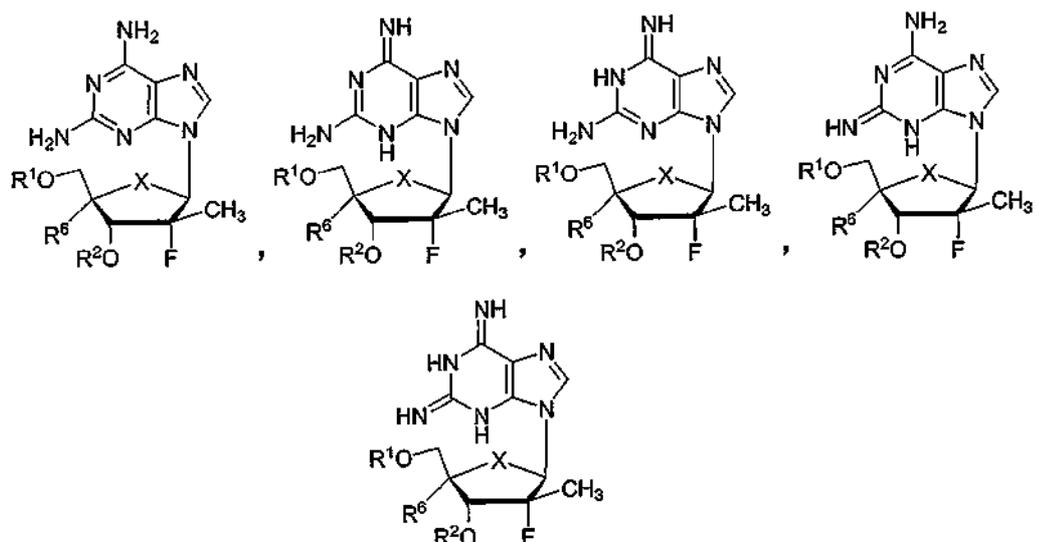


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A (2'R)-2'-deoxy-2'-fluoro-2'-C-methylguanosine:



A (2'R)-2-amino-2'-deoxy-2'-fluoro-2'-C-methyladenosine:



In each example above, the first drawn structure is the preferred form.

V. Prodrugs and Derivatives

The active compound can be administered as any salt or prodrug that upon administration to the recipient is capable of providing directly or indirectly the parent compound, or that exhibits activity itself. Nonlimiting examples are the pharmaceutically acceptable salts (alternatively referred to as "physiologically acceptable salts"), and a compound, which has been alkylated, acylated, or otherwise modified at the 5'-position, or on the purine or pyrimidine base (a type of "pharmaceutically acceptable prodrug"). Further, the modifications can affect the biological activity of the compound, in some cases increasing the activity over the parent compound. This can easily be assessed by preparing the salt or prodrug and testing its antiviral activity according to the methods described herein, or other methods known to those skilled in the art.

15 Pharmaceutically Acceptable Salts

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compound as a pharmaceutically acceptable salt may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed by addition of acids, which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorate, a-ketoglutarate, a-

glycerophosphate, formate, fumarate, propionate, glycolate, lactate, pyruvate, oxalate, maleate, and salicylate. Suitable inorganic salts may also be formed, including, sulfate, nitrate, bicarbonate, carbonate salts, hydrobromate and phosphoric acid. In a preferred embodiment, the salt is a mono- or di- hydrochloride salt.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made. In one embodiment, the salt is a hydrochloride, hydrobromide, or mesylate salt of the compound. In another embodiment, the pharmaceutically acceptable salt is a dihydrochloride, dihydrobromide, or dimesylate salt.

Nucleotide Prodrug Formulations

The nucleosides described herein can be administered as a nucleotide prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A number of nucleotide prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the mono-, di- or triphosphate of the nucleoside reduces polarity and allows passage into cells. Examples of substituent groups that can replace one or more hydrogens on the phosphate moiety are alkyl, aryl, steroids, carbohydrates, including sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones and N. Bisehoferger, Antiviral Research, 1995, 27:1-17. Any of these can be used in combination with the disclosed nucleosides to achieve a desired effect.

In an alternative embodiment, the nucleoside is delivered as a phosphonate or a SATE derivative.

The active nucleoside can also be provided as a 2'-, 3'- and/or 5'-phosphoether lipid or a 2'-, 3'- and/or 5'-ether lipid. Non-limiting examples are described include the following references, which are incorporated by reference herein: Kucera, L.S., N. Iyer, E. Leake, A. Raben, Modest E.K., D.L.W., and C.

Piantadosi. 1990. "Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation." *AIDS Res. Hum. Retro Viruses*. 6:491-501; Piantadosi, C., J. Marasco C.J., S.L. Morris-Natschke, K.L. Meyer, F. Gumus, J.R. Surles, K.S. Ishaq, L.S. Kucera, N. Iyer, CA. Wallen, S. Piantadosi, and E.J. Modest. 1991. "Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity." *J. Med Chem.* 34:1408.1414; Hosteller, K.Y., D.D. Richman, D.A. Carson, L.M. Stuhmiller, G.M. T. van Wijk, and H. van den Bosch. 1992. "Greatly enhanced inhibition of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 3'-deoxythymine diphosphate dimyristoylglycerol, a lipid prodrug of 3'-deoxythymine." *Antinziacrob. Agents Chemother.* 36:2025.2029; Hostetler, K.Y., L.M. Stuhmiller, H.B. Lenting, H. van den Bosch, and D.D. Richman, 1990. "Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides." *J. Biol. Chem.* 265:61127.

15 Nonlimiting examples of U.S. patents that disclose suitable lipophilic substituents that can be covalently incorporated into the nucleoside, preferably at the 2'-, 3'- and/or 5'-OH position of the nucleoside or lipophilic preparations, include U.S. Patent Nos. 5,149,794 (Sep. 22, 1992, Yatvin et al.); 5,194,654 (Mar. 16, 1993, Hostetler et al., 5,223,263 (June 29, 1993, Hostetler et al.); 5,256,641 (Oct. 26, 20 1993, Yatvin et al.); 5,411,947 (May 2, 1995, Hostetler et al.); 5,463,092 (Oct. 31, 1995, Hostetler et al.); 5,543,389 (Aug. 6, 1996, Yatvin et al.); 5,543,390 (Aug. 6, 1996, Yatvin et al.); 5,543,391 (Aug. 6, 1996, Yatvin et al.); and 5,554,728 (Sep. 10, 1996; Basava et al.), all of which are incorporated herein by reference. Foreign patent applications that disclose lipophilic substituents that can be attached to the 25 nucleosides of the present invention, or lipophilic preparations, include WO 89/02733, WO 90/00555, WO 91/16920, WO 91/18914, WO 93/00910, WO 94/26273, WO 96/15132, EP 0350287, EP 93917054.4, and WO 91/19721.

Aryl esters, especially phenyl esters, are also provided. Nonlimiting examples are disclosed in DeLambert et al., *J. Med. Chem.* 37: 498 (1994). Phenyl esters containing a carboxylic ester ortho to the phosphate are also provided. 30 Khaninei and Torrence, *J. Med. Chem.*; 39:41094115 (1996). In particular, benzyl esters, which generate the parent compound, in some cases using substituents at the

ortho- or para-position to accelerate hydrolysis, are provided. Examples of this class of prodrugs are described by Mitchell et al., *J. Chem. Soc. Perkin Trans. I* 2345 (1992); Brook, et al. WO 91/19721; and Glazier et al. WO 91/1 9721.

Cyclic and noncyclic phosphonate esters are also provided. Nonlimiting examples are disclosed in Hunston et al., *J. Med. Chem.* 27: 440-444 (1984) and Starrett et al. *J. Med. Chem.* 37: 1857-1864 (1994). Additionally, cyclic 3',5'-phosphate esters are provided. Nonlimiting examples are disclosed in Meier et al. *J. Med. Chem.* 22: 811-815 (1979). Cyclic 1',3'-propanyl phosphonate and phosphate esters, such as ones containing a fused aryl ring, i.e. the cyclosaligenyl ester, are also provided (Meier et al., *Bioorg. Med. Chem. Lett.* 7: 99-104 (1997)). Unsubstituted cyclic 1',3'-propanyl esters of the monophosphates are also provided (Farquhar et al., *J. Med. Chem.* 26: 1153 (1983); Farquhar et al., *J. Med. Chem.* 28: 1358 (1985)) were prepared. In addition, cyclic 1',3'-propanyl esters substituted with a pivaloyloxy methoxy group at C-1' are provided (Freed et al., *Biochem. Pharmac.* 38: 3193 (1989); Biller et al., U.S. Pat. No. 5,157,027).

Cyclic phosphoramidates are known to cleave in vivo by an oxidative mechanism. Therefore, in one embodiment of the present invention, a variety of substituted 1',3' propanyl cyclic phosphoramidates are provided. Non-limiting examples are disclosed by Zon, *Progress in Med. Chem.* 19, 1205 (1982). Additionally, a number of 2'- and 3'- substituted proesters are provided. 2'-Substituents include methyl, dimethyl, bromo, trifluoromethyl, chloro, hydroxy, and methoxy; 3'-substituents including phenyl, methyl, trifluoromethyl, ethyl, propyl, i-propyl, and cyclohexyl. A variety of 1'-substituted analogs are also provided.

Cyclic esters of phosphorus-containing compounds are also provided. Non-limiting examples are described in the following:

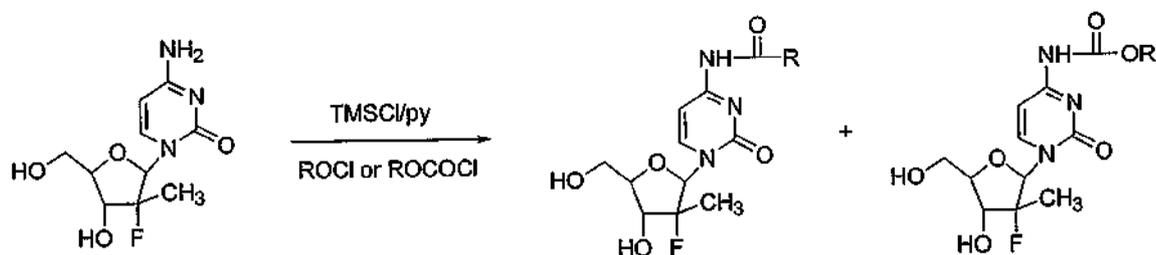
- di and tri esters of phosphoric acids as reported in Nifantsev et al., *Phosphorus, Sulfur Silicon and Related Elements*, 113: 1 (1996); Wijnberg et al., EP-180276 A1;
- phosphorus (III) acid esters. Kryuchkov et al., *Izy. Akad. Nauk SSSR, Ser. Khim.* 6:1244 (1987). Some of the compounds were claimed to be useful for

the asymmetric synthesis of L-Dopa precursors. Sylvain et al., DE3S 12781 A1;

- phosphoramidates. Shili et al., Bull. Inst. Chem. Acad. Sin, 41: 9 (1994); Edmundson et al., J. Chem. Res. Synop. 5:122 (1989); and
- 5 • phosphonates. Neidlein et al., Heterocycles 35: 1185 (1993).

N⁴-acyl Prodrugs

The invention also provides N⁴- acyl prodrugs. A non-limiting example of an N⁴-acyl derivative of (2'*R*)-2'-F-2'-C-methylcytidine is shown below:



10 wherein R can be any acyl group as described herein.

The invention also contemplates other embodiments, wherein the prodrug of a (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) includes biologically cleavable moieties at the 3' and/or 5' positions. Preferred moieties are natural or synthetic D or L amino acid esters, including D or L -valyl, though preferably L -

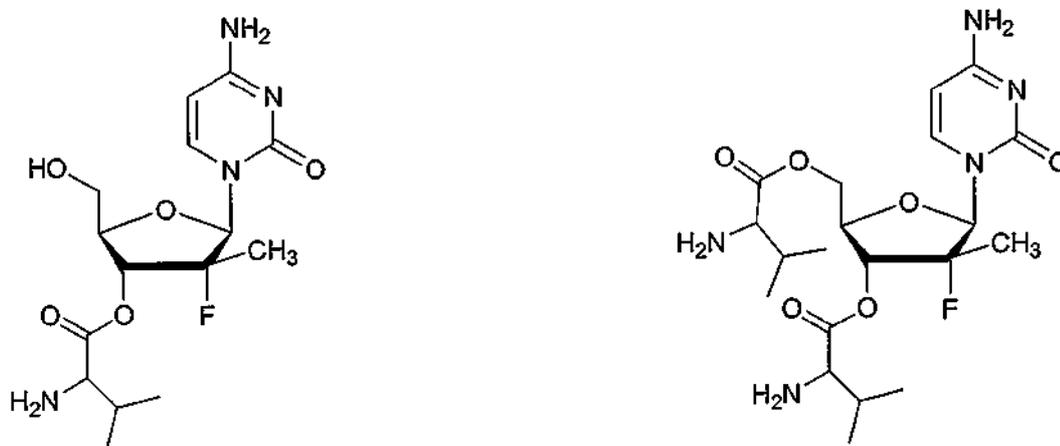
15 amino acids esters, such as L -valyl, and alkyl esters including acetyl. Therefore, this invention specifically includes 3'- L or D - amino acid ester and 3', 5'- L or D - diamino acid ester of (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) nucleosides, preferably L -amino acid, with any desired purine or pyrimidine base, wherein the parent drug optionally has an EC₅₀ of less than 15 micromolar, and

20 even more preferably less than 10 micromolar; 3'-(alkyl or aryl) ester or 3',5'- L - di(alkyl or aryl) ester of (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) with any desired purine or pyrimidine base, wherein the parent drug optionally has an EC₅₀ of less than 10 or 15 micromolar; and prodrugs of 3',5'-diesters of (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl nucleosides (β -D or β -L) wherein (i) the 3' ester is an

25 amino acid ester and the 5'-ester is an alkyl or aryl ester; (ii) both esters are amino acid esters; (iii) both esters are independently alkyl or aryl esters; and (iv) the 3'

ester is independently an alkyl or aryl ester and the 5'-ester is an amino acid ester, wherein the parent drug optionally has an EC_{50} of less than 10 or 15 micromolar.

Non-limiting examples of prodrugs falling within the invention are:



5

VI. Combination or Alternation Therapy

In another embodiment, for the treatment, inhibition, prevention and/or prophylaxis of any viral infection described herein, the active compound or its derivative or salt can be administered in combination or alternation with another antiviral agent. In general, in combination therapy, effective dosages of two or more agents are administered together, whereas during alternation therapy, an effective dosage of each agent is administered serially. The dosage will depend on absorption, inactivation and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens and schedules should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

It has been recognized that drug-resistant variants of flaviviruses, pestiviruses or HCV can emerge after prolonged treatment with an antiviral agent. Drug resistance most typically occurs by mutation of a gene that encodes for an enzyme used in viral replication. The efficacy of a drug against the viral infection can be prolonged, augmented, or restored by administering the compound in

combination or alternation with a second, and perhaps third, antiviral compound that induces a different mutation from that caused by the principle drug. Alternatively, the pharmacokinetics, biodistribution or other parameter of the drug can be altered by such combination or alternation therapy. In general, combination therapy is typically preferred over alternation therapy because it induces multiple simultaneous stresses on the virus.

For example, one skilled in the art will recognize that any antiviral drug or therapy can be used in combination or alternation with any nucleoside of the present invention. Any of the viral treatments described in the Background of the Invention can be used in combination or alternation with the compounds described in this specification. Nonlimiting examples of the types of antiviral agents or their prodrugs that can be used in combination with the compounds disclosed herein include: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

Further nonlimiting examples of the types of drugs or their prodrugs described above include: acyclovir (ACV), ganciclovir (GCV or DHPG) and its prodrugs (e.g. valyl-ganciclovir), E-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), (E)-5-vinyl-1- β -D-arabonosyluracil (VaraU), (E)-5-(2-bromovinyl)-1- β -D-arabinosyluracil (BV-araU), 1-(2-deoxy-2-fluoro- β -D-arabinosyl)-5-iodocytosine (D-FIAC), 1-(2-deoxy-2-fluoro- β -L-arabinosyl)-5-methyluracil (L-FMAU, or

clevudine), (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(*S*)-HPMPA], (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-2,6-diaminopurine [(*S*)-HPMPDAP], (*S*)-1-(3-hydroxy-2-phosphonyl-methoxypropyl)cytosine [(*S*)-HPMPC, or *cidofivir*], and (2*S*,4*S*)-1-[2-(hydroxymethyl)-1,3-dioxolan-4-yl]-5-iodouracil (L-5-IoddU), entecavir, , lamivudine (3TC), LdT, LdC, tenofovir, and adefovir, the (-)-enantiomer of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ((-)-FTC); the (-)-enantiomer of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (3TC); carbovir, acyclovir, famciclovir, penciclovir, AZT, DDI, DDC, L-(-)-FMAU, D4T, amdoxovir, Reverset, Racivir, abacavir, L-DDA phosphate prodrugs, and β -D-dioxolanyl-6-chloropurine (ACP), non-nucleoside RT inhibitors such as nevirapine, MKC-442, DMP-226 (sustiva), protease inhibitors such as indinavir, saquinavir, Kaletra, atazanavir; and anti-HIV compounds such as BILN-2061, ISIS 14803; viramidine, NM 283, VX-497, JKT-003, levovirin, isatoribine, albuferon, Peg-infergen, VX-950, R803, HCV-086, R1479 and DMP45.

15 Pharmaceutical Compositions

Hosts, including humans, infected with pestivirus, flavivirus, HCV infection, or any other condition described herein, or another organism replicating through a RNA-dependent RNA viral polymerase, or for treating any other disorder described herein, can be treated by administering to the patient an effective amount of the active compound or a pharmaceutically acceptable prodrug or salt thereof in the presence of a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

A preferred dose of the compound for a *Flaviviridae* infection, including hepatitis C virus, West Nile Virus and yellow fever virus and rhinovirus infection will be in the range from about 50 to about 2000 mg one to four times per day. Lower doses may be useful, and thus ranges can include from 50 – 1,000 mg one to four times per day. The effective dosage range of the pharmaceutically acceptable salts and prodrugs can be calculated based on the weight of the parent nucleoside to be delivered. If the salt or prodrug exhibits activity in itself the effective dosage can

be estimated as above using the weight of the salt or prodrug, or by other means known to those skilled in the art.

The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 25 to 3000 mg, preferably 50 to 2000 mg of active ingredient per unit dosage form. An oral dosage of 50-1000 mg is usually convenient, including in one or multiple dosage forms of 50, 100, 200, 250, 300, 400, 500, 600, 700, 800, 900 or 1000 mgs. Also contemplated are doses of 0.1-50 mg, or 0.1-20 mg or 0.1-10.0 mg. Furthermore, lower doses may be utilized in the case of administration by a non-oral route, as, for example, by injection or inhalation.

Ideally the active ingredient should be administered to achieve peak plasma concentrations (C_{max}) of the active compound of from about 5.0 to 70 μ M, preferably about 5.0 to 15 μ M. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

The concentration of active compound in the drug composition will depend on absorption, inactivation and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients

and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

5 The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is
10 a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

15 The compound can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

20 The compound or a pharmaceutically acceptable prodrug or salts thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, anti-inflammatories, or other antivirals, including other nucleoside compounds. Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes
25 or multiple dose vials made of glass or plastic.
30

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation.

Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearyl phosphatidyl ethanolamine, stearyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

VII. Biological Methods

Antiviral Testing Of Candidate Compounds With HCV Replicon System In Huh7 Cells.

Huh7 cells harboring the HCV replicon can be cultivated in DMEM media (high glucose, no pyruvate) containing 10% fetal bovine serum, 1X non-essential Amino Acids, Pen-Strep-Glu (100 units/liter, 100 microgram/liter, and 2.92 mg/liter, respectively) and 500 to 1000 microgram/milliliter G418. Antiviral screening assays can be done in the same media without G418 as follows: in order to keep cells in logarithmic growth phase, cells are seeded in a 96-well plate at low density,

for example 1000 cells per well. The test compound is added immediately after seeding the cells and incubate for a period of 3 to 7 days at 37°C in an incubator. Media is then removed, and the cells are prepared for total nucleic acid extraction (including replicon RNA and host RNA). Replicon RNA can then be amplified in a Q-RT-PCR protocol, and quantified accordingly. The observed differences in replicon HCV RNA levels compared to the untreated control is one way to express the antiviral potency of the test compound.

In another typical setting, a compound might reduce the viral RNA polymerase activity, but not the host RNA polymerase activity. Therefore, quantification of *r*RNA or beta-actin *m*RNA (or any other host RNA fragment) and comparison with RNA levels of the no-drug control is a relative measurement of the inhibitory effect of the test compound on cellular RNA polymerases.

Phosphorylation Assay of Nucleoside to Active Triphosphate

To determine the cellular metabolism of the compounds, Huh-7 cells are obtained from the American Type Culture Collection (Rockville, MD), and are grown in 225 cm² tissue culture flasks in minimal essential medium supplemented with non-essential amino acids, 1% penicillin-streptomycin. The medium is renewed every three days, and the cells are sub cultured once a week. After detachment of the adherent monolayer with a 10 minute exposure to 30 mL of trypsin-EDTA and three consecutive washes with medium, confluent Huh-7 cells are seeded at a density of 2.5 x 10⁶ cells per well in a 6-well plate and exposed to 10 μM of [³H] labeled active compound (500 dpm/pmol) for the specified time periods. The cells are maintained at 37 °C under a 5% CO₂ atmosphere. At the selected time points, the cells are washed three times with ice-cold phosphate-buffered saline (PBS). Intracellular active compound and its respective metabolites are extracted by incubating the cell pellet overnight at -20 °C with 60% methanol followed by extraction with an additional 20 μL of cold methanol for one hour in an ice bath. The extracts are then combined, dried under gentle filtered air flow and stored at -20 °C until HPLC analysis.

Bioavailability Assay in Cynomolgus Monkeys

Within 1 week prior to the study initiation, the cynomolgus monkey is surgically implanted with a chronic venous catheter and subcutaneous venous access port (VAP) to facilitate blood collection and underwent a physical examination including hematology and serum chemistry evaluations and the body weight was recorded. Each monkey (six total) receives approximately 250 μCi of ^3H -labeled compound combined with each dose of active compound at a dose level of 10 mg/kg at a dose concentration of 5 mg/mL, either via an intravenous bolus (3 monkeys, IV), or via oral gavage (3 monkeys, PO). Each dosing syringe is weighed before dosing to gravimetrically determine the quantity of formulation administered. Urine samples are collected via pan catch at the designated intervals (approximately 18-0 hours pre-dose, 0-4, 4-8 and 8-12 hours post-dosage) and processed. Blood samples are collected as well (pre-dose, 0.25, 0.5, 1, 2, 3, 6, 8, 12 and 24 hours post-dosage) via the chronic venous catheter and VAP or from a peripheral vessel if the chronic venous catheter procedure should not be possible. The blood and urine samples are analyzed for the maximum concentration (C_{max}), time when the maximum concentration is achieved (T_{max}), area under the curve (AUC), half life of the dosage concentration ($T_{1/2}$), clearance (CL), steady state volume and distribution (V_{ss}) and bioavailability (F).

Bone Marrow Toxicity Assay

Human bone marrow cells are collected from normal healthy volunteers and the mononuclear population are separated by Ficoll-Hypaque gradient centrifugation as described previously by Sommadossi J-P, Carlisle R. "Toxicity of 3'-azido-3'-deoxythymidine and 9-(1,3~dihydroxy-2-propoxymethyl)guanine for normal human hematopoietic progenitor cells in vitro" Antimicrobial Agents and Chemotherapy 1987; 31:452-454; and Sommadossi J-P, Schinazi RF, Chu CK, Xie M-Y. "Comparison of cytotoxicity of the (-)- and (+)-enantiomer of 2',3'-dideoxy-3'-thiacytidine in normal human bone marrow progenitor cells" Biochemical Pharmacology 1992; 44:1921-1925. The culture assays for CFU-GM and BFU-E are performed using a bilayer soft agar or methylcellulose method. Drugs are diluted in tissue culture medium and filtered. After 14 to 18 days at 37 °C in a humidified atmosphere of 5% CO_2 in air, colonies of greater than 50 cells are counted using an

inverted microscope. The results are presented as the percent inhibition of colony formation in the presence of drug compared to solvent control cultures.

Mitochondria Toxicity Assay

Fifty microliters of 2X drug dilutions were added per well in a 96 well plate. A “no drug” (media only) control was used to determine maximum amount of mitochondrial DNA produced and ribosomal DNA. 3TC @ 10 μ M was used as a negative control, and ddC @ 10 μ M was used as a toxic control. Ribosomal DNA levels were used to determine specific toxicity to mitochondria or generally cytotoxicity. HepG2 cells (5,000 cells/well at 50 μ l) were added to the plate. The plate was incubated at 37°C in a humidified 5% CO₂ atmosphere for 7 days. After incubation, the supernatant was removed and stored for lactic acid quantification, and total DNA was extracted from cells as described in the RNeasy 96 handbook (February 1999), pages 22-23. No DNA digestions were performed, therefore total RNA and DNA were extracted.

The extracted DNA was amplified and the change in mitochondrial DNA and ribosomal DNA for each sample was determined. The fold difference in mitochondrial DNA normalized for ribosomal DNA relative to control was calculated.

Lactic acid quantification was performed by the D-Lactic Acid/ L-Lactic acid test kit (Boehringer Mannheim/ R-Biopharm/ Roche). The total amount of lactic acid produced for each sample was found as well as the fold change in lactic acid production (% of lactic acid / % of rDNA) as described in the manufacturers instructions.

Cytotoxicity Assay

50 μ l of 2X drug dilutions were added per well in a 96 well plate. Final concentrations of drug ranged from 1 to 100 μ M. A “no drug” (media only) control was used to determine the minimum absorbance values and a “cells + media only” control was used for maximum absorbance value. A solvent control was also used. Cells were then added (PBM: 5 x 10⁴ cells/well; CEM : 2.5 x 10³ cells/well; Vero, HepG2, Huh-7, and Clone A: 5 x 10³ cells/well) and incubated at 37°C in a

humidified 5% CO₂ atmosphere for 3-5 days (PBM :5 days; CEM : 3 days, all others :4 days). After incubation, 20 µl of MTS dye was added from Cell Titer Aqueous One Solution Cell Proliferation Assay to each well and the plate was re-incubated for 2-4 hours. The absorbance (490 nm) was then read on an ELISA plate reader using the media only/ no cell wells as blanks. Percent inhibition was found and used to calculate the CC₅₀.

In vivo Toxicity in Mice

In vivo toxicity was also determined following injections into female Swiss mice of the various nucleosides disclosed in the present invention. Intraperitoneal injections were given on days 0, day 1, day 2, day 3, and day 5 of varying doses of the particular nucleoside. Separate animals were injected with vehicle as control groups. In these studies, each dosing group contained 5-10 mice. The average weight change in each of the mice was measured as a sign of toxicity of the compound.

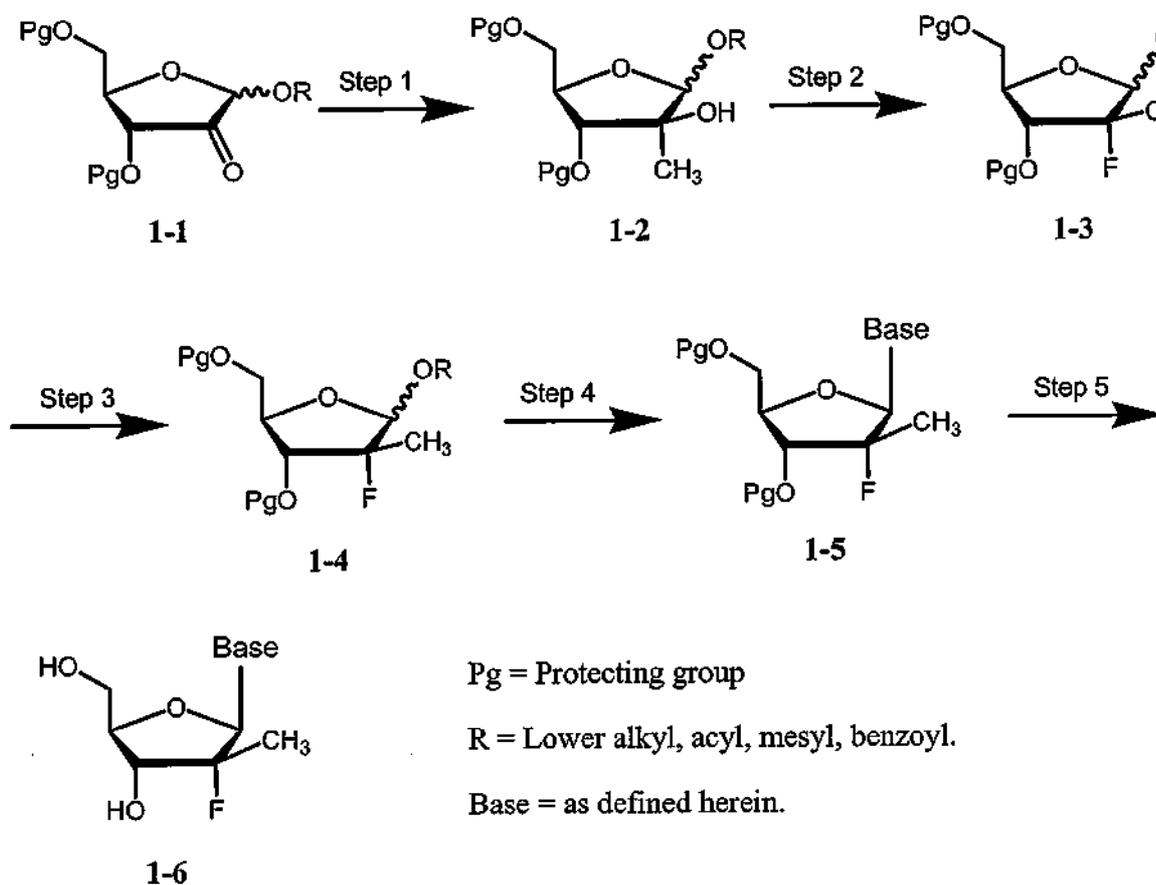
(BVDV) Yield Reduction Assay]

Madin-Darby Bovine Kidney (MDBK) cells were grown in Dulbecco's modified eagle medium supplemented with 10% horse serum and 100µg/ml penicillin-streptomycin. Cells were seeded in a 96-well plate at 5×10^3 cells /well and incubated for 72h at 37°C in a humidified 5% CO₂ atmosphere. Cells were infected with either cytopathic (NADL strain) or noncytopathic (SD-1 strain) BVDV at a virus dilution of 10⁻² and incubated for 45 min. Cell monolayers were washed three times with medium. Fresh medium-containing test compounds in dose response concentrations or ribavirin, as a positive control, were added to cultures and medium containing no drug was added to the no-drug controls. After 72h incubation, supernatant was collected and viral RNA was extracted using the QIAmp Viral RNA Mini Kit (Qiagen, CA). Viral load was determined by Q-RT-PCR using primers specific for either NADL or SD-1 (1).

VIII. Synthetic Protocol

The following non-limiting embodiments illustrate some general methodologies to obtain the nucleosides of the present invention. Two representative general methods for the preparation of compounds of the present invention are outlined in Schemes 1 and 2 while more specific examples of these general methods are provided in Scheme 3 (Example 1), Scheme 4 (Example 2),
5 Scheme 5 (Example 3), and Scheme 6 (Example 4). Scheme 1 represents a generalized process starting from a (2*R*) 2-deoxy-2-methyl-2-fluoro-carbohydrate and forms the nucleosides of the present invention by condensing with a nucleobase. Scheme 2 starts from a pre-formed, purine or pyrimidine nucleoside, optionally substituted at C-4' and constructs the C-2' (*R*) methyl, fluoro nucleosides of the
10 present invention. While these schemes illustrate the syntheses of compounds of the present invention of general formulas (I) and (II) wherein there is a furanose ring in the β -D-ribo configuration, this is not intended to be a limitation on the scope of the process invention in any way, and they should not be so construed. Those skilled in the art of nucleoside and nucleotide synthesis will readily appreciate that known
15 variations of the conditions and processes of the following preparative procedures and known manipulations of the nucleobase can be used to prepare these and other compounds of the present invention. Additionally, the L-enantiomers corresponding to the compounds of the invention can be prepared following the same methods, beginning with the corresponding L-carbohydrate building block or nucleoside L-
20 enantiomer as the starting material.

1. Glycosylation of the nucleobase with an appropriately modified sugar

Scheme 1

5 Step 1 in Scheme 1 introduces the 2-methyl group by using an appropriate
alkylating agent such as methyllithium, trimethylaluminum, or methylmagnesium
bromide in an anhydrous solvent such as tetrahydrofuran (THF), chloroform, or
diethyl ether. Compounds 1-1 through 1-4 can be purely α or β or they may exist as
an anomeric mixture containing both α and β anomers in any ratio. However, the
10 preferred anomeric configuration of structure 1-1 is β .

 Step 2 introduces the fluorine atom at the 2- position of the alkyl furanoside.
This can be achieved by treatment of the tertiary alcohol, 1-2, with a commercially
available fluorinating reagent such as (diethylamino)sulfur trifluoride (DAST) or
Deoxofluor in an anhydrous, aprotic solvent such as tetrahydrofuran, chloroform,
15 dichloromethane, or toluene. Preferably the stereochemistry proceeds with inversion
of configuration at C-2. That is, starting from a C-2 hydroxyl "up" (or

arabinofuranoside) in structure 1-2, the C-2 fluorine is "down" in the intermediate ribofuranoside 1-3.

In step 3, the optional protecting groups (Pg) can be deprotected and reprotected to groups more suitable for the remaining manipulations (T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999). For example, benzyl ethers (Bn) may be difficult to remove in the protected nucleoside, 1-5 and may be deprotected and replaced with a group more facile to remove from the nucleoside of structural type 1-5. Furthermore, the anomeric position (C-1) can also be optionally manipulated to a suitable group for the coupling reaction with the nucleobase (step 4). Several methods for anomeric manipulations are established to those skilled in the art of nucleoside synthesis. Some non-limiting examples by treatment of the alkyl furanoside (1-3, R = alkyl) with a mixture of acetic anhydride, acetic acid, and a catalytic amount of sulfuric acid (acetolysis) to provide structure 1-4 where R = Ac, with optional protecting groups. Also, the alkyl group in 1-3 may be converted to an acetate, benzoate, mesylate, tosylate, triflate, or tosylate, for example, by first hydrolyzing the 1-*Oalkyl* group to a 1-hydroxyl group by using a mineral acid consisting of but not limited to sulfuric acid, hydrochloric acid, and hydrobromic acid or an organic acid consisting of but not limited to trifluoroacetic acid, acetic acid, and formic acid (at ambient temperature or elevated temperature). The reducing sugar could then be converted to the desired carbohydrate by treatment with acetyl chloride, acetic anhydride, benzyol chloride, benzoic anhydride, methanesulfonyl chloride, triflic anhydride, triflyl chloride, or tosyl chloride in the presence of a suitable base such as triethylamine, pyridine, or dimethylaminopyridine.

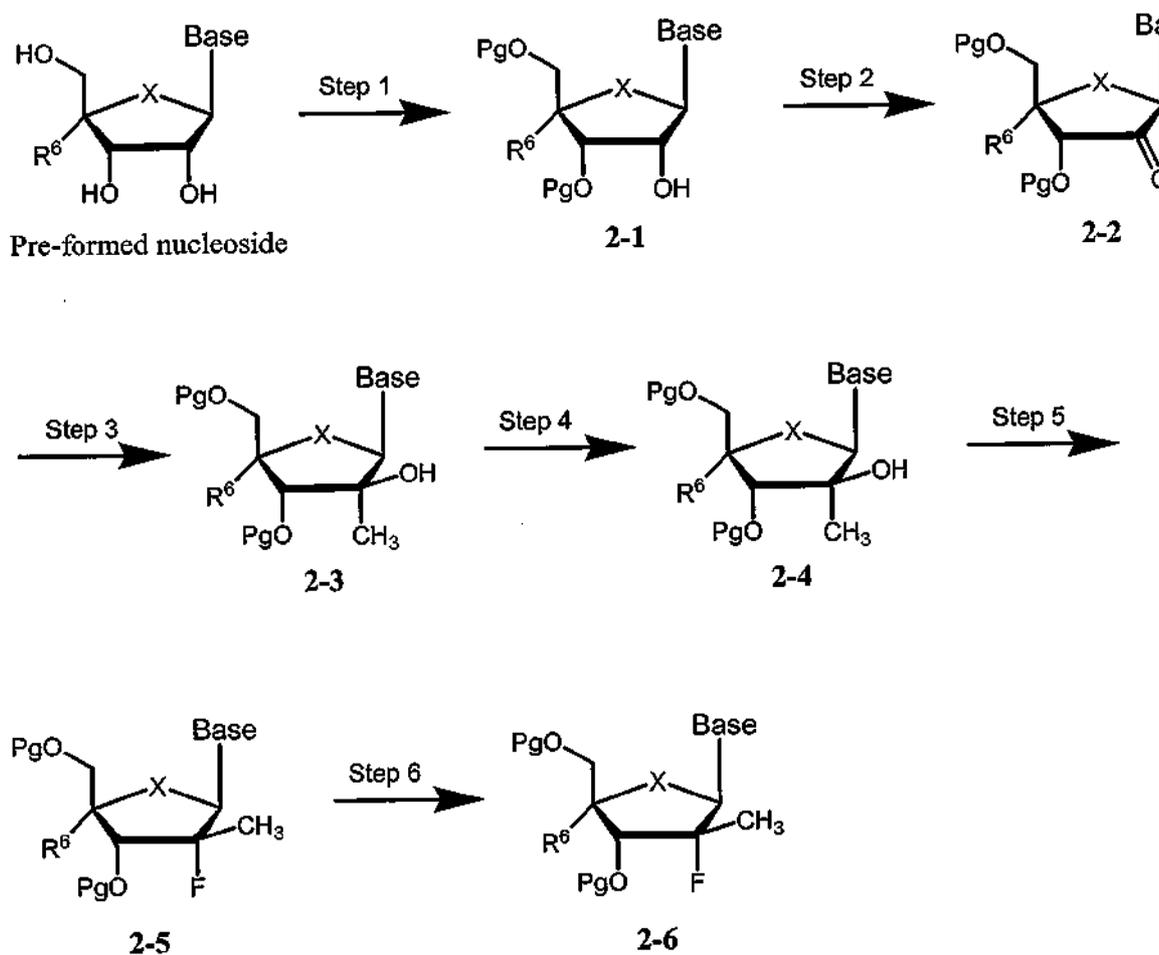
The nucleosidic linkage is constructed by treatment of intermediate 1-3 or 1-4 with the appropriate persilylated nucleobase in the presence of a Lewis acid such as tin tetrachloride, titanium tetrachloride, trimethylsilyltriflate, or a mercury (II) reagent (HgO/HgBr₂) usually at an elevated temperature in an aprotic solvent such as toluene, acetonitrile, benzene, or a mixture of any or all of these solvents.

The optional protecting groups in the protected nucleosides or structural formula 1-5 can be cleaved following established deprotection methodologies (T.W.

Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999).

2. Modification of a pre-formed nucleoside

Scheme 2



Pg = Protecting group

Base = as defined herein (optionally protected)

X = as defined herein

R⁶ = as defined herein

5

The starting material for this process is an appropriately substituted purine or pyrimidine nucleoside with a 2'-OH and 2'-H. The nucleoside can be purchased or

can be prepared by any known means including standard coupling techniques. The nucleoside can be optionally protected with suitable protecting groups, preferably with acyl or silyl groups, by methods well known to those skilled in the art, as taught by T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999.

The purine or pyrimidine nucleoside can then be oxidized at the 2'-position with the appropriate oxidizing agent in a compatible solvent at a suitable temperature to yield the 2'-modified nucleoside. Possible oxidizing agents are a mixture of dimethylsulfoxide, trifluoroacetic anhydride or acetic anhydride (a Swern/Moffat oxidation), chromium trioxide or other chromate reagent, Dess-Martin periodinane, or by ruthenium tetroxide/sodium periodate.

The optionally protected nucleoside 2'-ketone is then alkylated using such alkylating agents methyl lithium, trimethylaluminum, methylmagnesium bromide, or similar reagents in an anhydrous solvent such tetrahydrofuran (THF), chloroform, or diethyl ether usually at temperatures below 0 °C. Compounds of the structural formula 2-3 are preferred to have the 2'(*S*) or 2'-methyl "down", 2'-OH "up" configuration.

The nucleoside of structure 2-3 can be deprotected and reprotected with a number of protecting groups such as an *O*-acyl (alkyl or aryl), *O*-sulfonyl, or an *N*-acyl (alkyl or aryl) for the base. This optional reprotection step need not be limited to protecting groups that function as chemical protecting groups. Other protecting groups such as long chain acyl groups of between 6 and 18 carbon units or amino acids can be introduced independently on the nucleobase or the sugar. The protecting groups can serve as prodrugs of the active substance.

Step 5 introduces the fluorine atom at the 2' position of the pre-formed nucleoside. This can be achieved by treatment of the tertiary alcohol, 2-4, with a commercially available fluorinating reagent such as (diethylamino)sulfur trifluoride (DAST) or Deoxofluor in an anhydrous, aprotic solvent such as tetrahydrofuran, chloroform, dichloromethane, or toluene. Preferably the stereochemistry proceeds with inversion of configuration at the 2' position. That is, starting from a C-2' hydroxyl "up" (or arabinonucleoside) in structure 2-4, the C-2' fluorine is "down" in

the intermediate nucleoside 2-5. The absolute configuration of a nucleoside of structure 2-4 is (2'S) while the absolute configuration of a nucleoside of structure 2-5 is (2'R).

5 Subsequently, the nucleosides of structural type 2-5 can be deprotected by methods well known to those skilled in the art, as taught by T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999.

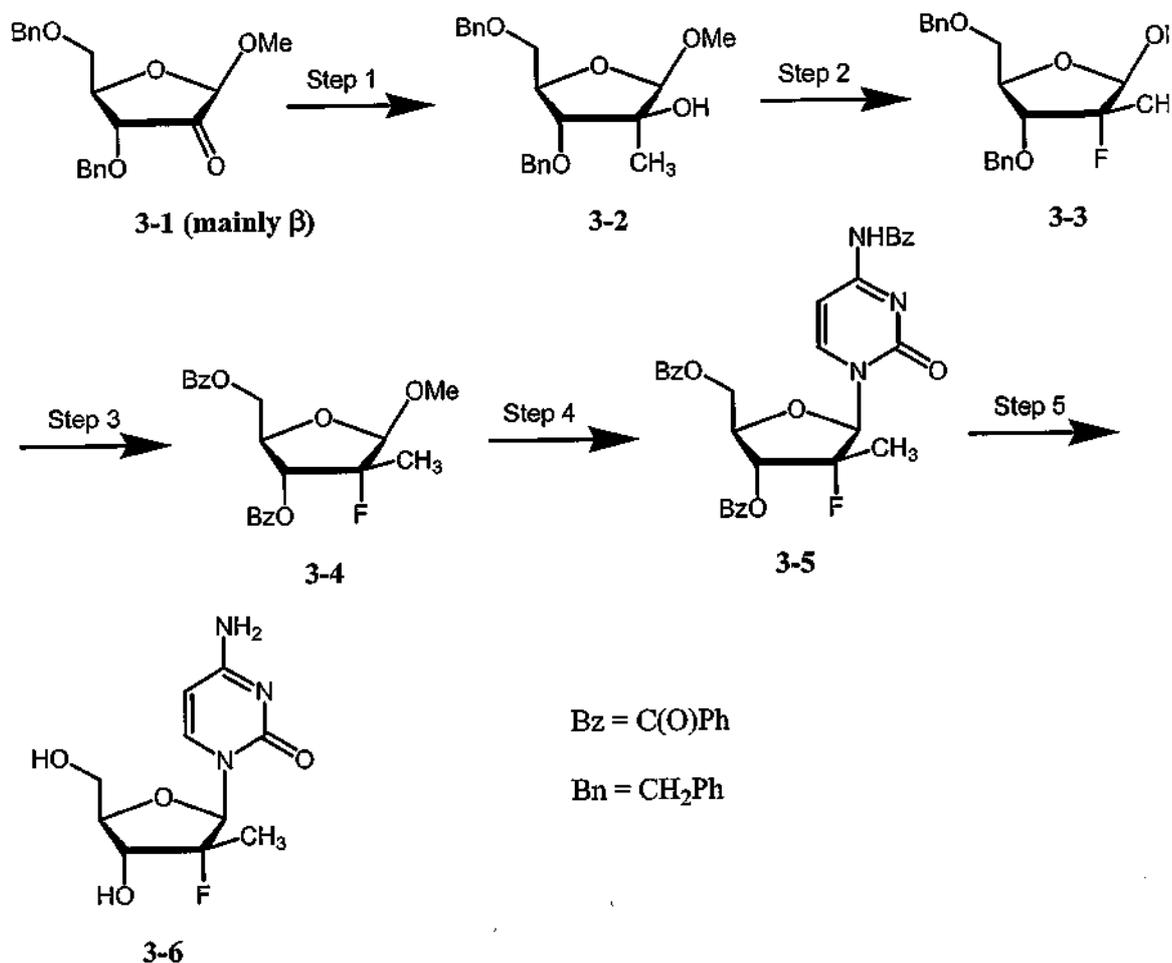
10 The following working examples provide a further understanding of the method of the present invention and further exemplify the general examples in Schemes 1 and 2 above. These examples are of illustrative purposes, and are not meant to limit the scope of the invention. Equivalent, similar or suitable solvents, reagents or reaction conditions may be substituted for those particular solvents, reagents or reaction conditions described without departing from the general scope of the method.

15

EXAMPLES

Example 1

Synthesis of (2'R)-2'-Deoxy-2'-Fluoro-2'-C-Methylcytidine Starting from a Carbohydrate

Scheme 3

Step 1: Compound 3-1 (7.7 g, 0.022 mmol) was dissolved in anhydrous diethyl ether and cooled to -78 °C. To this solution was added MeLi (30 mL, 1.6 M in diethyl ether). After the reaction was complete, the mixture was treated with ammonium chloride (1 M, 65 mL) and the organic phase was separated, dried (Na₂SO₄), filtered, and concentrated to dryness. Silica gel chromatography followed by crystallization from diethyl ether-hexanes afforded pure compound 3-2 (6.31 g).

¹H NMR (400 MHz, CDCl₃): δ 1.40 (s, 3H), 3.41 (s, 3H), 3.49 (dd, 1H, $J = 10.3, 6.89$ Hz), 3.57 (dd, 1H, $J = 10.3, 3.88$ Hz), 3.84 (d, 1H, $J = 7.3$ Hz), 4.03 (m, 1H), 4.48 (s, 1H), 4.58 (m, 3H), 4.83 (d, 1H, $J = 11.6$ Hz), 7.31–7.36 (m, 10H); ¹³C NMR (100 MHz, CDCl₃): δ 18.4, 55.4, 72.2, 73.4, 79.5, 80.2, 84.7, 107.4, 127.7, 127.8, 127.83, 128.5, 138.2, 138.3.

Step 2: Compound 3-2 was dissolved in CH₂Cl₂ and was treated with DAST (4.0 mL, 30.3 mmol) at room temperature. The solution was stirred at room temp overnight. The so-obtained mixture was poured into sat NaHCO₃ (100 mL) and washed with sat NaHCO₃ (1 x 15 mL). The organic layer was further worked up in the usual manner. Silica gel chromatography (1:5 EtOAc-hexanes) gave crude compound 3-3 (0.671 g) that was sufficiently pure for the next step. ¹H NMR (400 MHz, CDCl₃): δ 1.43 (d, 3H, *J* = 22.8 Hz), 3.35 (s, 3H), 3.49 (dd, 1H, *J* = 10.5, 5.4 Hz), 3.55 (dd, 1H, *J* = 10.5, 4.1 Hz), 3.87 (dd, 1H, *J* = 23.5, 7.5 Hz), 4.26 (m, 1H), 4.56 (d, 2H, *J* = 6.9 Hz), 4.66 (d, 2H, *J* = 8.2 Hz), 4.72 (d, 1H, *J* = 10.8 Hz), 7.29–7.36 (m, 10H); ¹³C NMR (100 MHz, CDCl₃): δ 17.0 (d, *J* = 24.4 Hz), 55.2, 77.1, 73.4, 73.8, 77.3, 80.3, 81.2 (d, *J* = 16 Hz), 99.7 (d, *J* = 178.9 Hz), 106.8 (d, *J* = 32.0 Hz), 127.7, 127.8, 128.1, 128.3, 128.5, 128.6, 137.8, 138.3; ¹⁹F NMR (100 MHz, CDCl₃): δ -8.2 (m, 1F).

Step 3: Compound 3-3 (0.39 g, 1.1 mmol) was dissolved in 1:2 EtOH-EtOAc and treated with Pd/C (~0.1 g) and cyclohexene (~1 mL). The mixture was heated to reflux overnight and then filtered through celite. The solvent was removed *in vacuo* and the residue was dissolved in pyridine (~5 mL). To this solution was added benzoyl chloride (0.22 mL, 1.83 mmol) and the mixture was stirred at room temp overnight. The pyridine was removed *in vacuo* and the residue was partitioned between CH₂Cl₂ and sat NaHCO₃ (10.0 mL). The organic phase was dried (Na₂SO₄), filtered, and the solution was concentrated to dryness. Column chromatography provided 0.350 g of pure compound 3-4. ¹H NMR (400 MHz, CDCl₃): δ 1.53 (d, 3H, *J* = 22.4 Hz), 3.39 (s, 3H), 4.46 (dd, 1H, *J* = 11.6, 4.7 Hz), 4.58 (m, 1H), 4.65 (dd, 1H, *J* = 11.6, 3.9 Hz), 4.87 (d, 1H, *J* = 9.9 Hz), 5.64 (dd, 2H, *J* = 24.1, 7.8 Hz), 7.29–7.36 (m, 10H); ¹⁹F NMR (100 MHz, CDCl₃): δ -7.5 (m, 1F).

Step 4: A solution of bis(trimethylsilyl)-*N*-benzoylcytosine (0.28 g, 0.77 mmol) and compound 3-4 (0.20 g, 0.5 mmol) in 1,2 dichloroethane (2 mL) and toluene (2 mL) was treated with TMSOTf (0.15 mL, 0.77 mmol). After most of the starting material disappeared as judged by TLC, the solution was cooled to room temp, washed with water (1 x 5 mL), brine (1 x 5 mL), dried (Na₂SO₄), filtered, and concentrated to dryness. Flash chromatography followed by crystallization from CH₂Cl₂-hexanes afforded compound 3-5 (68 mg). mp 241 °C; ¹H NMR (400 MHz,

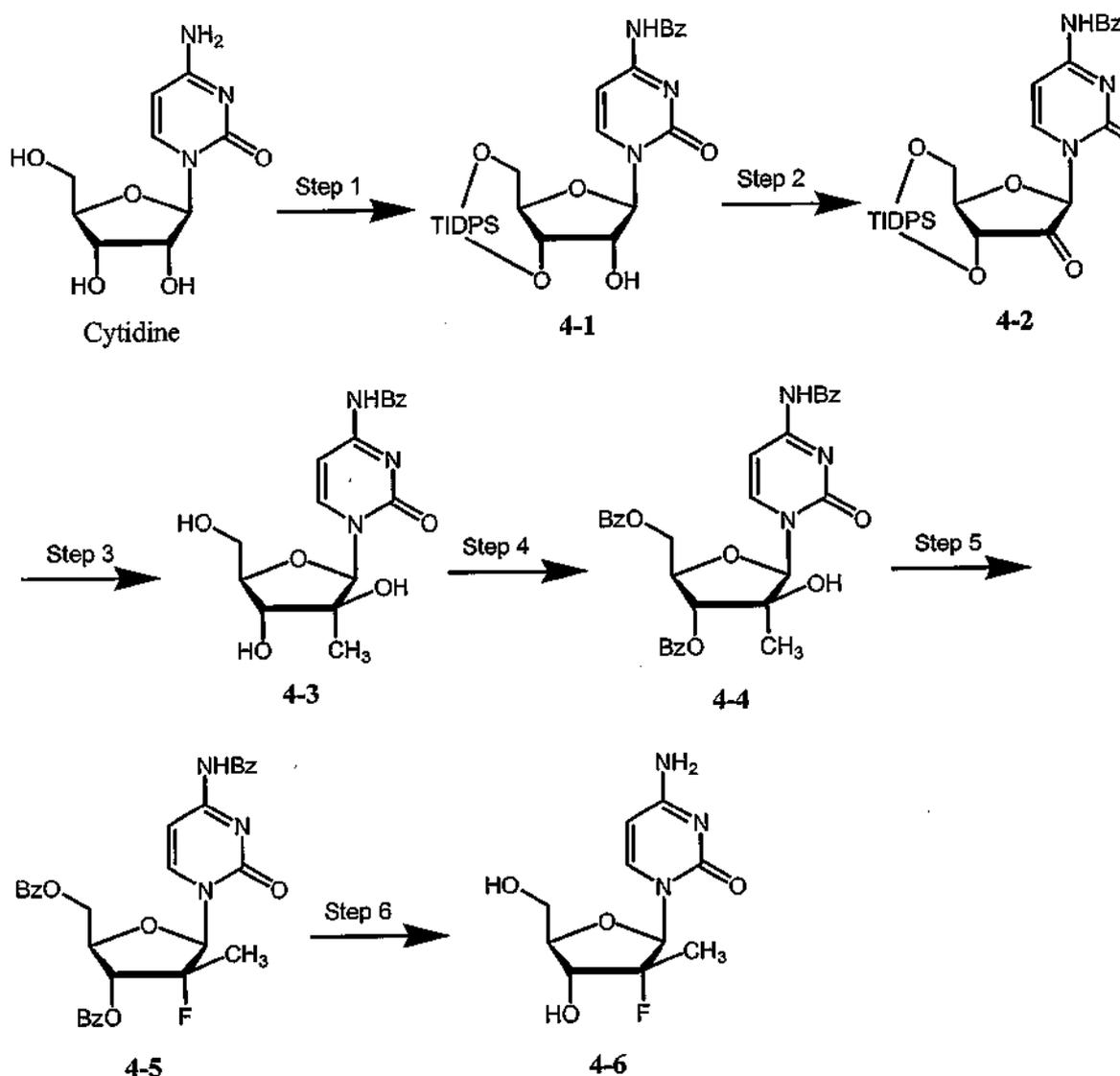
CDCl₃): δ 1.49 (d, 3H, *J* = 22.4 Hz), 4.64 (dd, 1H, *J* = 12.9, 3.4 Hz), 4.73 (app d, 1H, *J* = 9.5 Hz), 4.89 (dd, 1H, *J* = 12.7, 2.2 Hz), 5.56 (dd, 1H, *J* = 20.7, 8.6 Hz), 6.52 (d, 1H, *J* = 15.9 Hz), 7.38–7.67 (m, 10H), 7.89 (d, 2H, *J* = 6.9 Hz), 8.07–8.11 (m, 5H), 8.67 (s, 1H); ¹⁹F NMR (100 MHz, CDCl₃): δ 2.85 (m, 1F).

- 5 **Step 5:** Compound 3-5 (40 mg, 0.05 mmol) was dissolved in methanolic ammonia and stirred at room temp for 48 h. The solution was concentrated to dryness and chromatographed (SiO₂) eluting with 1:4 EtOH-CH₂Cl₂. The yield was about 12 mg of pure (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methylcytidine, 3-6. ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.16 (d, 3H, *J* = 22.0 Hz), 3.61 (dd, 1H, *J* = 11.6, 5.2 Hz),
- 10 3.60–3.83 (m, 3H, *J* = 10.5, 5.4 Hz), 5.24 (s, 1H, exchangeable with D₂O), 5.59 (s, 1H, exchangeable with D₂O), 5.71 (d, 1H, *J* = 7.3 Hz), 6.08 (d, 1H, *J* = 19.0 Hz), 7.24 (d, 1H, *J* = 17.7 Hz, exchangeable with D₂O), 7.87 (d, 1H); ¹⁹F NMR (100 MHz, DMSO-*d*₆): δ 4.13 (m, 1F).

Example 2

- 15 *Synthesis of (2'*R*)-2'-Deoxy-2'-Fluoro-2'-C-Methylcytidine Starting from Cytidine*

Scheme 4



- 5 **Step 1:** To a suspension of cytidine (100 g, 0.411 mol) in DMF (2.06 L) is added benzoic anhydride (102.4 g, 0.452 mol). The mixture was stirred at room temperature for 20 h. The DMF was removed *in vacuo* and the residue was triturated with diethyl ether. The resulting solid was collected by suction filtration and washed with diethyl ether (2 x 200 mL). Further drying *in vacuo* at room
- 10 temperature gave the *N*⁴ benzamide (140.6 g, 98.3%). A portion of this material

(139.3 g, 0.401 mol) was dissolved in anhydrous pyridine (1.2 L) and was treated with 1,3-dichloro-1,1,3,3-tetraisopropyl-disiloxane (141.4 mL, 0.441 mol) at room temp. The solution was stirred at room temperature overnight. The mixture was concentrated to near dryness *in vacuo* and coevaporated with toluene (3 x 200 mL).
5 The residue was treated with EtOAc (1.8 L) and washed with HCl (2 x 200 mL, 0.05 N), NaHCO₃ (5 %, 2 x 400 mL). The organic layer was washed dried (Na₂SO₄), filtered, and evaporated to dryness. Compound 4-1 (256.5 g, >100%) was isolated as a white foam and used without further purification.

Step 2: Compound 4-1 (236.5 g, 0.40 mol) was dissolved in dry THF (1.22
10 L). Anhydrous dmso (180.8 mL, 2.1 mol) was added and the resulting solution was cooled to between -20 °C and -15 °C. Trifluoroacetic anhydride (90.6 mL, 0.64 mol) was added dropwise over 45 minutes and the solution was stirred between -20 °C and -15 °C for 2 hrs after which anhydrous triethylamine (223.5 mL, 1.6 mol) was added over 20 min. The crude reaction containing ketone 4-2 was dissolved in
15 EtOAc (500 mL), and the resulting solution was washed with H₂O (3 x 400 mL), dried (Na₂SO₄) and the solvents were removed *in vacuo* to give a yellow solid that was purified on a silica gel column eluting with a stepwise gradient of Et₂O (0–60%) in hexanes followed by a stepwise gradient of EtOAc (50–100%) in hexanes. The crude ketone so-obtained (~192 g) was crystallized from petroleum ether to give
20 ketone 4-2 (138.91 g, 57.5% from cytidine) as a white solid and 22 g of unreacted starting material, 4-1, as a yellow solid.

Step 3: Compound 4-2 (48.57 g, 8.26 mmol) was dissolved in anhydrous toluene (~400 mL) and the solvent was removed *in vacuo* with exclusion of moisture. The residue was then further dried *in vacuo* (oil pump) for another 2 h.
25 With strict exclusion of moisture, the residual foam was dissolved in anhydrous diethyl ether (1.03 L) under argon. The resulting solution was cooled to -78 °C under argon and MeLi (1.6 M, 258.0 mL, 0.413 mol) was added dropwise *via* additional funnel. After the addition was complete, the mixture was stirred for 2 h at -78 °C. Aqueous 1 M NH₄Cl (500 mL) was added slowly. After warming to room
30 temperature, the mixture was washed with H₂O (2 x 500 mL), dried (Na₂SO₄), and then concentrated to dryness to give a brown foam (~60 g, >100%).

The reaction was performed two more times using 37.62 g and 56.4 g of compound 4-2. The combined crude products (128.0 g, 0.212 mol) were dissolved in THF (1.28 L) and treated with concd HOAc (23 mL, 0.402 mol). To the solution was added TBAF (384.0 mL, 1 M in THF). The solution was stirred at room temp
5 for 0.75 h and the mixture was treated with silica gel (750 g) and concentrated to dryness. The powder was placed on a silica gel column packed in CH₂Cl₂. Elution with 1:7 EtOH-CH₂Cl₂ afforded a dark waxy solid that was pre-adsorbed on silica gel (300 g) and chromatographed as before. Compound 4-3 (46.4 g, 53.0 % from 4-2) was isolated as an off-white solid. ¹H NMR (DMSO-d₆): δ 1.20 (s, 3H, CH₃),
10 3.62–3.69 (m, 2H), 3.73–3.78 (m, 2H), 5.19 (t, 1H, *J* = 5.4 Hz, OH-5'), 5.25 (s, 1H, OH-2'), 5.52 (d, 1H, *J* = 5.0 Hz, OH-3'), 5.99 (s, 1H, H-1'), 7.32 (d, 1H, *J* = 5.8 Hz), 7.50 (Ψt, 2H, *J* = 7.7 Hz), 7.62 (Ψt, 1H, *J* = 7.3 Hz), 8.00 (d, 2H, *J* = 7.3 Hz), 8.14 (d, 1H, *J* = 6.9 Hz), 11.22 (s, 1H, NH). Anal. Calcd for C₁₇H₁₉N₃O₆ • 0.5 H₂O: C, 55.13; H, 5.44; N, 11.35. Found: C, 55.21; H, 5.47; N, 11.33.

Step 4: Compound 4-3 (46.0 g, 0.13 mol) was dissolved in anhydrous pyridine and concentrated to dryness *in vacuo*. The resulting syrup was dissolved in anhydrous pyridine under argon and cooled to 0 °C with stirring. The brown solution was treated with benzoyl chloride (30 mL, 0.250 mol) dropwise over 10 min. The ice bath was removed and stirring continued for 1.5 h whereby TLC
20 showed no remaining starting material. The mixture was quenched by the addition of water (5 mL) and concentrated to dryness. The residue was dissolved in a minimal amount of CH₂Cl₂ and washed with satd NaHCO₃ (1 x 500 mL) and H₂O (1 x 500 mL). The organic phase was dried (Na₂SO₄) and filtered, concentrated to dryness and chromatographed on silica gel eluting with a stepwise gradient of
25 EtOAc-hexanes (25-60%) to provide compound 4-4 as yellow foam (48.5 g, 67%). ¹H NMR (CDCl₃): δ 1.64 (s, 3H, CH₃), 4.50 (m, 1H, H-4), 4.78–4.85 (m, 2H, H-5', 5a'), 5.50 (d, 1H, *J* = 3.4 Hz, H-3'), 6.42 (s, 1H, H-1'), 7.44–7.54 (m, 7H, Ar), 7.57–7.66 (m, 3H, Ar), 7.94 (d, 2H, *J* = 7.8 Hz), 8.05–8.09 (m, 4H, Ar), 8.21 (d, 1H, *J* = 7.3 Hz). Anal. Calcd for C₃₁H₂₇N₃O₈: C, 65.37; H, 4.78; N, 7.38. Found: C, 65.59; H, 4.79; N, 7.16.

Step 5: Compound 4-4 (7.50 g, 0.013 mol) was dissolved in anhydrous toluene (150 mL) under argon and cooled to -20 °C. DAST (2.5 mL, 18.9 mmol)

was added slowly and the cooling bath was removed after the addition was complete. Stirring was continued for 1 h and the mixture was poured into satd NaHCO₃ (100 mL) and washed until gas evolution ceased. The organic phase was dried (Na₂SO₄), concentrated, and purified by silica gel chromatography eluting with 1:1 EtOAc-hexanes. Yield was 1.22 g (16.3%) of pure 4-5 as a white solid. mp 241 °C (CH₂Cl₂-hexanes); ¹H NMR (CDCl₃): δ 1.49 (d, 3H, *J* = 22.4 Hz, CH₃), 4.64 (dd, 1H, *J* = 3.44, 12.9 Hz, H-5'), 4.73 (d, 1H, *J* = 9.5 Hz, H-4'), 4.90 (dd, 1H, *J* = 2.4, 12.7 Hz, H-5a'), 5.56 (dd, 1H, *J* = 8.6, 20.7 Hz, H-3'), 6.52 (d, 1H, *J* = 18.0 Hz, H-1'), 7.47–7.57 (m, 7H, Ar), 7.62–7.71 (m, 3H, Ar), 7.89 (d, 2H, *J* = 6.9 Hz), 8.07–8.11 (m, 5H, Ar), 8.67 (bs, 1H, NH). ¹⁹F NMR (CDCl₃): δ 3.3 (m). Anal. Calcd for C₃₁H₂₆FN₃O₇ • 0.7 H₂O: C, 63.74; H, 4.72; N, 7.20. Found: C, 63.71; H, 4.54; N, 7.20.

Step 6: Compound 4-5 (6.30 g, 0.011 mol) was suspended in methanolic ammonia (*ca* 7 N, 150 mL) and stirred at room temperature overnight. The solvent was removed *in vacuo*, co-evaporated with methanol (1 x 20 mL), and pre-adsorbed onto silica gel. The white powder was placed onto a silica gel column (packed in CHCl₃) and the column was eluted with 9% EtOH in CHCl₃, then 17% EtOH and finally 25% EtOH in CHCl₃. Concentration of the fractions containing the product, filtration through a 0.4 μm disk, and lyophilization from water afforded compound 4-6, 2.18 g (76%). ¹H NMR (DMSO-*d*₆): δ 1.17 (d, 3H, *J* = 22.3 Hz, CH₃), 3.63 (dd, 1H, *J* = 2.7, 13.7 Hz, H-5'), 3.70–3.84 (m, 3H, H-3', H-4', H-5a'), 5.24 (app s, 1H, OH-3'), 5.60 (d, 1H, *J* = 5.4 Hz, H-5'), 5.74 (d, 1H, *J* = 7.71 Hz, H-5), 6.07 (d, 1H, *J* = 18.9 Hz, H-1'), 7.31 (s, 1H, NH₂), 7.42 (s, 1H, NH₂), 7.90 (d, 1H, *J* = 7.3 Hz, H-6). ¹⁹F NMR (DMSO-*d*₆): δ 2.60 (m). Anal. Calcd for C₁₀H₁₄FN₃O₄ • 1.4 H₂O: C, 44.22; H, 5.95; N, 14.77. Found: C, 42.24; H, 5.63; N, 14.54. Compound 4-6 (0.10 g, 0.386 mmol) was converted to the hydrochloride salt by dissolving in water (2 mL) and adjusting the pH to approximately 3.0 with 1 M HCl. The water was removed *in vacuo* and the residue was crystallized from aqueous EtOH to give 4-6 as the hydrochloride salt (71.0 mg). mp 243 °C (dec); ¹H NMR (DMSO-*d*₆): δ 1.29 (d, 3H, *J* = 22.6 Hz, CH₃), 3.65 (dd, 1H, *J* = 2.3, 12.7 Hz, H-5'), 3.76–3.90 (m, 3H, H-3', H-4', H-5a'), 5.96 (d, 1H, *J* = 17.3 Hz, H-1'), 6.15 (d, 1H, *J* = 7.9 Hz, H-5), 8.33 (d, 1H, *J* = 7.9 Hz, H-6), 8.69 (s, 1.5H, NH), 9.78 (s, 1.5H, NH). ¹⁹F

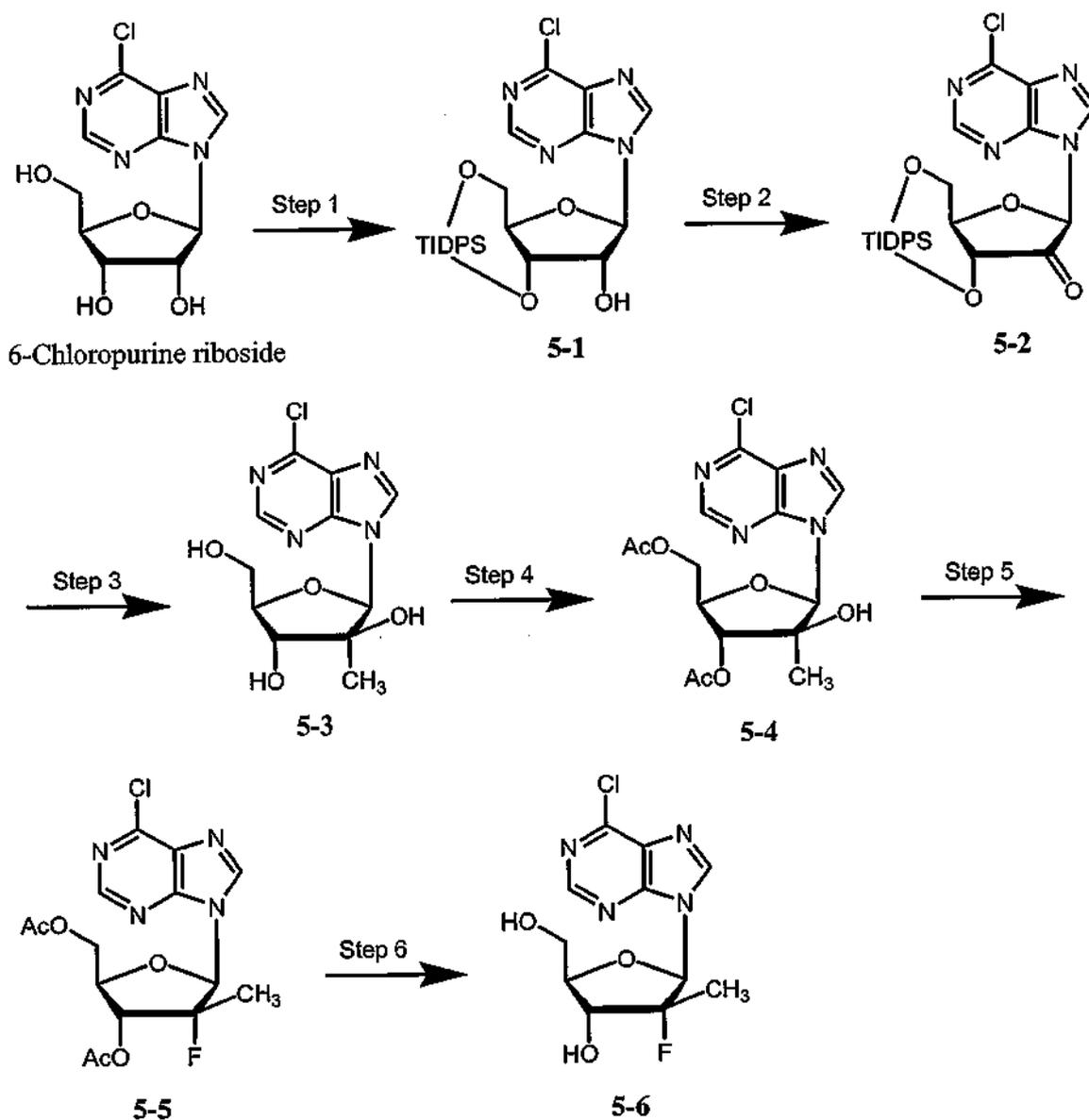
NMR (DMSO- d_6): δ 1.69 (m). Anal. Calcd for $C_{10}H_{14}FN_3O_4 \cdot HCl$: C, 40.62; H, 5.11; N, 14.21. Found: C, 40.80; H, 5.09; N, 14.23.

Example 3

Synthesis of (2'R)-6-Chloro-2'-Deoxy-2'-Fluoro-2'-C-Methylpurine Starting from 6-Chloropurine Riboside.

5

Scheme 5



TIDPS = 1,3-(1,1,3,3-Tetraisopropylidisiloxanylidene)

Step 1: The nucleoside, 6-chloropurine riboside, (3.18 g, 11.09 mmol) was dissolved in anhydrous pyridine (300 mL) and was treated dropwise with 1,3-dichloro-1,1,3,3-tetraisopropyl-disiloxane (4.08 mL, 12.75 mmol) at 0 °C under an argon atmosphere. The solution was brought to room temp and stirred overnight. The mixture was concentrated to near dryness *in vacuo*, dissolved in a minimal amount of chloroform, and washed with HCl (100 mL, 0.05 N) and NaHCO₃ (5%, 100 mL). The organic layer was dried (Na₂SO₄), filtered, and evaporated to dryness to afford compound 5-1 as an amber glass (6.10 g, >100%) that was used without further purification. ¹H NMR (CDCl₃): δ 1.01–1.13 (m, 24H), 4.03–4.18 (m, 3H), 4.58 (d, 1H, *J* = 5.2 Hz), 5.01 (m, 1H), 6.07 (s, 1H), 8.31 (s, 1H), 8.71 (s, 1H).

Step 2: Compound 5-1 (7.13 g, 13.47 mmol) was dissolved in dry THF (35 mL). Anhydrous DMSO (5.11 mL, 72.06 mmol) was added and the resulting solution was cooled to between -20°C and -15°C. Trifluoroacetic anhydride (3.06 mL, 21.69 mmol) was added dropwise over 45 minutes and the solution was stirred between -20 °C and -15 °C for 2 hrs after which anhydrous triethylamine (8.08 mL, 57.92 mmol) was added over 20 min. The crude reaction containing ketone 5-2 was dissolved in Et₂O (25 mL), and the resulting solution was washed with H₂O (2 x 50 mL), dried (Na₂SO₄) and the solvents were removed *in vacuo* to give a yellow solid that was purified on a silica gel column eluting with a stepwise stepwise gradient of 0–50% petroleum ether-diethyl ether afforded compound 5-2 as a mixture with the corresponding geminal diol. The glass was dissolved in CH₂Cl₂ and stirred over an excess of MgSO₄ for 36 h. The mixture, free from the geminal diol, was filtered, and evaporated to dryness to afford compound 5-2 as an amber glass (7.0 g, 97%). ¹H NMR (CDCl₃): δ 1.01–1.13 (m, 24H), 4.09–4.22 (m, 3H), 5.55 (d, 1H, *J* = 9.6 Hz), 5.80 (s, 1H), 8.19 (s, 1H), 8.61 (s, 1H).

Step 3: A solution of compound 5-2 (7.0 g, 13.26 mmol) in anhydrous tetrahydrofuran (45 mL) was cooled to -78 °C with stirring under an argon atmosphere. To the solution was added methylmagnesium bromide (15.85 mL, 3.0 M in ethyl ether) dropwise over a 30 min period. After stirring for an additional 3 h at -78 °C, the reaction was quenched by the careful addition of aqueous 1 M NH₄Cl (50.0 mL). After warming to room temperature, the mixture was washed with H₂O

(2 x 500 mL), dried (Na_2SO_4), and concentrated to dryness to give a brown foam (3.8 g) that was dissolved in tetrahydrofuran (50 mL) and treated with a solution of TBAF (18.9 mL, 1 M solution in THF) and glacial acetic acid (0.85 mL) at room temp. The solution was stirred at room temp for 2h, concentrated to dryness, and purified by silica gel chromatography to give compound 5-3 (2.0 g, 50%).

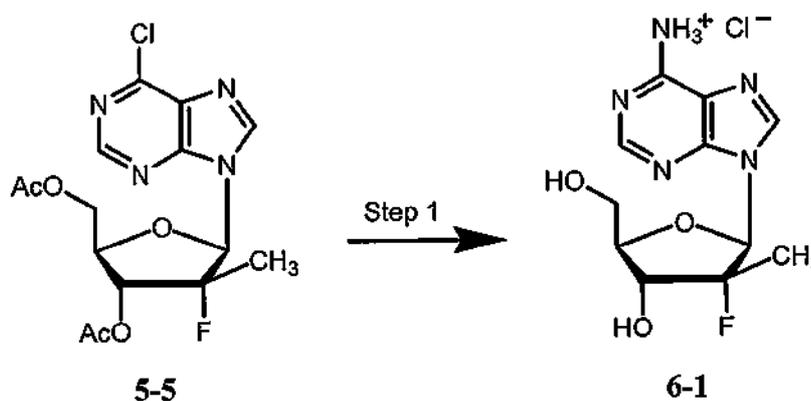
Step 4: Compound 5-3 (0.491 g, 1.63 mmol) was dissolved in pyridine (3 mL) and treated with acetic anhydride (0.38 mL, 4.08 mL) at room temp. The solution was stirred at room temp for 2 h after which time, the solution was concentrated to dryness and treated with diethyl ether (10 mL) and water (5 mL). The organic layer was further washed with water (2 x 10 mL), dried (Na_2SO_4), filtered, and evaporated to dryness to give compound 5-4 as a foam (0.450 g, 91.0%). ^1H NMR (CDCl_3): δ 1.39 (s, 3H), 2.15 (s, 3H), 2.21 (s, 3H), 4.27 (m, 1H), 4.49 (dd, 1H, $J = 4.2, 11.9$ Hz), 4.57 (dd, 1H, $J = 6.16, 11.9$ Hz), 5.14 (d, 1H, $J = 3.1$ Hz), 6.25 (s, 1H), 8.54 (s, 1H), 8.75 (s, 1H).

Step 5: Compound 5-4 (0.100 g, 0.259 mmol) was dissolved in anhydrous toluene (3.0 mL) under argon and cooled to -20 °C. DAST (0.2 mL, 1.55 mmol) was added slowly and the cooling bath was removed after the addition was complete. Stirring was continued for 1 h and the mixture was poured into satd NaHCO_3 (100 mL) and washed until gas evolution ceased. The organic phase was dried (Na_2SO_4), concentrated, and purified by silica gel chromatography eluting with 30% Et_2O -petroleum ether gave pure 5-5 (0.028 g, 27.9%). ^1H NMR (CDCl_3): δ 1.24 (d, 3H, $J = 22.8$ Hz), 2.20 (s, 3H), 2.22 (s, 3H), 4.41–4.55 (m, 3H), 4.47 (dd, 1H, $J = 9.2, 22.0$ Hz), 6.37 (d, 1H, $J = 17.6$ Hz), 8.45 (s, 1H), 8.82 (s, 1H).

Step 6: Compound 5-5 (0.018 g, 0.047 mmol) was dissolved in methanol (5 mL) and treated with a solution of sodium methoxide (3.6 mg, 0.67 mmol) in methanol (5 mL). The solution was stirred at room temp for 1 h, neutralized with concd acetic acid and chromatographed on silica gel eluting with a stepwise gradient of Et_2O /methanol (0-5%) to afford compound 5-6 (0.010 g, 70.9%). ^1H NMR (CDCl_3): δ 1.23 (d, 3H, $J = 22.4$ Hz), 4.04 (dd, 1H, $J = 2.11, 12.5$ Hz), 4.17 (dd, 1H, $J = 1.5, 9.2$ Hz), 4.25 (dd, 1H, $J = 1.9, 12.3$ Hz), 4.61 (dd, 1H, $J = 9.2, 22.3$ Hz), 6.37 (d, 1H, $J = 17.3$ Hz), 8.70 (s, 1H), 8.78 (s, 1H).

Example 4

Synthesis of (2'R)-2'-Deoxy-2'-Fluoro-2'-C-Methyladenosine Starting from (2'R)-6-Chloro-2'-Deoxy-2'-Fluoro-2'-C-Methylpurine

Scheme 6

5

Step 1: Compound **5-5** (0.100 g, 0.26 mmol) was heated in a pressure tube with methanolic ammonia (ca. 7 N, 25 mL) at 80 °C for 12 h. The crude reaction was pre-adsorbed onto silica gel and purified by column chromatography eluting with a stepwise gradient of Et₂O-MeOH (0-5%). The impure product was converted to the hydrochloride salt by dissolving the compound in a minimal amount of ethanol and treating the solution with 0.5 mL of a 0.6 M HCl solution. Concentration to near dryness gave compound **6-1** as off-white crystals (0.020g, 24.2%). ¹H NMR (CD₃OD): δ 1.19 (d, 3H, *J* = 22.3 Hz), 3.88 (dd, 1H, *J* = 2.7, 12.7 Hz), 4.06 (dd, 1H, *J* = 2.1, 12.5 Hz), 4.11 (app d, 1H, *J* = 9.2 Hz), 4.35 (dd, 1H, *J* = 9.4, 24.5 Hz), 6.35 (d, 1H, *J* = 16.5 Hz), 8.43 (s, 1H), 8.85 (s, 1H).

15

Example 5

Antiviral Activity of (2'R)-2'-Deoxy-2'-Fluoro-2'-C-Methylcytidine

HCV Replicon Assay

The anti-flavivirus activity of the compounds was determined as described by Stuyver, et al. ("Ribonucleoside analogue that blocks replication of bovine viral diarrhea and hepatitis C viruses in culture", *Antimicrobial Agents and Chemotherapy* 47:244-254 (2003)). The compound was dissolved in DMSO and

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added to the culture media at final concentrations ranging from 3 to 100 μM . A 4-days incubation resulted in dose-dependant reduction of the replicon HCV RNA (Figure 1A). A 1-log reduction of replicon RNA (or EC_{90} value) was reached at approximately 2.5 μM . Measurement of the reduction of rRNA gave an indication of the inhibitory effect on cellular polymerases. Subtraction of this cellular toxicity value from the antiviral values resulted in the therapeutic index line and EC_{90} value. Based on these calculations, an average EC_{90} value, corrected for cellular toxicity, of approximately 2.5 μM was obtained. Figure 1A shows the dose-dependant reduction of the replicon HCV RNA based on the treatment with (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine. The viral reduction was compared to the reduction of cellular RNA levels (ribosomal RNA) to obtain therapeutic index values. EC_{90} represents the effective concentration 90% at 96 hours following the dose dependant administration of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine. Figure 1B shows the prolonged reduction in replicon HCV RNA up to 7 days following treatment with 5 and 25 μM .

The activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine in the replicon system is summarized in Table 1. The EC_{90} values for (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine as well as 2'-C-methylcytidine and 2'-C-methyladenosine are shown for three separate replicon clones (HCV-WT (Wild Type), 9-13 and 21-5) as well as two other clones (S282T and rRNA). The EC_{90} values for (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine were in the range of 1.6 to 4.6 μM for the replicon clones. In contrast the EC_{90} values for 2'-C-methylcytidine were in the range of 6.6-37.4 μM . Interestingly, the EC_{90} values for 2'-C-methyladenosine were comparable to those of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine. The activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine and 2'-C-methylcytidine in other replicons tested is shown in Table 2.

Polymerase Assay

Table 3 shows the potency of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine-5'-triphosphate (TP) in the NS5B polymerase assay. The inhibitory concentration 50% was determined to be in the range of 1.7 to 7.7 μM .

Toxicity

A summary of the toxicity data for (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine using the mitochondrial toxicity assay is shown in Tables 6 and 7. Table 7 shows the lack of effects of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine and 2'-C-methylcytidine on mitochondrial DNA synthesis and lack of effects on lactic acid increase in this assay. Results shows the relative lack of toxicity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine. Table 6 shows a cytotoxicity analysis in various cell lines (Clone A, Huh7, HepG2, MDBK, PBM, CEM, Vero, MRC-5). Cytotoxic concentration 50% (CC₅₀) was greater than 75-100 μ M in all clones tested for (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine as well as 2'-C-methylcytidine. In contrast is the relative toxicity of 2'-C-methyladenosine.

The effects the nucleoside analogs tested on human bone marrow cells is depicted in Table 9. As shown, the IC₅₀ values for 2'-methyl-2'-fluorocytidine were significantly higher (98.2, BFU-E) and 93.9 (CFU-GM) as compared to 2'-methylcytidine or AZT. Results show that 2'-methyl-2'-fluorocytidine was significantly less toxic than compared to the other nucleoside compounds.

Animal Studies

Figure 2 depicts the average weight change (%) of female Swiss mice in vivo the toxicity analysis of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine at various doses. Intraperitoneal injections were given on days 0 to day 5 of the 0, 3.3, 10, 33, 100 mg/kg. Each dosing group contained 5 mice and no mice died during the 30-day study. No significant toxicity was observed in the mice.

Figure 3 and Table 6 summarize the pharmacokinetic parameters of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine in Rhesus monkeys given a single dose (33.3 mg/kg) oral (Table 6, Figure 3) or intravenous dose (Figure 3) of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine.

Other Antiviral Activity

Summary of the range of antiviral activity of (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methylcytidine is shown in Table 4. Table shows that in addition to HCV virus (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methylcytidine shows activity against Rhinovirus, West Nile virus, Yellow Fever virus, and Dengue virus.

5 Table 5 shows the lack of activity of (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methylcytidine on HCV surrogate models BVDV as well as other viruses including HIV, HBV and Corona virus. In contrast, 2'-*C*-methylcytidine and 2'-*C*-methyladenosine show greater activity in the HCV surrogate model, BVDV. These results show the necessity for screening this series of compounds against the HCV replicon system versus surrogate HCV systems.

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Table 1: Summary of the Anti-HCV Replicon Activity of (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methylcytidine*

Replicon	(2' <i>R</i>)-2'-deoxy-2'-fluoro-2'- <i>C</i> -methylcytidine	2'- <i>C</i> -methylcytidine	2'- <i>C</i> -methyladenosine
HCV-WT 1b	4.6 ± 2.0	21.9 ± 4.3	2.1 ± 0.27
S282T mut. 1b	30.7 ± 11.7	37.4 ± 12.1	>100
9-13 (subgenomic)	4.6 ± 2.3	13.0	0.7
21-5 (full-length)	1.6 ± 0.7	6.6	0.6

* Values represent EC₉₀ (μM)

Table 2: Activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine and 2'-C-methylcytidine in other Replicons

Replicon	(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine			2'-C-methylcytidine		
	EC ₉₀ (μ M)	IC ₉₀ (μ M)		EC ₉₀ (μ M)	IC ₉₀ (μ M)	
		GAPDH	MTT		GAPDH	MTT
1b (Ntat)	3.8	>100	>100	27.2	>100	>100
1b (Btat)	11.5	>100	>100	31.1	>100	>100
1a (pp1aSI-7)	34.7	>100	>100	35.0	>100	>100

Table 3: HCV 1b NS5B Polymerase Assay (IC₅₀, μ M)

	(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine TP	2'-C-methylcytidine TP	2'-C-methyladenosine TP
Wild-Type NS5B	1.7 \pm 0.4 ^a	6.0 \pm 0.5	20.6 \pm 5.2
	7.7 \pm 1.2 ^b		
S282T	2.0 ^a	26.9 \pm 5.5	>100
	8.3 \pm 2.4 ^c		

5 ^a Values determined using batch 1; ^b Value determined using batch 2 and 3; and ^c Value determined using batch 2.

Table 4: Summary of Antiviral Activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine

Virus	Cell	EC ₅₀ , CPE (μ M)	EC ₅₀ , NR ^a (μ M)	CC ₅₀ , CPE (μ M)	CC ₅₀ , NR ^a (μ M)
West Nile	Vero	32	12	>100	32
Dengue Type 2	Vero	32/55	>100/>100	>100	>100
Yellow Fever	Vero	19/3.2	32/12	>100	>100
Influenza A (H1N1)	MDCK	>100	>100	>100	>100
Influenza A (H3N2)	MDCK	>100	>100	>100	>100
Influenza B	MDCK	>100	>100	>100	>100
Rhinovirus Type 2	KB	25	20	>100	>100
VEE	Vero	>100	>100	>100	>100
SARSCoV	Vero	>100	>100	>100	>100

^aNR = Neutral Red.

5

Table 5: Summary of Antiviral Activity of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine

Virus	(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine (EC ₉₀ , μ M)	2'-C-methylcytidine (EC ₉₀ , μ M)	2'-C-methyladenosine (EC ₉₀ , μ M)
BVDVncp	>22	0.5	1.2
BVDVcp	>100	2	1.5
RSV	>100	>100	>100
HIV ^a	>100	ND	ND
HBV	>10	>10	ND
Coronavirus 229E	>100	ND	ND

ND = Not determined.

Table 6: Cytotoxicity Studies^a

Cell Line	(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine CC ₅₀ , μ M	2'-C-methylcytidine CC ₅₀ , μ M	2'-C-methyladenosine CC ₅₀ , μ M
CloneA	>100	>100	37
Huh7	>100	>100	30
HepG2	75	>100	58
MDBK	>100	>100	
PBM	>100		
CEM	>100		
Vero	>100		
MRC-5	>100		

^aResults determined using MTS assay.

5

Table 7: Mitochondrial Toxicity Study

Compound	mtDNA Synthesis (IC ₅₀ , μ M)	Lactic Acid Increase
(2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine	>25	No effect $\geq 33\mu$ M
2'-C-methylcytidine	>25	No effect $\geq 33\mu$ M

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Table 8: Preliminary PK Parameters in Rhesus Monkeys Following a Single Oral Dose of (2'R)-2'-deoxy-2'-fluoro-2'-C-methylcytidine at 33.3 mg/kg

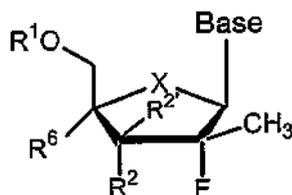
Parameter	Units	Mean \pm SD
C _{max}	μ M	9.6 \pm 2.7
T _{max}	hours	2 \pm 1
AUC _{0-last}	μ Mxh	44.2 \pm 22.2
T 1/2	hours	3.9 \pm 0.1
Bioavailability	F%	21 \pm 11

Table 9: Effect of Nucleoside Analogs on Human Bone Marrow Cells

Compound (β -D-analog)	BFU-E	CFU-GM
	IC ₅₀ (μ M)	
2'-fluoro-2'-C-methylcytidine	98.2	93.9
2'-C-methylcytidine	20.1	13.2
AZT	0.08	0.95

WE CLAIM:

1. A (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) of the formula:



5 wherein

Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I;

10 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

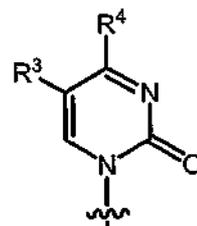
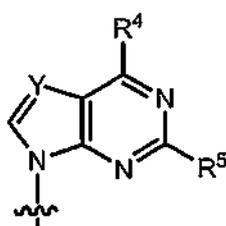
25 R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄

alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂; and

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof.

2. The (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β-D or β-L) of claim 1 or its pharmaceutically acceptable salt or prodrug thereof, wherein Base is selected from the group consisting of:



(a)

(b)

wherein

Y is N or CH.

5

R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

10

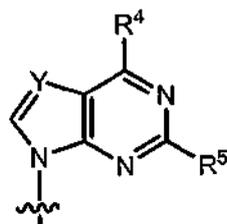
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R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

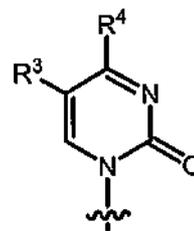
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3. The (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of claim 1 or its pharmaceutically acceptable salt or prodrug thereof,

wherein Base is selected from the group consisting of (a) or (b):



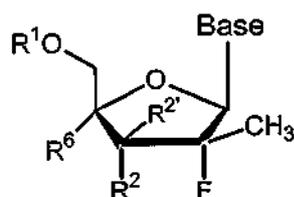
(a)



(b)

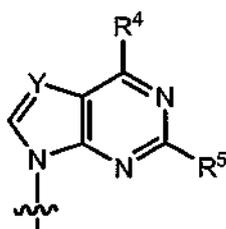
and wherein R^1 is H, R^2 is OH, $R^{2'}$ is H, R^3 is H, and R^4 is NH_2 or OH, and R^5 is NH_2 .

4. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the
5 formula:

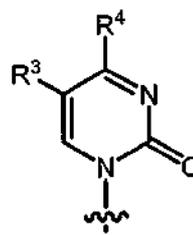


wherein

Base is selected from the group consisting of



(a)



(b)

10

Y is N or CH;

15

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other

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pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

5 R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkynyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkynyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

30 R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆

5 such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

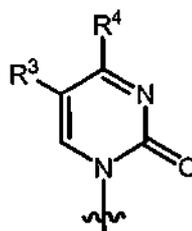
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

10 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

15 or its pharmaceutically acceptable salt or prodrug thereof.

5. The (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of claim 4 or its pharmaceutically acceptable salt or prodrug thereof, wherein

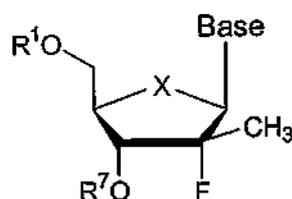
Base is



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and R¹ is H, R² is OH, R^{2'} is H, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

6. A (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) or its pharmaceutically acceptable salt or prodrug thereof of the structure:



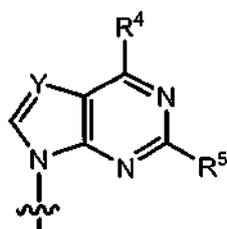
wherein Base is a purine or pyrimidine base;

5 X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and,

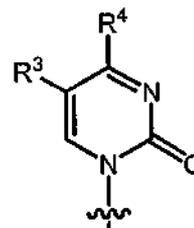
R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group.

7. The (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) of claim 6 or its pharmaceutically acceptable salt or prodrug thereof,

wherein Base is selected from the group consisting of:



(a)



(b)

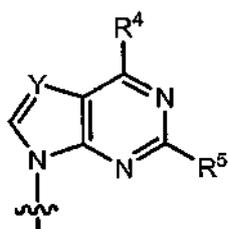
Y is N or CH;

5 R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

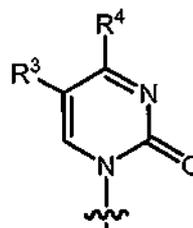
15 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

20 8. The (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of claim 6 or its pharmaceutically acceptable salt or prodrug thereof,

wherein Base is selected from the group consisting of (a) or (b):



(a)

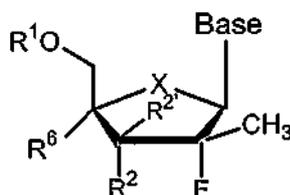


(b)

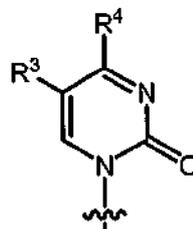
and wherein R^1 and R^7 are H, R^3 is H, and R^4 is NH_2 or OH , and R^5 is NH_2 .

5

9. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the
10 formula:



wherein Base is



- 15 X is O, S, CH_2 , Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)_2 ,
wherein W is F, Cl, Br, or I;

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl,

including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked

together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

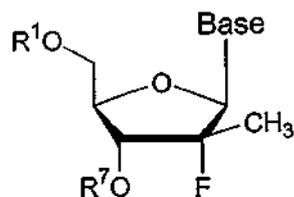
5 R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-
10 C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

15 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

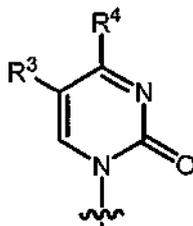
20 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof.

25 10. A (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β-D or β-L) of the formula



wherein Base is



5 R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its
10 peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other
15 pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R^1 is H or phosphate; R^2 is H or phosphate; R^1 and R^2 or R^7 can also be linked with cyclic phosphate group;

20 R^3 and R^4 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆

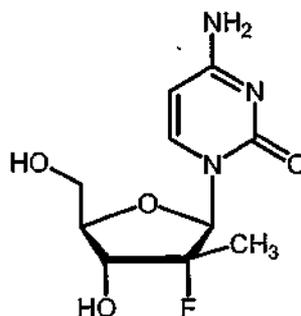
such as $C\equiv CH$, halogenated (F, Cl, Br, I) lower alkynyl of C_2 - C_6 , lower alkoxy of C_1 - C_6 such as CH_2OH and CH_2CH_2OH , halogenated (F, Cl, Br, I) lower alkoxy of C_1 - C_6 , CO_2H , CO_2R' , $CONH_2$, $CONHR'$, $CONR'_2$, $CH=CHCO_2H$, $CH=CHCO_2R'$;

5

R' is an optionally substituted alkyl of C_1 - C_{12} (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C_2 - C_6 , optionally substituted lower alkenyl of C_2 - C_6 , or optionally substituted acyl;

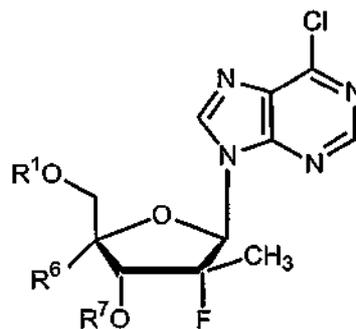
10 or its pharmaceutically acceptable salt or prodrug thereof.

11. A (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:



15

12. A (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:



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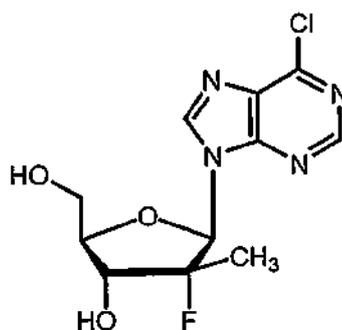
wherein

5 R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7 can also be linked with cyclic phosphate group; and,

10 R^6 is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH_3 , OCH_3 , OCH_2CH_3 , hydroxy methyl (CH_2OH), fluoromethyl (CH_2F), azido (N_3), $CHCN$, CH_2N_3 , CH_2NH_2 , CH_2NHCH_3 , $CH_2N(CH_3)_2$, alkyne (optionally substituted), or fluoro.

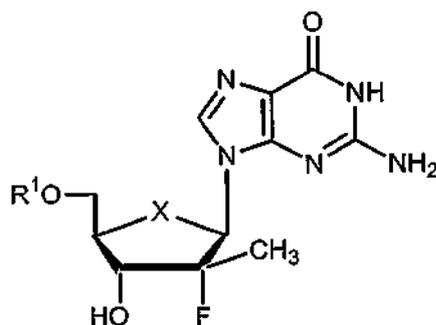
20

13. A (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:



25

14. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:



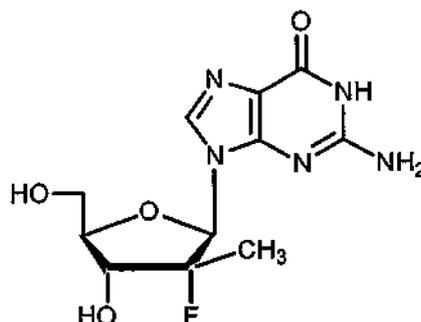
wherein

5 X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and

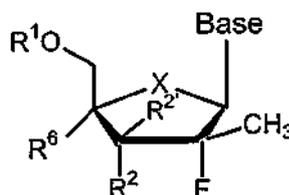
10 R¹ is H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate.

20

15. A (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:



16. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the formula:



5

wherein

Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I;

10 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its
15 peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other
20 pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound

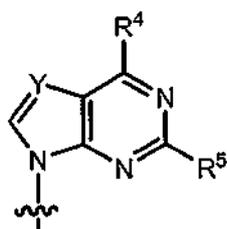
wherein R^1 is H or phosphate; R^2 is H or phosphate; R^1 and R^2 or R^7 can also be linked with cyclic phosphate group;

R^2 and $R^{2'}$ are independently H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{1-4} alkynyl, vinyl, N_3 , CN, Cl, Br, F, I, NO_2 , $C(O)O(C_{1-4}$ alkyl), $C(O)O(C_{1-4}$ alkyl), $C(O)O(C_{1-4}$ alkynyl), $C(O)O(C_{1-4}$ alkenyl), $O(C_{1-4}$ acyl), $O(C_{1-4}$ alkyl), $O(C_{1-4}$ alkenyl), $S(C_{1-4}$ acyl), $S(C_{1-4}$ alkyl), $S(C_{1-4}$ alkynyl), $S(C_{1-4}$ alkenyl), $SO(C_{1-4}$ acyl), $SO(C_{1-4}$ alkyl), $SO(C_{1-4}$ alkynyl), $SO(C_{1-4}$ alkenyl), $SO_2(C_{1-4}$ acyl), $SO_2(C_{1-4}$ alkyl), $SO_2(C_{1-4}$ alkynyl), $SO_2(C_{1-4}$ alkenyl), $O_3S(C_{1-4}$ acyl), $O_3S(C_{1-4}$ alkyl), $O_3S(C_{1-4}$ alkenyl), NH_2 , $NH(C_{1-4}$ alkyl), $NH(C_{1-4}$ alkenyl), $NH(C_{1-4}$ alkynyl), $NH(C_{1-4}$ acyl), $N(C_{1-4}$ alkyl)₂, $N(C_{1-18}$ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N_3 , CN, one to three halogen (Cl, Br, F, I), NO_2 , $C(O)O(C_{1-4}$ alkyl), $C(O)O(C_{1-4}$ alkyl), $C(O)O(C_{1-4}$ alkynyl), $C(O)O(C_{1-4}$ alkenyl), $O(C_{1-4}$ acyl), $O(C_{1-4}$ alkyl), $O(C_{1-4}$ alkenyl), $S(C_{1-4}$ acyl), $S(C_{1-4}$ alkyl), $S(C_{1-4}$ alkynyl), $S(C_{1-4}$ alkenyl), $SO(C_{1-4}$ acyl), $SO(C_{1-4}$ alkyl), $SO(C_{1-4}$ alkynyl), $SO(C_{1-4}$ alkenyl), $SO_2(C_{1-4}$ acyl), $SO_2(C_{1-4}$ alkyl), $SO_2(C_{1-4}$ alkynyl), $SO_2(C_{1-4}$ alkenyl), $O_3S(C_{1-4}$ acyl), $O_3S(C_{1-4}$ alkyl), $O_3S(C_{1-4}$ alkenyl), NH_2 , $NH(C_{1-4}$ alkyl), $NH(C_{1-4}$ alkenyl), $NH(C_{1-4}$ alkynyl), $NH(C_{1-4}$ acyl), $N(C_{1-4}$ alkyl)₂, $N(C_{1-4}$ acyl)₂, OR^7 ; R^2 and $R^{2'}$ can be linked together to form a vinyl optionally substituted by one or two of N_3 , CN, Cl, Br, F, I, NO_2 ;

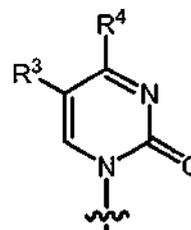
R^6 is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH_3 , OCH_3 , OCH_2CH_3 , hydroxy methyl (CH_2OH), fluoromethyl (CH_2F), azido (N_3), $CHCN$, CH_2N_3 , CH_2NH_2 , CH_2NHCH_3 , $CH_2N(CH_3)_2$, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, a pharmaceutically acceptable carrier.

17. The composition of claim 16, wherein Base is selected from the group consisting of:



(a)



(b)

5 wherein

Y is N or CH.

10 R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

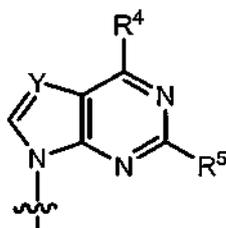
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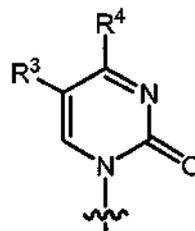
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

18. The composition of claim 16, wherein

Base is selected from the group consisting of (a) or (b):



(a)

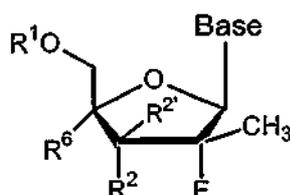


(b)

and wherein R^1 is H, R^2 is OH, $R^{2'}$ is H, R^3 is H,
and R^4 is NH_2 or OH, and R^5 is NH_2 .

5

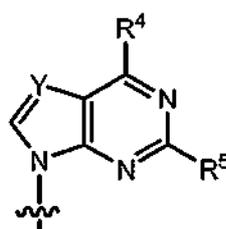
19. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the formula:



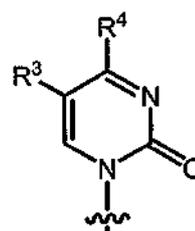
10

wherein

Base is selected from the group consisting of



(a)



(b)

Y is N or CH;

15

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl,

including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

5 R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

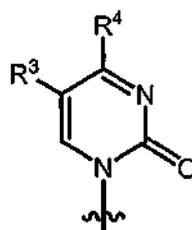
15 R^7 is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

20 R^6 is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof in a pharmaceutically acceptable carrier.

20. The composition of claim 19, wherein

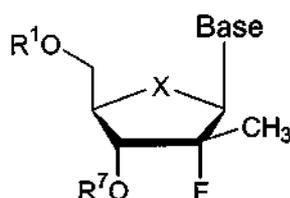
25 Base is



115

and R^1 is H, R^2 is OH, $R^{2'}$ is H, R^3 is H, R^4 is NH_2 or OH, and R^6 is H.

- 5 21. A pharmaceutical composition comprising a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier, of the structure:



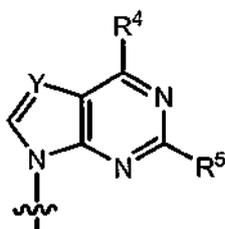
wherein Base is a purine or pyrimidine base;

- 10 X is O, S, CH_2 , Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), $C(W)_2$,
wherein W is F, Cl, Br, or I; and,

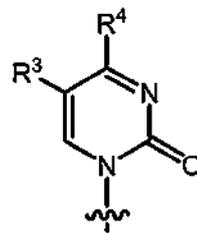
- 15 R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound
20 wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7
25 can also be linked with cyclic phosphate group.

22. The composition of claim 21, wherein

Base is selected from the group consisting of:



(a)



(b)

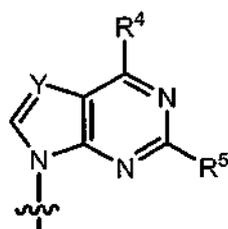
5 Y is N or CH;

10 R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

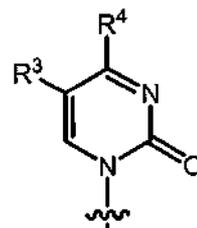
20 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

23. The composition of claim 21, wherein

Base is selected from the group consisting of (a) or (b):



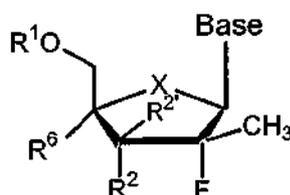
(a)



(b)

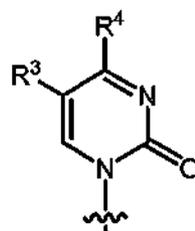
and wherein R^1 and R^7 are H, R^3 is H, and R^4 is NH_2 or OH, and R^5 is NH_2 .

- 5 24. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the formula:



wherein

Base is



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X is O, S, CH_2 , Se, NH, N-alkyl, CHW (R, S, or racemic), C(W)_2 , wherein W is F, Cl, Br, or I;

15

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl,

wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃

and $\text{CH}_2\text{CH}_2\text{F}$, lower alkenyl of $\text{C}_2\text{-C}_6$ such as $\text{CH}=\text{CH}_2$,
 halogenated (F, Cl, Br, I) lower alkenyl of $\text{C}_2\text{-C}_6$ such as
 $\text{CH}=\text{CHCl}$, $\text{CH}=\text{CHBr}$ and $\text{CH}=\text{CHI}$, lower alkynyl of $\text{C}_2\text{-C}_6$
 such as $\text{C}\equiv\text{CH}$, halogenated (F, Cl, Br, I) lower alkynyl of $\text{C}_2\text{-C}_6$,
 lower alkoxy of $\text{C}_1\text{-C}_6$ such as CH_2OH and $\text{CH}_2\text{CH}_2\text{OH}$,
 halogenated (F, Cl, Br, I) lower alkoxy of $\text{C}_1\text{-C}_6$, CO_2H ,
 $\text{CO}_2\text{R}'$, CONH_2 , CONHR' , CONR'_2 , $\text{CH}=\text{CHCO}_2\text{H}$,
 $\text{CH}=\text{CHCO}_2\text{R}'$;

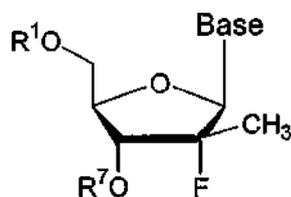
R' is an optionally substituted alkyl of $\text{C}_1\text{-C}_{12}$ (particularly when the
 alkyl is an amino acid residue), cycloalkyl, optionally
 substituted alkynyl of $\text{C}_2\text{-C}_6$, optionally substituted lower
 alkenyl of $\text{C}_2\text{-C}_6$, or optionally substituted acyl; and

R^6 is an optionally substituted alkyl (including lower alkyl), cyano
 (CN), CH_3 , OCH_3 , OCH_2CH_3 , hydroxy methyl (CH_2OH),
 fluoromethyl (CH_2F), azido (N_3), CHCN , CH_2N_3 , CH_2NH_2 ,
 CH_2NHCH_3 , $\text{CH}_2\text{N}(\text{CH}_3)_2$, alkyne (optionally substituted), or
 fluoro;

or its pharmaceutically acceptable salt or prodrug thereof and a pharmaceutically
 acceptable carrier.

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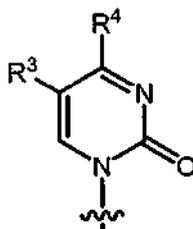
25. A pharmaceutical composition comprising a (2'R)-2'-deoxy-2'-fluoro-
 2'-C-methyl nucleoside ($\beta\text{-D}$ or $\beta\text{-L}$) of the formula:



25

wherein

Base is



5 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group;

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R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

20

25

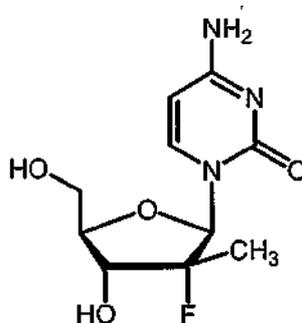
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally

substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier.

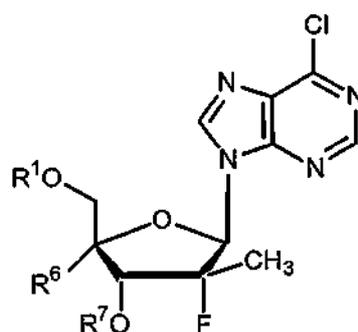
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26. A pharmaceutical composition comprising a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier of the formula:



10

27. A pharmaceutical composition comprising a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L), or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier, of the formula:



15

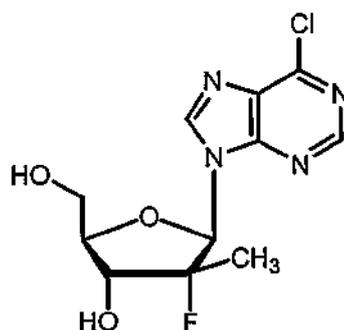
wherein

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl,

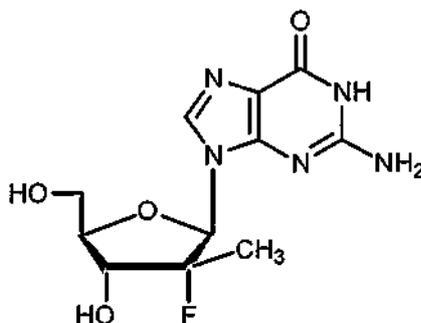
including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group; and,

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro.

28. A pharmaceutical composition comprising a (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier, of the formula:

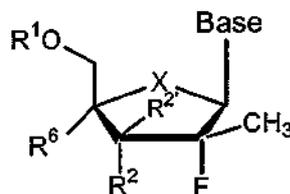


30. A pharmaceutical composition comprising a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof, in a pharmaceutically acceptable carrier, of the structure:



5

31. Use of an an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) of the formula:



10

wherein

Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I;

15

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid,

20

including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

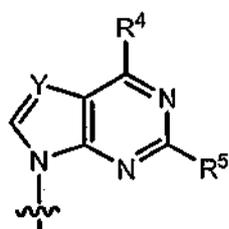
R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂,

CH_2NHCH_3 , $\text{CH}_2\text{N}(\text{CH}_3)_2$, alkyne (optionally substituted), or fluoro;

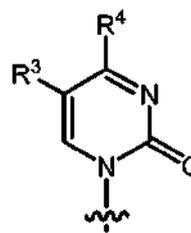
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host.

32. The use of claim 31,

wherein Base is selected from the group consisting of:



(a)



(b)

10

Y is N or CH.

15

R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR' , SH, SR' , NH_2 , NHR' , NR'_2 , lower alkyl of $\text{C}_1\text{-C}_6$, halogenated (F, Cl, Br, I) lower alkyl of $\text{C}_1\text{-C}_6$ such as CF_3 and $\text{CH}_2\text{CH}_2\text{F}$, lower alkenyl of $\text{C}_2\text{-C}_6$ such as $\text{CH}=\text{CH}_2$, halogenated (F, Cl, Br, I) lower alkenyl of $\text{C}_2\text{-C}_6$ such as $\text{CH}=\text{CHCl}$, $\text{CH}=\text{CHBr}$ and $\text{CH}=\text{CHI}$, lower alkynyl of $\text{C}_2\text{-C}_6$ such as $\text{C}\equiv\text{CH}$, halogenated (F, Cl, Br, I) lower alkynyl of $\text{C}_2\text{-C}_6$, lower alkoxy of $\text{C}_1\text{-C}_6$ such as CH_2OH and $\text{CH}_2\text{CH}_2\text{OH}$, halogenated (F, Cl, Br, I) lower alkoxy of $\text{C}_1\text{-C}_6$, CO_2H , $\text{CO}_2\text{R}'$, CONH_2 , CONHR' , CONR'_2 , $\text{CH}=\text{CHCO}_2\text{H}$, $\text{CH}=\text{CHCO}_2\text{R}'$; and,

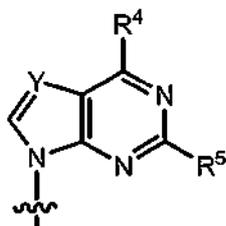
20

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

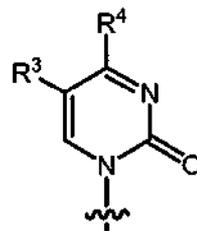
5

33. The use of claim 31, wherein

Base is selected from the group consisting of (a) or (b):



(a)



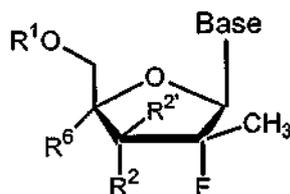
(b)

10

and wherein R¹ is H, R² is OH, R^{2'} is H, R³ is H, and R⁴ is NH₂ or OH, and R⁵ is NH₂.

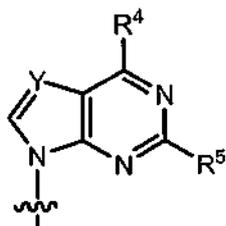
34. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:

15

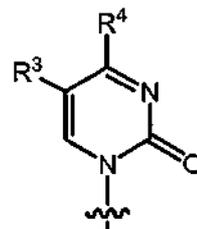


wherein

Base is selected from the group consisting of



(a)



(b)

and wherein Y is N or CH;

R¹ and R⁷ are independently H, phosphate, including monophosphate,
 5 diphosphate, triphosphate, or a stabilized phosphate prodrug,
 H-phosphonate, including stabilized H-phosphonates, acyl,
 including optionally substituted phenyl and lower acyl, alkyl,
 including lower alkyl, O-substituted carboxyalkylamino or its
 10 peptide derivatives, sulfonate ester, including alkyl or
 arylalkyl sulfonyl, including methanesulfonyl and benzyl,
 wherein the phenyl group is optionally substituted, a lipid,
 including a phospholipid, an L or D-amino acid (or racemic
 mixture), a carbohydrate, a peptide, a cholesterol, or other
 pharmaceutically acceptable leaving group which when
 15 administered *in vivo* is capable of providing a compound
 wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and
 R² or R⁷ can also be linked with cyclic phosphate group;

R² and R²¹ are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl,
 vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄
 20 alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄
 acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄
 alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄
 alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl),
 SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄
 25 acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄
 alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl),
 N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl
 and vinyl are optionally substituted by N₃, CN, one to three

5 halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

10 R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

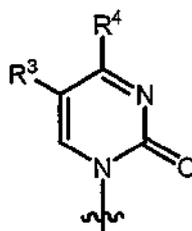
20 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

25 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host.

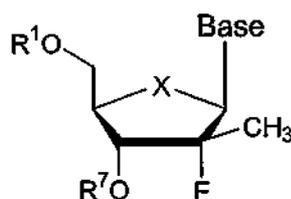
5 35. The use of claim 34, wherein

Base is



10 and R¹ is H, R² is OH, R^{2'} is H, R³ is H, R⁴ is
NH₂ or OH, and R⁶ is H.

36. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β-D or β-L) of the structure:



15 wherein Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I; and,

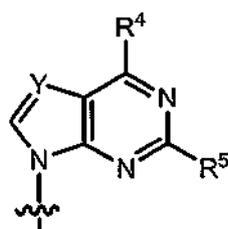
20 R¹ and R⁷ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,

including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group; and

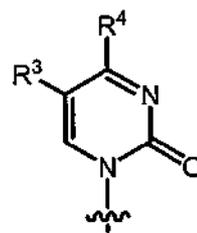
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host.

37. The use of claim 36, wherein

Base is selected from the group consisting of:



(a)



(b)

Y is N or CH;

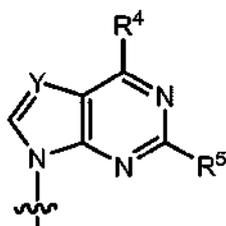
R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as

5
 CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆
 such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-
 C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH,
 halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H,
 CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H,
 CH=CHCO₂R'; and,

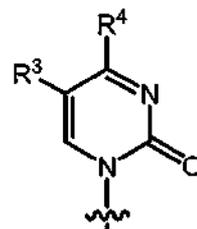
10
 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the
 alkyl is an amino acid residue), cycloalkyl, optionally
 substituted alkynyl of C₂-C₆, optionally substituted lower
 alkenyl of C₂-C₆, or optionally substituted acyl.

38. The use of claim 36, wherein

Base is selected from the group consisting of (a) or (b):



(a)

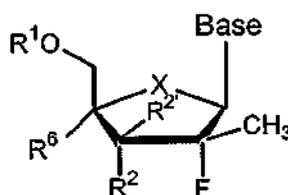


(b)

15

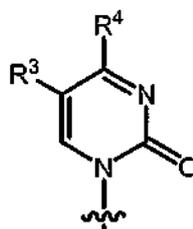
and wherein R¹ and R⁷ are H, R³ is H, and R⁴ is
 NH₂ or OH, and R⁵ is NH₂.

20
 39. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-
 2'-C-methyl nucleoside (β-D or β-L) of the formula:



wherein

Base is



5 X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I;

10 R¹ and R⁷ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
15 arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid (or racemic
mixture), a carbohydrate, a peptide, a cholesterol, or other
pharmaceutically acceptable leaving group which when
administered *in vivo* is capable of providing a compound
wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and
R² or R⁷ can also be linked with cyclic phosphate group;

20 R² and R²¹ are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl,
vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄
alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄
acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄
alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄
25 alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl),
SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄
acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄

alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl),
 N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl
 and vinyl are optionally substituted by N₃, CN, one to three
 halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄
 5 alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄
 acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄
 alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄
 alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl),
 SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄
 10 acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄
 alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl),
 N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked
 together to form a vinyl optionally substituted by one or two
 of N₃, CN, Cl, Br, F, I, NO₂;

15 R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH,
 OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆,
 halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃
 and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂,
 halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as
 20 CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆
 such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-
 C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH,
 halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H,
 CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H,
 25 CH=CHCO₂R';

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the
 alkyl is an amino acid residue), cycloalkyl, optionally
 substituted alkynyl of C₂-C₆, optionally substituted lower
 alkenyl of C₂-C₆, or optionally substituted acyl;

30 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano
 (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH),

fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

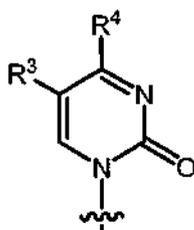
5 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier for the treatment or prophylaxis of hepatitis C infection in a host

40. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β-D or β-L) of the formula:



wherein

Base is



15 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or

20 arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a

carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group;

5

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

10

15

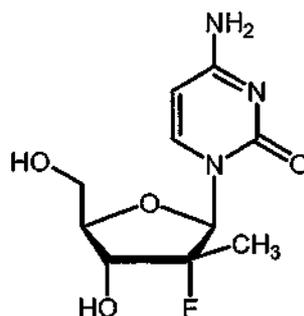
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

20

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host

25

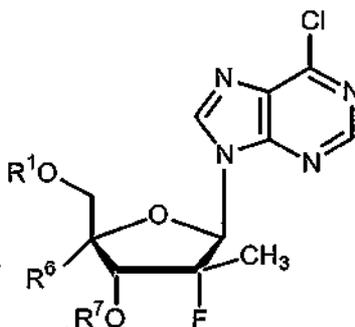
41. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:



or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier for the treatment or prophylaxis of hepatitis C infection in a host.

5

42. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) of the formula:



wherein

10

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when

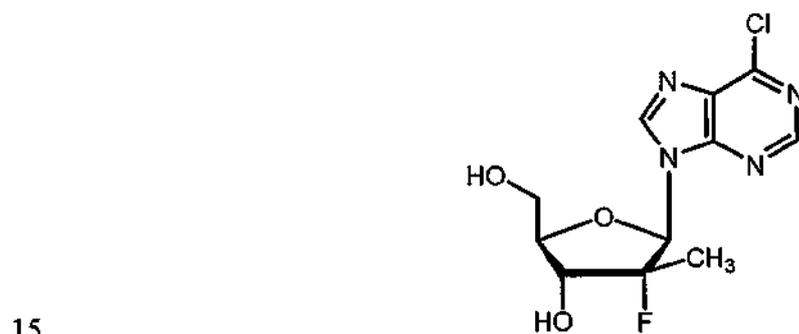
20

administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group; and,

5 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

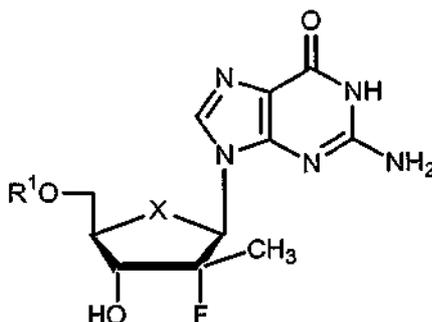
10 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier for the treatment or prophylaxis of hepatitis C infection in a host

43. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β-D) of the formula:



or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host

20 44. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β-D) of the formula:



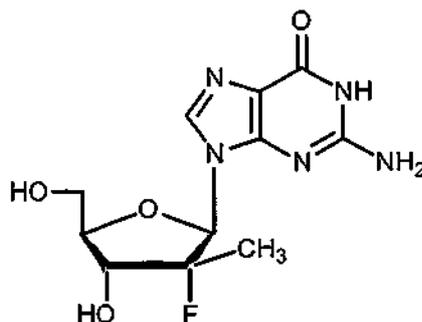
wherein

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I; and

5 R¹ is H, phosphate, including monophosphate, diphosphate,
triphosphate, or a stabilized phosphate prodrug, H-
phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
10 peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid, a
carbohydrate, a peptide, a cholesterol, or other
15 pharmaceutically acceptable leaving group which when
administered *in vivo* is capable of providing a compound
wherein R¹ is H or phosphate;

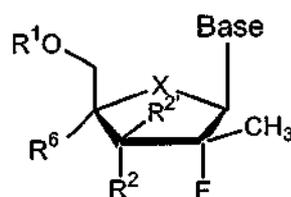
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a
pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C
20 infection in a host

45. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-
2'-*C*-methyl nucleoside (β-D) of the formula:



or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of hepatitis C infection in a host.

- 5 46. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) of the formula:



wherein

- 10 Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I;

- 15 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other

pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R^1 is H or phosphate; R^2 is H or phosphate; R^1 and R^2 or R^7 can also be linked with cyclic phosphate group;

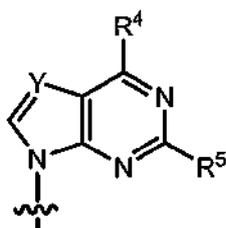
5 R^2 and $R^{2'}$ are independently H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{1-4} alkynyl, vinyl, N_3 , CN, Cl, Br, F, I, NO_2 , $C(O)O(C_{1-4}$ alkyl), $C(O)O(C_{1-4}$ alkyl), $C(O)O(C_{1-4}$ alkynyl), $C(O)O(C_{1-4}$ alkenyl), $O(C_{1-4}$ acyl), $O(C_{1-4}$ alkyl), $O(C_{1-4}$ alkenyl), $S(C_{1-4}$ acyl), $S(C_{1-4}$ alkyl), $S(C_{1-4}$ alkynyl), $S(C_{1-4}$ alkenyl), $SO(C_{1-4}$ acyl), $SO(C_{1-4}$ alkyl), $SO(C_{1-4}$ alkynyl), $SO(C_{1-4}$ alkenyl), $SO_2(C_{1-4}$ acyl), $SO_2(C_{1-4}$ alkyl), $SO_2(C_{1-4}$ alkynyl), $SO_2(C_{1-4}$ alkenyl), $O_3S(C_{1-4}$ acyl), $O_3S(C_{1-4}$ alkyl), $O_3S(C_{1-4}$ alkenyl), NH_2 , $NH(C_{1-4}$ alkyl), $NH(C_{1-4}$ alkenyl), $NH(C_{1-4}$ alkynyl), $NH(C_{1-4}$ acyl), $N(C_{1-4}$ alkyl)₂, $N(C_{1-18}$ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N_3 , CN, one to three halogen (Cl, Br, F, I), NO_2 , $C(O)O(C_{1-4}$ alkyl), $C(O)O(C_{1-4}$ alkyl), $C(O)O(C_{1-4}$ alkynyl), $C(O)O(C_{1-4}$ alkenyl), $O(C_{1-4}$ acyl), $O(C_{1-4}$ alkyl), $O(C_{1-4}$ alkenyl), $S(C_{1-4}$ acyl), $S(C_{1-4}$ alkyl), $S(C_{1-4}$ alkynyl), $S(C_{1-4}$ alkenyl), $SO(C_{1-4}$ acyl), $SO(C_{1-4}$ alkyl), $SO(C_{1-4}$ alkynyl), $SO(C_{1-4}$ alkenyl), $SO_2(C_{1-4}$ acyl), $SO_2(C_{1-4}$ alkyl), $SO_2(C_{1-4}$ alkynyl), $SO_2(C_{1-4}$ alkenyl), $O_3S(C_{1-4}$ acyl), $O_3S(C_{1-4}$ alkyl), $O_3S(C_{1-4}$ alkenyl), NH_2 , $NH(C_{1-4}$ alkyl), $NH(C_{1-4}$ alkenyl), $NH(C_{1-4}$ alkynyl), $NH(C_{1-4}$ acyl), $N(C_{1-4}$ alkyl)₂, $N(C_{1-4}$ acyl)₂, OR^7 ; R^2 and $R^{2'}$ can be linked together to form a vinyl optionally substituted by one or two of N_3 , CN, Cl, Br, F, I, NO_2 ;

25 R^6 is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH_3 , OCH_3 , OCH_2CH_3 , hydroxy methyl (CH_2OH), fluoromethyl (CH_2F), azido (N_3), $CHCN$, CH_2N_3 , CH_2NH_2 , CH_2NHCH_3 , $CH_2N(CH_3)_2$, alkyne (optionally substituted), or fluoro;

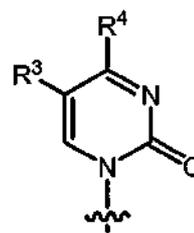
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

47. The method of claim 46,

5 wherein Base is selected from the group consisting of:



(a)



(b)

Y is N or CH.

10 R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

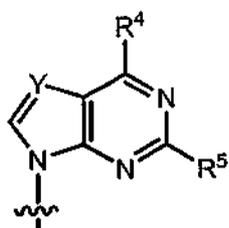
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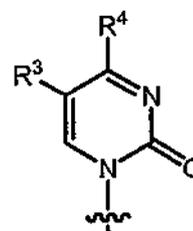
wherein R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

48. The method of claim 46, wherein

Base is selected from the group consisting of (a) or (b):



(a)



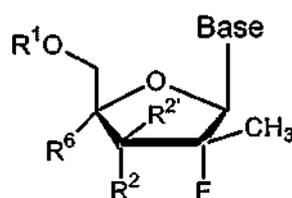
(b)

5

and wherein R^1 is H, R^2 is OH, $R^{2'}$ is H, R^3 is H, and R^4 is NH_2 or OH, and R^5 is NH_2 .

49. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the formula:

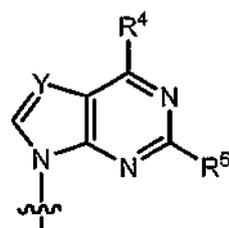
10



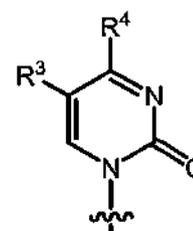
wherein

Base is selected from the group consisting of

15



(a)



(b)

and wherein Y is N or CH;

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋

4 acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two
 5 of N₃, CN, Cl, Br, F, I, NO₂;

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂,
 10 halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H,
 15 CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

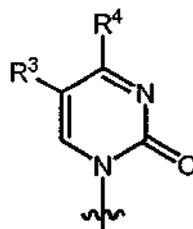
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower
 20 alkenyl of C₂-C₆, or optionally substituted acyl;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or
 25 fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

50. The method of claim 49, wherein

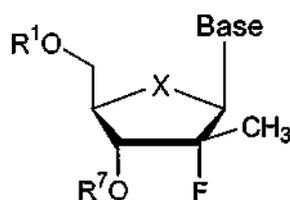
Base is



5 and R¹ is H, R² is OH, R^{2'} is H, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

51. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β-D or β-L) or its pharmaceutically acceptable salt or prodrug thereof of the structure:

10



wherein Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and,

15 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid,

20

pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

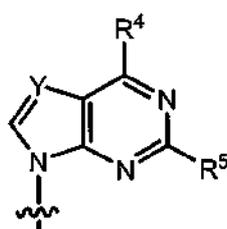
5 R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

30 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

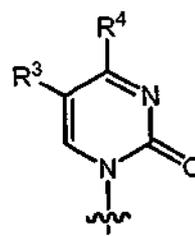
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

47. The method of claim 46,

5 wherein Base is selected from the group consisting of:



(a)



(b)

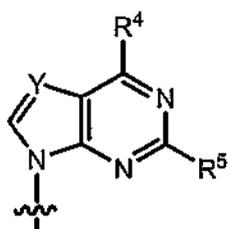
Y is N or CH.

10 R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as
 15 CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H,
 20 CH=CHCO₂R'; and,

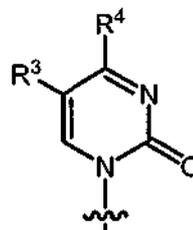
wherein R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

48. The method of claim 46, wherein

Base is selected from the group consisting of (a) or (b):



(a)

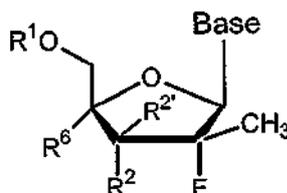


(b)

5

and wherein R^1 is H, R^2 is OH, $R^{2'}$ is H, R^3 is H,
and R^4 is NH_2 or OH, and R^5 is NH_2 .

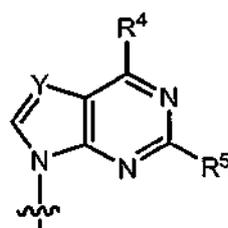
49. A method for the treatment or prophylaxis of a rhinovirus infection
10 comprising administering to a host an antivirally effective amount of a (2'R)-2'-
deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the formula:



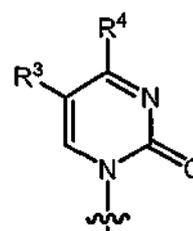
wherein

15

Base is selected from the group consisting of



(a)



(b)

and wherein Y is N or CH;

5 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

20 R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋

4 acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two
 5 of N₃, CN, Cl, Br, F, I, NO₂;

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂,
 10 halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

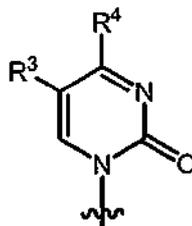
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;
 20

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or
 25 fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

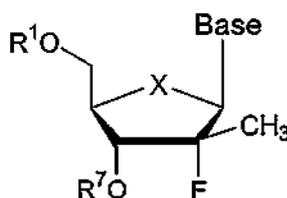
50. The method of claim 49, wherein

Base is



5 and R¹ is H, R² is OH, R^{2a} is H, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

51. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) or its pharmaceutically acceptable salt or prodrug thereof of the structure:



wherein Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and,

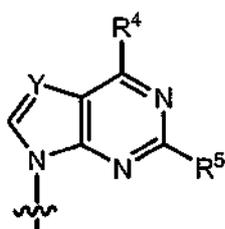
15 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid,

20

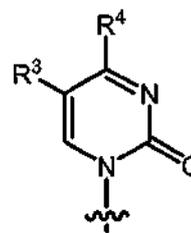
including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group; optionally in a pharmaceutically acceptable carrier.

52. The method of claim 51, wherein

Base is selected from the group consisting of:



(a)



(b)

Y is N or CH;

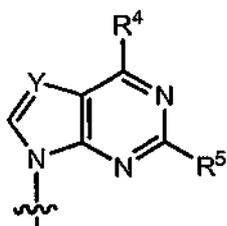
R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

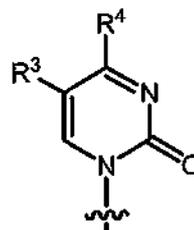
5

53. The method of claim 51, wherein

Base is selected from the group consisting of (a) or (b):



(a)

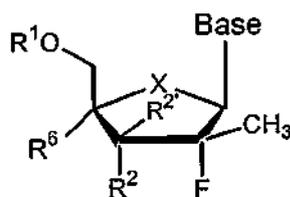


(b)

10

and wherein R¹ and R⁷ are H, R³ is H, and R⁴ is NH₂ or OH, and R⁵ is NH₂.

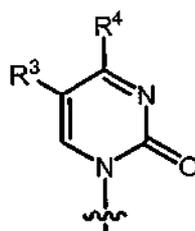
54. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:



15

wherein

Base is



149

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I;

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl),

5 SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

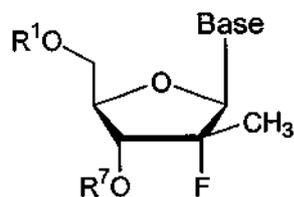
10 R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

20 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

25 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

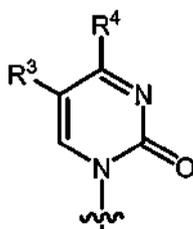
55. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the formula:



5

wherein

Base is



10 R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or
 15 arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when
 20 administered *in vivo* is capable of providing a compound wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7 can also be linked with cyclic phosphate group;

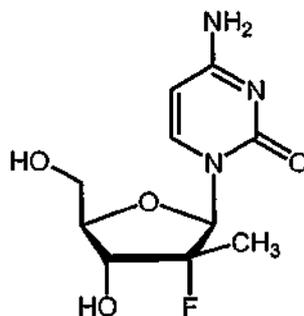
R^3 and R^4 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆,

5 halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

10 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

15 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier.

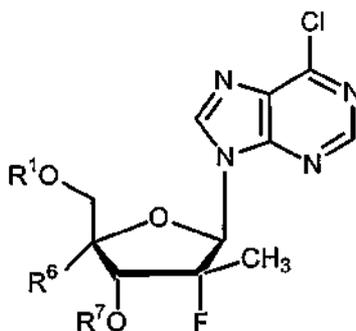
56. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:



optionally in a pharmaceutically acceptable carrier.

57. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'R)-2'-

deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:



wherein

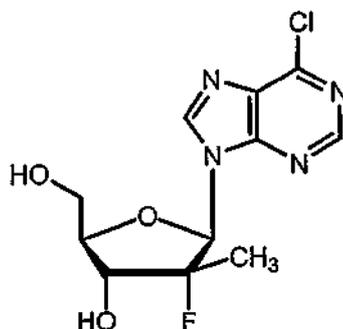
5 R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its
10 peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other
15 pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7 can also be linked with cyclic phosphate group; and,

20 R^6 is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH_3 , OCH_3 , OCH_2CH_3 , hydroxy methyl (CH_2OH), fluoromethyl (CH_2F), azido (N_3), $CHCN$, CH_2N_3 , CH_2NH_2 , CH_2NHCH_3 , $CH_2N(CH_3)_2$, alkyne (optionally substituted), or fluoro; and

optionally in a pharmaceutically acceptable carrier.

25

58. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:

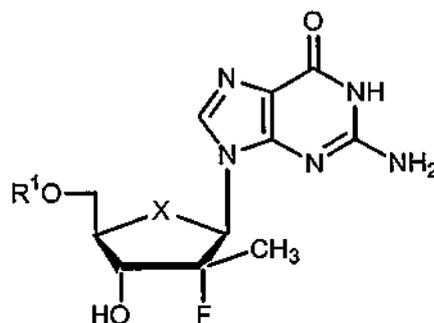


5

optionally in a pharmaceutically acceptable carrier.

59. A method for the treatment or prophylaxis of a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:

10



wherein

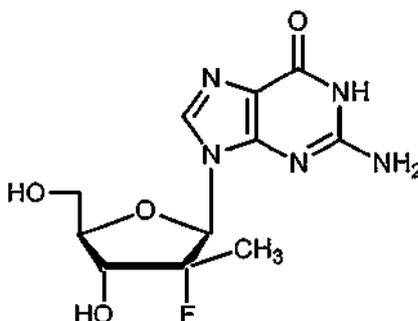
X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂,
 15 wherein W is F, Cl, Br, or I; and

R¹ is H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl,

including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate;

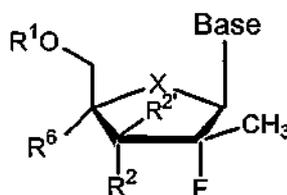
optionally in a pharmaceutically acceptable carrier.

60. A method for the treatment or prophylaxis of a a rhinovirus infection comprising administering to a host an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) or its pharmaceutically acceptable salt or prodrug thereof of the formula:



optionally in a pharmaceutically acceptable carrier.

61. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) of the formula:



wherein

Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I;

5 R¹ and R⁷ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
10 peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid (or racemic
mixture), a carbohydrate, a peptide, a cholesterol, or other
15 pharmaceutically acceptable leaving group which when
administered *in vivo* is capable of providing a compound
wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and
R² or R⁷ can also be linked with cyclic phosphate group;

20 R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl,
vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄
4 alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄
acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄
alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄
alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl),
25 SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄
4 acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄
alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl),
N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl
and vinyl are optionally substituted by N₃, CN, one to three
30 halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄
alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄

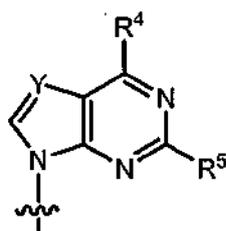
acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkynyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

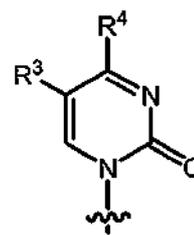
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

62. The use of claim 61,

wherein Base is selected from the group consisting of:



(a)



(b)

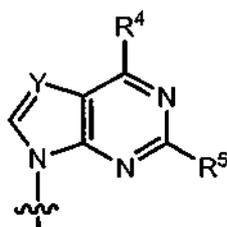
Y is N or CH.

R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

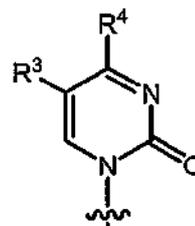
R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

63. The use of claim 61, wherein

Base is selected from the group consisting of (a) or (b):



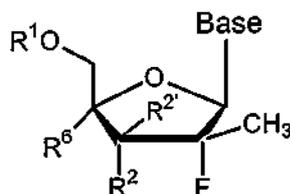
(a)



(b)

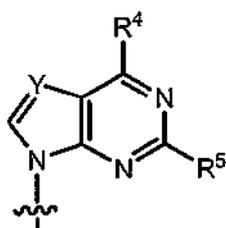
and wherein R^1 is H, R^2 is OH, $R^{2'}$ is H, R^3 is H, and R^4 is NH₂ or OH, and R^5 is NH₂.

64. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the formula:

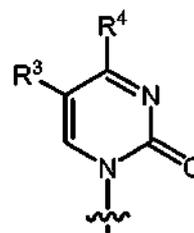


wherein

Base is selected from the group consisting of



(a)



(b)

5

Y is N or CH;

10

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R^1 is H or phosphate; R^2 is H or phosphate; R^1 and R^2 or R^7 can also be linked with cyclic phosphate group;

15

20

R^2 and $R^{2'}$ are independently H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{1-4} alkynyl, vinyl, N_3 , CN, Cl, Br, F, I, NO_2 , $C(O)O(C_{1-4}$ alkyl), $C(O)O(C_{1-4}$

4 alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

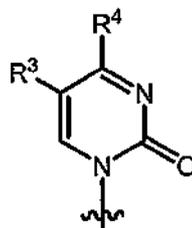
R⁷ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

5 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

10 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

65. The use of claim 64, wherein

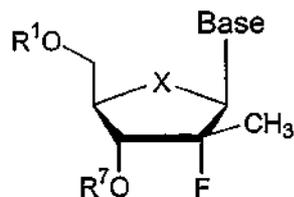
15 Base is



and R¹ is H, R² is OH, R^{2'} is H, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

20

66. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the structure:



wherein Base is a purine or pyrimidine base;

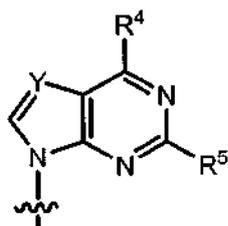
X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I; and,

5 R¹ and R⁷ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
10 peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid, a
carbohydrate, a peptide, a cholesterol, or other
15 pharmaceutically acceptable leaving group which when
administered *in vivo* is capable of providing a compound
wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷
can also be linked with cyclic phosphate group and

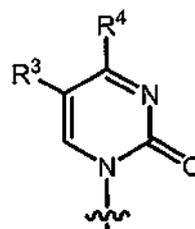
20 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a
pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow
fever virus infection in a host

67. The use of claim 66, wherein

Base is selected from the group consisting of:



(a)



(b)

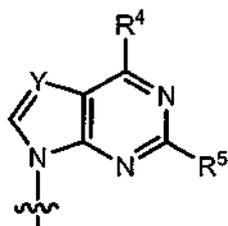
Y is N or CH;

5 R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

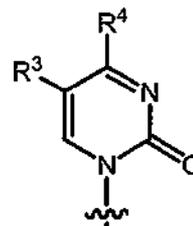
15 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

20 68. The use of claim 66, wherein

Base is selected from the group consisting of (a) or (b):



(a)

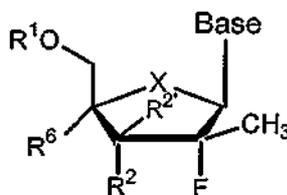


(b)

and wherein R^1 and R^7 are H, R^3 is H, and R^4 is NH_2 or OH, and R^5 is NH_2 .

5

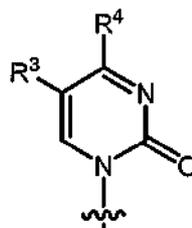
69. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the formula:



wherein

10

Base is



X is O, S, CH_2 , Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), $C(W)_2$, wherein W is F, Cl, Br, or I;

15

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or

5 arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

10 R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl); SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆,

5 halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

10 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

15 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

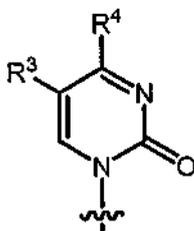
20 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host

70. Use of antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:



wherein

Base is



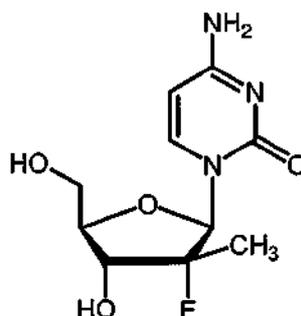
5 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or
10 arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when
15 administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group;

20 R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-
25 C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

- 5 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

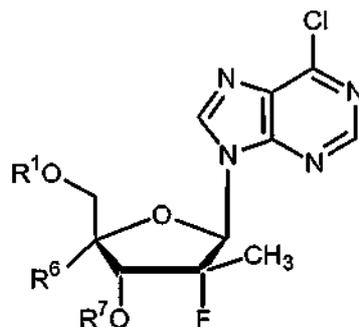
- 10 71. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β-D) of the formula:



or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

15

72. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β-D) of the formula:



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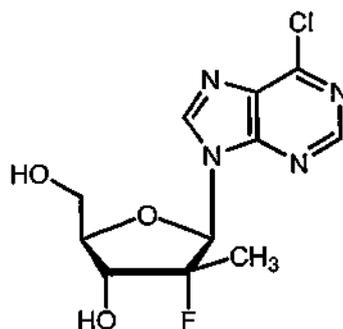
wherein

5 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, 10 including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ 15 can also be linked with cyclic phosphate group; and,

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or 20 fluoro, and

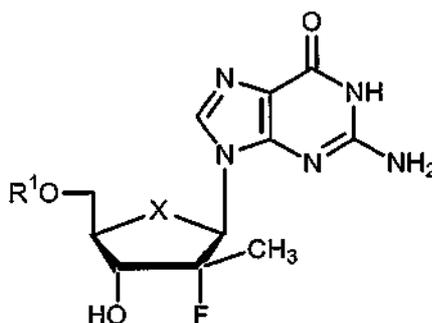
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

25 73. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D) of the formula:



or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

- 5 74. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) of the formula:



wherein

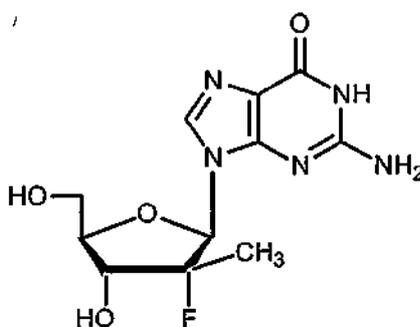
- X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂,
10 wherein W is F, Cl, Br, or I; and

- R¹ and R² are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
15 including lower alkyl, O-substituted carboxyalkylamino or its
peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid, a
20 carbohydrate, a peptide, a cholesterol, or other

pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate;

5 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

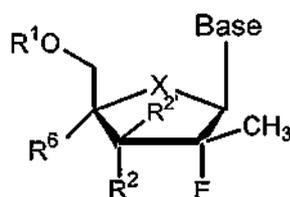
75. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) of the formula:



10

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a yellow fever virus infection in a host.

15 76. Use an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) of the formula:



wherein

Base is a purine or pyrimidine base;

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I;

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R²¹ are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl),

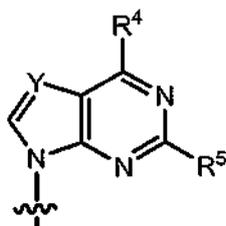
5 $\text{SO}_2(\text{C}_{1-4} \text{ alkyl}), \text{SO}_2(\text{C}_{1-4} \text{ alkynyl}), \text{SO}_2(\text{C}_{1-4} \text{ alkenyl}), \text{O}_3\text{S}(\text{C}_{1-4} \text{ acyl}), \text{O}_3\text{S}(\text{C}_{1-4} \text{ alkyl}), \text{O}_3\text{S}(\text{C}_{1-4} \text{ alkenyl}), \text{NH}_2, \text{NH}(\text{C}_{1-4} \text{ alkyl}), \text{NH}(\text{C}_{1-4} \text{ alkenyl}), \text{NH}(\text{C}_{1-4} \text{ alkynyl}), \text{NH}(\text{C}_{1-4} \text{ acyl}), \text{N}(\text{C}_{1-4} \text{ alkyl})_2, \text{N}(\text{C}_{1-4} \text{ acyl})_2, \text{OR}^7$; R^2 and R^{21} can be linked together to form a vinyl optionally substituted by one or two of $\text{N}_3, \text{CN}, \text{Cl}, \text{Br}, \text{F}, \text{I}, \text{NO}_2$;

10 R^6 is an optionally substituted alkyl (including lower alkyl), cyano (CN), $\text{CH}_3, \text{OCH}_3, \text{OCH}_2\text{CH}_3$, hydroxy methyl (CH_2OH), fluoromethyl (CH_2F), azido (N_3), $\text{CHCN}, \text{CH}_2\text{N}_3, \text{CH}_2\text{NH}_2, \text{CH}_2\text{NHCH}_3, \text{CH}_2\text{N}(\text{CH}_3)_2$, alkyne (optionally substituted), or fluoro;

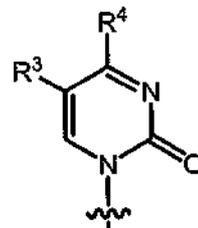
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

15 77. The use of claim 76,

wherein Base is selected from the group consisting of:



(a)



(b)

20 Y is N or CH.

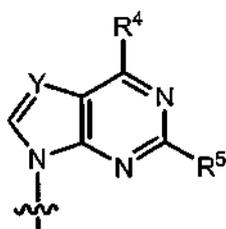
R^3, R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, $\text{OR}', \text{SH}, \text{SR}', \text{NH}_2, \text{NHR}', \text{NR}'_2$, lower alkyl of $\text{C}_1\text{-C}_6$, halogenated (F, Cl, Br, I) lower alkyl of $\text{C}_1\text{-C}_6$ such as CF_3 and $\text{CH}_2\text{CH}_2\text{F}$, lower alkenyl of $\text{C}_2\text{-C}_6$ such as $\text{CH}=\text{CH}_2$,

5 halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

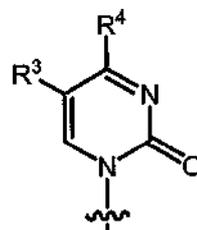
10 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

78. The use of claim 76, wherein

Base is selected from the group consisting of (a) or (b):



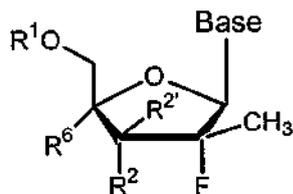
(a)



(b)

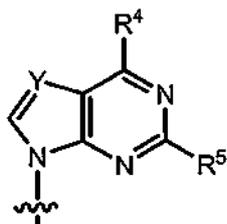
and wherein R¹ is H, R² is OH, R^{2'} is H, R³ is H, and R⁴ is NH₂ or OH, and R⁵ is NH₂.

20 79. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:

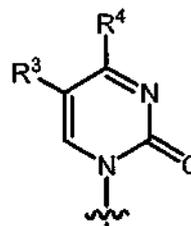


wherein

Base is selected from the group consisting of



(a)



(b)

5

Y is N or CH;

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

10

15

20

R² and R²¹ are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄

5 acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2a} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

20 R³, R⁴ and R⁵ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

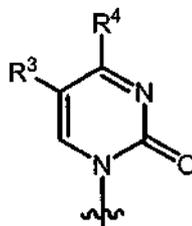
R⁷ is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

5 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

10 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

80. The use of claim 79, wherein

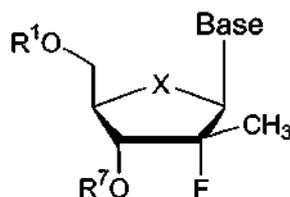
15 Base is



and R¹ is H, R² is OH, R^{2'} is H, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

20

81. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the structure:



wherein Base is a purine or pyrimidine base;

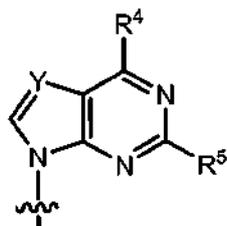
X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂,
wherein W is F, Cl, Br, or I; and,

5 R¹ and R⁷ are independently H, phosphate, including monophosphate,
diphosphate, triphosphate, or a stabilized phosphate prodrug,
H-phosphonate, including stabilized H-phosphonates, acyl,
including optionally substituted phenyl and lower acyl, alkyl,
including lower alkyl, O-substituted carboxyalkylamino or its
10 peptide derivatives, sulfonate ester, including alkyl or
arylalkyl sulfonyl, including methanesulfonyl and benzyl,
wherein the phenyl group is optionally substituted, a lipid,
including a phospholipid, an L or D-amino acid, a
carbohydrate, a peptide, a cholesterol, or other
15 pharmaceutically acceptable leaving group which when
administered *in vivo* is capable of providing a compound
wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷
can also be linked with cyclic phosphate group;

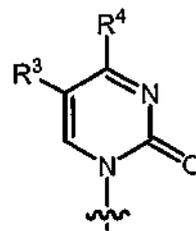
20 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a
pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile
virus infection in a host.

82. The use of claim 81, wherein

Base is selected from the group consisting of:



(a)



(b)

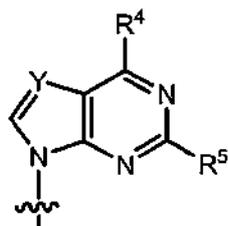
Y is N or CH;

5 R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

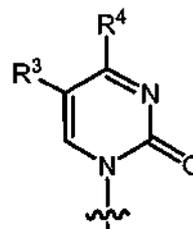
15 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

20 83. The use of claim 81, wherein

Base is selected from the group consisting of (a) or (b):



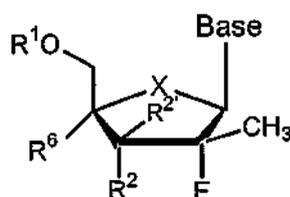
(a)



(b)

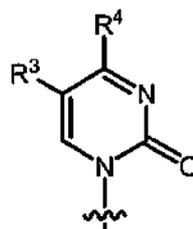
and wherein R^1 and R^7 are H, R^3 is H, and R^4 is NH_2 or OH, and R^5 is NH_2 .

- 5 84. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L) of the formula:



wherein

Base is



10

X is O, S, CH_2 , Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), $C(W)_2$, wherein W is F, Cl, Br, or I;

15

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl,

wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

R³ and R⁴ are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃

5 and $\text{CH}_2\text{CH}_2\text{F}$, lower alkenyl of $\text{C}_2\text{-C}_6$ such as $\text{CH}=\text{CH}_2$, halogenated (F, Cl, Br, I) lower alkenyl of $\text{C}_2\text{-C}_6$ such as $\text{CH}=\text{CHCl}$, $\text{CH}=\text{CHBr}$ and $\text{CH}=\text{CHI}$, lower alkynyl of $\text{C}_2\text{-C}_6$ such as $\text{C}\equiv\text{CH}$, halogenated (F, Cl, Br, I) lower alkynyl of $\text{C}_2\text{-C}_6$, lower alkoxy of $\text{C}_1\text{-C}_6$ such as CH_2OH and $\text{CH}_2\text{CH}_2\text{OH}$, halogenated (F, Cl, Br, I) lower alkoxy of $\text{C}_1\text{-C}_6$, CO_2H , $\text{CO}_2\text{R}'$, CONH_2 , CONHR' , CONR'_2 , $\text{CH}=\text{CHCO}_2\text{H}$, $\text{CH}=\text{CHCO}_2\text{R}'$;

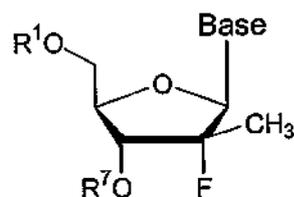
10 R' is an optionally substituted alkyl of $\text{C}_1\text{-C}_{12}$ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of $\text{C}_2\text{-C}_6$, optionally substituted lower alkenyl of $\text{C}_2\text{-C}_6$, or optionally substituted acyl;

15 R^6 is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH_3 , OCH_3 , OCH_2CH_3 , hydroxy methyl (CH_2OH), fluoromethyl (CH_2F), azido (N_3), CHCN , CH_2N_3 , CH_2NH_2 , CH_2NHCH_3 , $\text{CH}_2\text{N}(\text{CH}_3)_2$, alkyne (optionally substituted), or fluoro;

20 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

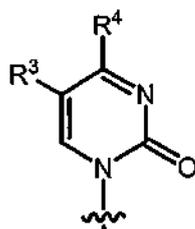
25

85. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside ($\beta\text{-D}$ or $\beta\text{-L}$) of the formula:



wherein

Base is



5

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7 can also be linked with cyclic phosphate group;

10

15

20

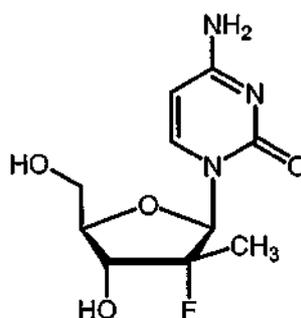
R^3 and R^4 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as

5 CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

10 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

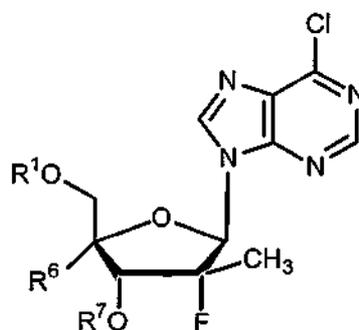
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

15 86. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β-D) of the formula:



20 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

87. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D) of the formula:



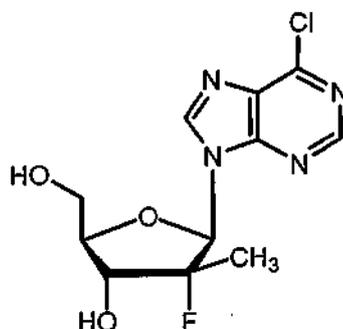
wherein

5 R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its
10 peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other
15 pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7 can also be linked with cyclic phosphate group; and,

20 R^6 is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH_3 , OCH_3 , OCH_2CH_3 , hydroxy methyl (CH_2OH), fluoromethyl (CH_2F), azido (N_3), $CHCN$, CH_2N_3 , CH_2NH_2 , CH_2NHCH_3 , $CH_2N(CH_3)_2$, alkyne (optionally substituted), or fluoro and,

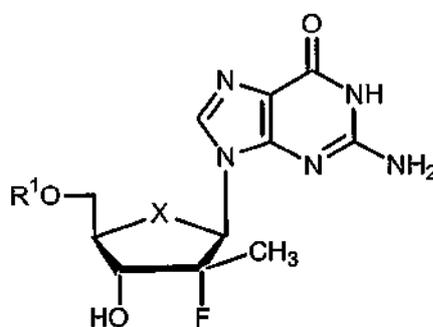
25 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

88. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) of the formula:



5 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

89. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) of the formula:



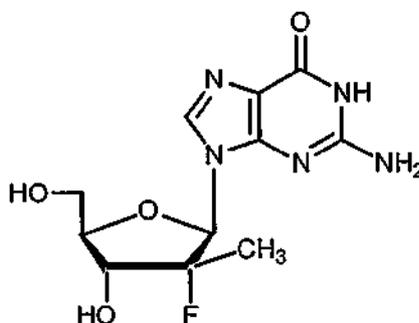
wherein

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and

15 R¹ is H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl,

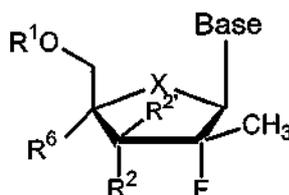
- 5 including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate;
- 10 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

- 15 90. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) of the formula:



or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

91. Use an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) of the formula:



wherein

Base is a purine or pyrimidine base;

- X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂,
 10 wherein W is F, Cl, Br, or I;

- R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

- R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄

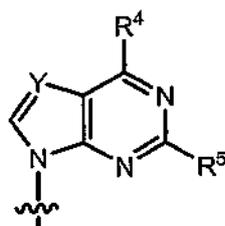
5 acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R²¹ can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

20 R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

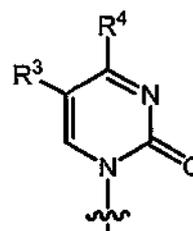
25 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

92. The use of claim 91,

wherein Base is selected from the group consisting of:



(a)



(b)

Y is N or CH.

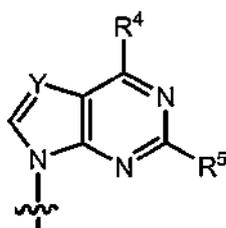
5 R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as
 10 CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H,
 15 CH=CHCO₂R'; and,

R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

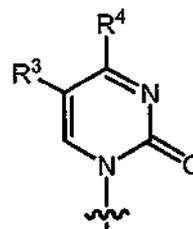
20

93. The use of claim 91, wherein

Base is selected from the group consisting of (a) or (b):



(a)

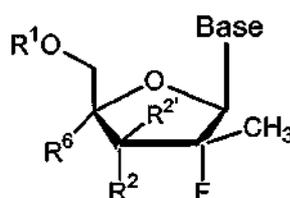


(b)

and wherein R^1 is H, R^2 is OH, $R^{2'}$ is H, R^3 is H, and R^4 is NH_2 or OH, and R^5 is NH_2 .

5

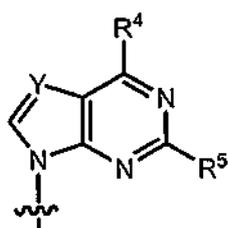
94. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D) of the formula:



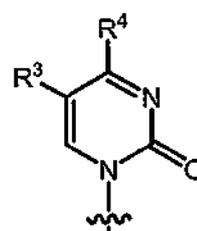
10

wherein

Base is selected from the group consisting of



(a)



(b)

Y is N or CH;

15

R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl,

including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₄ acyl)₂, OR⁷; R² and R^{2'} can be linked together to form a vinyl optionally substituted by one or two of N₃, CN, Cl, Br, F, I, NO₂;

5 R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

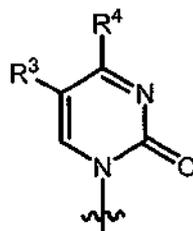
15 R^7 is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl;

20 R^6 is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or fluoro;

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

25 95. The use of claim 94, wherein

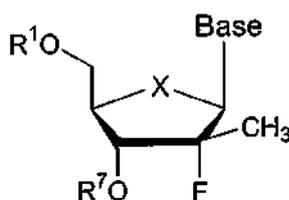
Base is



and R¹ is H, R² is OH, R^{2'} is H, R³ is H, R⁴ is NH₂ or OH, and R⁶ is H.

5

96. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) of the structure:



wherein Base is a purine or pyrimidine base;

10

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and,

15

R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound

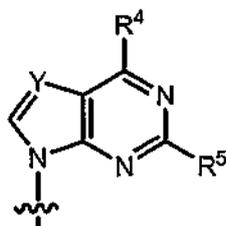
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wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7 can also be linked with cyclic phosphate group;

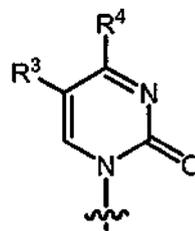
or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

97. The use of claim 96, wherein

Base is selected from the group consisting of:



(a)



(b)

10

Y is N or CH;

15

R^3 , R^4 and R^5 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R'; and,

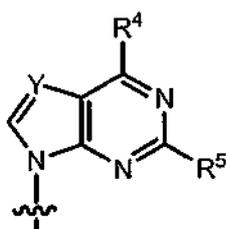
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R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally

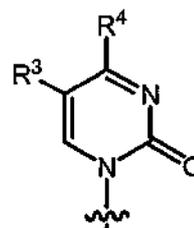
substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

98. The use of claim 96, wherein

5 Base is selected from the group consisting of (a) or (b):



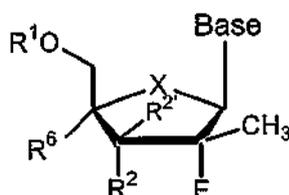
(a)



(b)

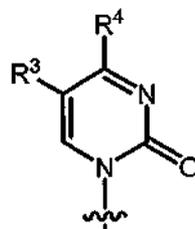
and wherein R¹ and R⁷ are H, R³ is H, and R⁴ is NH₂ or OH, and R⁵ is NH₂.

10 99. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D or β-L) of the formula:



wherein

Base is



15

X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I;

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R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid (or racemic mixture), a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate; R² is H or phosphate; R¹ and R² or R⁷ can also be linked with cyclic phosphate group;

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R² and R^{2'} are independently H, C₁₋₄ alkyl, C₁₋₄ alkenyl, C₁₋₄ alkynyl, vinyl, N₃, CN, Cl, Br, F, I, NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl), N(C₁₋₄ alkyl)₂, N(C₁₋₁₈ acyl)₂, wherein alkyl, alkynyl, alkenyl and vinyl are optionally substituted by N₃, CN, one to three halogen (Cl, Br, F, I), NO₂, C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkyl), C(O)O(C₁₋₄ alkynyl), C(O)O(C₁₋₄ alkenyl), O(C₁₋₄ acyl), O(C₁₋₄ alkyl), O(C₁₋₄ alkenyl), S(C₁₋₄ acyl), S(C₁₋₄ alkyl), S(C₁₋₄ alkynyl), S(C₁₋₄ alkenyl), SO(C₁₋₄ acyl), SO(C₁₋₄ alkyl), SO(C₁₋₄ alkynyl), SO(C₁₋₄ alkenyl), SO₂(C₁₋₄ acyl), SO₂(C₁₋₄ alkyl), SO₂(C₁₋₄ alkynyl), SO₂(C₁₋₄ alkenyl), O₃S(C₁₋₄ acyl), O₃S(C₁₋₄ alkyl), O₃S(C₁₋₄ alkenyl), NH₂, NH(C₁₋₄ alkyl), NH(C₁₋₄ alkenyl), NH(C₁₋₄ alkynyl), NH(C₁₋₄ acyl),

$N(C_{1-4} \text{ alkyl})_2$, $N(C_{1-4} \text{ acyl})_2$, OR^7 ; R^2 and R^{2a} can be linked together to form a vinyl optionally substituted by one or two of N_3 , CN , Cl , Br , F , I , NO_2 ;

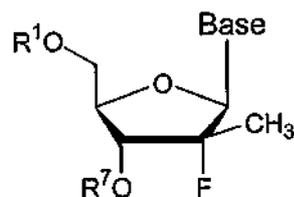
5 R^3 and R^4 are independently H , halogen including F , Cl , Br , I , OH , OR' , SH , SR' , NH_2 , NHR' , NR'_2 , lower alkyl of C_1-C_6 , halogenated (F , Cl , Br , I) lower alkyl of C_1-C_6 such as CF_3 and CH_2CH_2F , lower alkenyl of C_2-C_6 such as $CH=CH_2$, halogenated (F , Cl , Br , I) lower alkenyl of C_2-C_6 such as $CH=CHCl$, $CH=CHBr$ and $CH=CHI$, lower alkynyl of C_2-C_6
10 such as $C\equiv CH$, halogenated (F , Cl , Br , I) lower alkynyl of C_2-C_6 , lower alkoxy of C_1-C_6 such as CH_2OH and CH_2CH_2OH , halogenated (F , Cl , Br , I) lower alkoxy of C_1-C_6 , CO_2H , CO_2R' , $CONH_2$, $CONHR'$, $CONR'_2$, $CH=CHCO_2H$, $CH=CHCO_2R'$;

15 R^5 is an optionally substituted alkyl of C_1-C_{12} (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C_2-C_6 , optionally substituted lower alkenyl of C_2-C_6 , or optionally substituted acyl;

20 R^6 is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH_3 , OCH_3 , OCH_2CH_3 , hydroxy methyl (CH_2OH), fluoromethyl (CH_2F), azido (N_3), $CHCN$, CH_2N_3 , CH_2NH_2 , CH_2NHCH_3 , $CH_2N(CH_3)_2$, alkyne (optionally substituted), or fluoro;

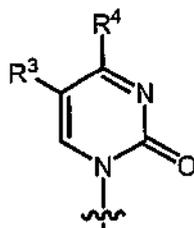
25 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

100. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) of the formula:



wherein

Base is



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R^1 and R^7 are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R^1 or R^7 is independently H or phosphate; R^1 and R^7 can also be linked with cyclic phosphate group;

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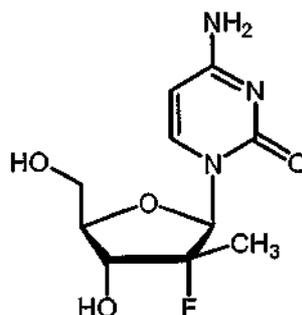
R^3 and R^4 are independently H, halogen including F, Cl, Br, I, OH, OR', SH, SR', NH₂, NHR', NR'₂, lower alkyl of C₁-C₆, halogenated (F, Cl, Br, I) lower alkyl of C₁-C₆ such as CF₃ and CH₂CH₂F, lower alkenyl of C₂-C₆ such as CH=CH₂, halogenated (F, Cl, Br, I) lower alkenyl of C₂-C₆ such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C₂-C₆ such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C₂-C₆, lower alkoxy of C₁-C₆ such as

CH₂OH and CH₂CH₂OH, halogenated (F, Cl, Br, I) lower alkoxy of C₁-C₆, CO₂H, CO₂R', CONH₂, CONHR', CONR'₂, CH=CHCO₂H, CH=CHCO₂R';

5 R' is an optionally substituted alkyl of C₁-C₁₂ (particularly when the alkyl is an amino acid residue), cycloalkyl, optionally substituted alkynyl of C₂-C₆, optionally substituted lower alkenyl of C₂-C₆, or optionally substituted acyl.

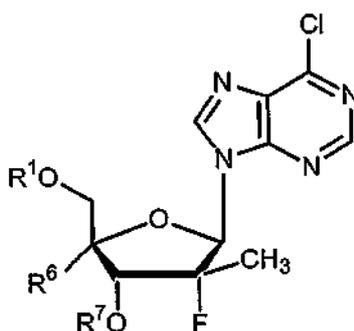
10 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

101. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:



15 or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

20 102. Use of an antivirally effective amount of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β-D) of the formula:



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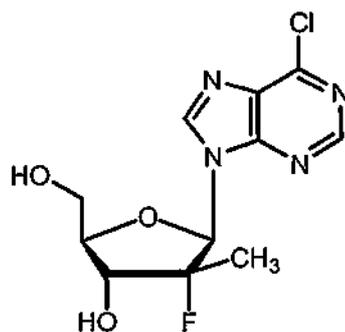
wherein

5 R¹ and R⁷ are independently H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid, 10 including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ or R⁷ is independently H or phosphate; R¹ and R⁷ can also be linked with cyclic phosphate group; and, 15

R⁶ is an optionally substituted alkyl (including lower alkyl), cyano (CN), CH₃, OCH₃, OCH₂CH₃, hydroxy methyl (CH₂OH), fluoromethyl (CH₂F), azido (N₃), CHCN, CH₂N₃, CH₂NH₂, CH₂NHCH₃, CH₂N(CH₃)₂, alkyne (optionally substituted), or 20 fluoro and,

or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

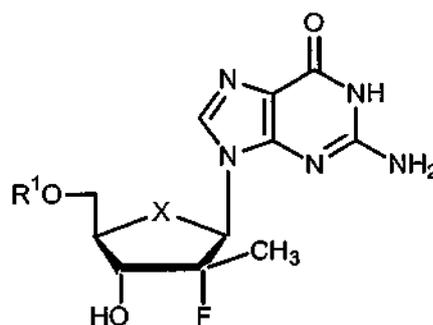
103. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro- 25 2'-C-methyl nucleoside (β-D) of the formula:



or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

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104. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) of the formula:



wherein

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X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and

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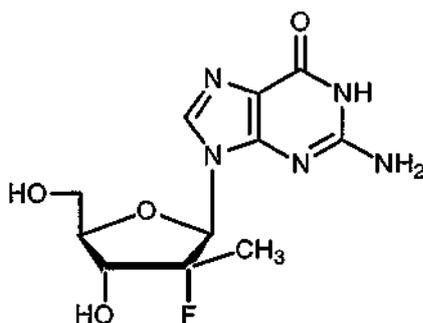
R¹ is H, phosphate, including monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, H-phosphonate, including stabilized H-phosphonates, acyl, including optionally substituted phenyl and lower acyl, alkyl, including lower alkyl, O-substituted carboxyalkylamino or its peptide derivatives, sulfonate ester, including alkyl or arylalkyl sulfonyl, including methanesulfonyl and benzyl, wherein the phenyl group is optionally substituted, a lipid,

including a phospholipid, an L or D-amino acid, a carbohydrate, a peptide, a cholesterol, or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R¹ is H or phosphate;

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or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

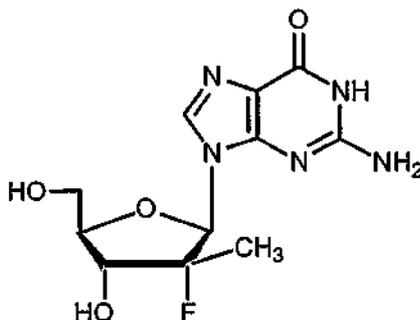
10 105. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) of the formula:



or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a Dengue virus infection in a host.

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106. Use of an antivirally effective amount of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D) of the formula:



or its pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier, for the treatment or prophylaxis of a West Nile virus infection in a host.

5 107. The use claim 31, wherein the antivirally effective amount of (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an
10 interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B
15 polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazole; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an
20 IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

 108. The use claim 41, wherein the antivirally effective amount of (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of:
25 interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase
30 inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a

thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside
prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant
including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-
aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles;
5 thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an
IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

109. The use of claim 43, wherein the antivirally effective amount of
(2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or
10 alternation with at least one treatment selected from the group consisting of:
interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,
interferon beta, interferon gamma, interferon tau and interferon omega; an
interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha
or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
15 protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase
inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B
polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a
thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside
prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant
20 including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-
aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles;
thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an
IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

110. The use of claim 45, wherein the antivirally effective amount of
(2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or
alternation with at least one treatment selected from the group consisting of:
interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,
interferon beta, interferon gamma, interferon tau and interferon omega; an
interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha
25 or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
30

protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside
5 prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

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111. The use of claim 46, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,
15 interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B
20 polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles;
25 thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

112. The use of claim 56, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or
30 alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,

interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

113. The use of claim 58, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

30

114. The use of claim 60, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

115. The use of claim 61, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles;

thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

116. The use of claim 71, wherein the antivirally effective amount of
5 (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or
alternation with at least one treatment selected from the group consisting of:
interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,
interferon beta, interferon gamma, interferon tau and interferon omega; an
interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha
10 or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase
inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B
polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a
thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside
15 prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant
including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-
aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles;
thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an
IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

20

117. The use of claim 73, wherein the antivirally effective amount of
(2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or
alternation with at least one treatment selected from the group consisting of:
interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,
25 interferon beta, interferon gamma, interferon tau and interferon omega; an
interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha
or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase
inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B
30 polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a
thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside

prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an
5 IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

118. The use of claim 75, wherein the antivirally effective amount of (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of:
10 interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase
15 inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-
20 aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

119. The use of claim 76, wherein the antivirally effective amount of (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of:
25 interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
30 protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase

inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

10 120. The use of claim 86, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an
15 interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a
20 thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an
25 IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

 121. The use of claim 88, wherein the antivirally effective amount of (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or alternation with at least one treatment selected from the group consisting of:
30 interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an

interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B
5 polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles;
10 thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

122. The use of claim 90, wherein the antivirally effective amount of (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside is administered in combination or
15 alternation with at least one treatment selected from the group consisting of: interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon, interferon beta, interferon gamma, interferon tau and interferon omega; an interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
20 protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant
25 including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles; thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

30 123. The use of claim 91, wherein the antivirally effective amount of (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside is administered in combination or

alternation with at least one treatment selected from the group consisting of:
interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,
interferon beta, interferon gamma, interferon tau and interferon omega; an
interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha
5 or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase
inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B
polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a
thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside
10 prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant
including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-
aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles;
thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an
IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

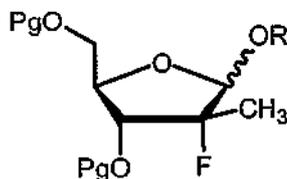
15 124. The use of claim 101, wherein the antivirally effective amount of
(2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or
alternation with at least one treatment selected from the group consisting of:
interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,
interferon beta, interferon gamma, interferon tau and interferon omega; an
20 interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha
or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase
inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B
polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a
25 thiazolidine derivative; a benzanilide, a ribozyme; another nucleoside, nucleoside
prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant
including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-
aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazoles;
thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an
30 IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

125. The use of claim 103, wherein the antivirally effective amount of
(2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or

alternation with at least one treatment selected from the group consisting of:
interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,
interferon beta, interferon gamma, interferon tau and interferon omega; an
interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha
5 or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase
inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B
polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a
thiazolidine derivative; a benzamide, a ribozyme; another nucleoside, nucleoside
10 prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant
including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-
aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazole;
thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an
IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

15 126. The use of claim 105, wherein the antivirally effective amount of
(2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside is administered in combination or
alternation with at least one treatment selected from the group consisting of:
interferon, including interferon alpha 2a, interferon alpha 2b, a pegylated interferon,
interferon beta, interferon gamma, interferon tau and interferon omega; an
20 interleukin, including interleukin 10 and interleukin 12; ribavirin; interferon alpha
or pegylated interferon alpha in combination with ribavirin or levovirin; levovirin; a
protease inhibitor including an NS3 inhibitor, a NS3-4A inhibitor; a helicase
inhibitor; a polymerase inhibitor including HCV RNA polymerase and NS5B
polymerase inhibitor; gliotoxin; an IRES inhibitor; and antisense oligonucleotide; a
25 thiazolidine derivative; a benzamide, a ribozyme; another nucleoside, nucleoside
prodrug or nucleoside derivative; a 1-amino-alkylcyclohexane; an antioxidant
including vitamin E; squalene; amantadine; a bile acid; N-(phosphonoacetyl)-L-
aspartic acid; a benzenedicarboxamide; polyadenylic acid; a benzimidazole;
thymosin; a beta tubulin inhibitor; a prophylactic vaccine; an immune modulator, an
30 IMPDH inhibitor; silybin-phosphatidylcholine phytosome; and mycophenolate.

127. A method of synthesizing a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) comprising glycosylation of a nucleobase with an intermediate structure:

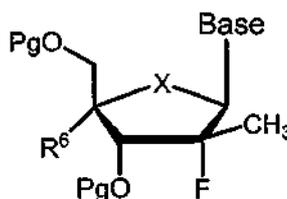


1-4

5 wherein R is lower alkyl, acyl, benzoyl, or mesyl; and Pg is any acceptable protecting group consisting of but not limited to C(O)-alkyl, C(O)Ph, C(O)aryl, CH₃, CH₂-alkyl, CH₂-alkenyl, CH₂Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-alkyl, SO₂-aryl, *tert*-butyldimethylsilyl, *tert*-butyldiphenylsilyl, or both Pg's may come together to for a 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene).

10

128. A method of synthesizing a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L) comprising selective deprotection of either Pg in an intermediate of the structure:



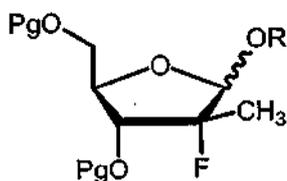
2-5

15

wherein, X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and Pg is independently any pharmaceutically acceptable protecting group selected from the group consisting of C(O)-alkyl, C(O)Ph, C(O)aryl, CH₃, CH₂-alkyl, CH₂-alkenyl, CH₂Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-alkyl, SO₂-aryl, *tert*-butyldimethylsilyl, *tert*-butyldiphenylsilyl, or both Pg's may come together to for a 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene).

20

129. An intermediate in the synthesis of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L), wherein the intermediate is of the structure:

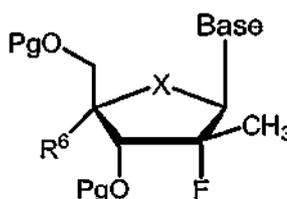


1-4

5 wherein R is lower alkyl, acyl, benzoyl, or mesyl; and Pg is any acceptable protecting group consisting of but not limited to C(O)-alkyl, C(O)Ph, C(O)aryl, CH₃, CH₂-alkyl, CH₂-alkenyl, CH₂Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-alkyl, SO₂-aryl, *tert*-butyldimethylsilyl, *tert*-butyldiphenylsilyl, or both Pg's may come together to for a 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene).

10

130. An intermediate in the synthesis of a (2'*R*)-2'-deoxy-2'-fluoro-2'-*C*-methyl nucleoside (β -D or β -L), wherein the intermediate is of the structure:



2-5

15 wherein, X is O, S, CH₂, Se, NH, N-alkyl, CHW (*R*, *S*, or racemic), C(W)₂, wherein W is F, Cl, Br, or I; and Pg is independently any pharmaceutically acceptable protecting group selected from the group consisting of C(O)-alkyl, C(O)Ph, C(O)aryl, CH₃, CH₂-alkyl, CH₂-alkenyl, CH₂Ph, CH₂-aryl, CH₂O-alkyl, CH₂O-aryl, SO₂-alkyl, SO₂-aryl, *tert*-butyldimethylsilyl, *tert*-butyldiphenylsilyl, or both Pg's may
20 come together to for a 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene).

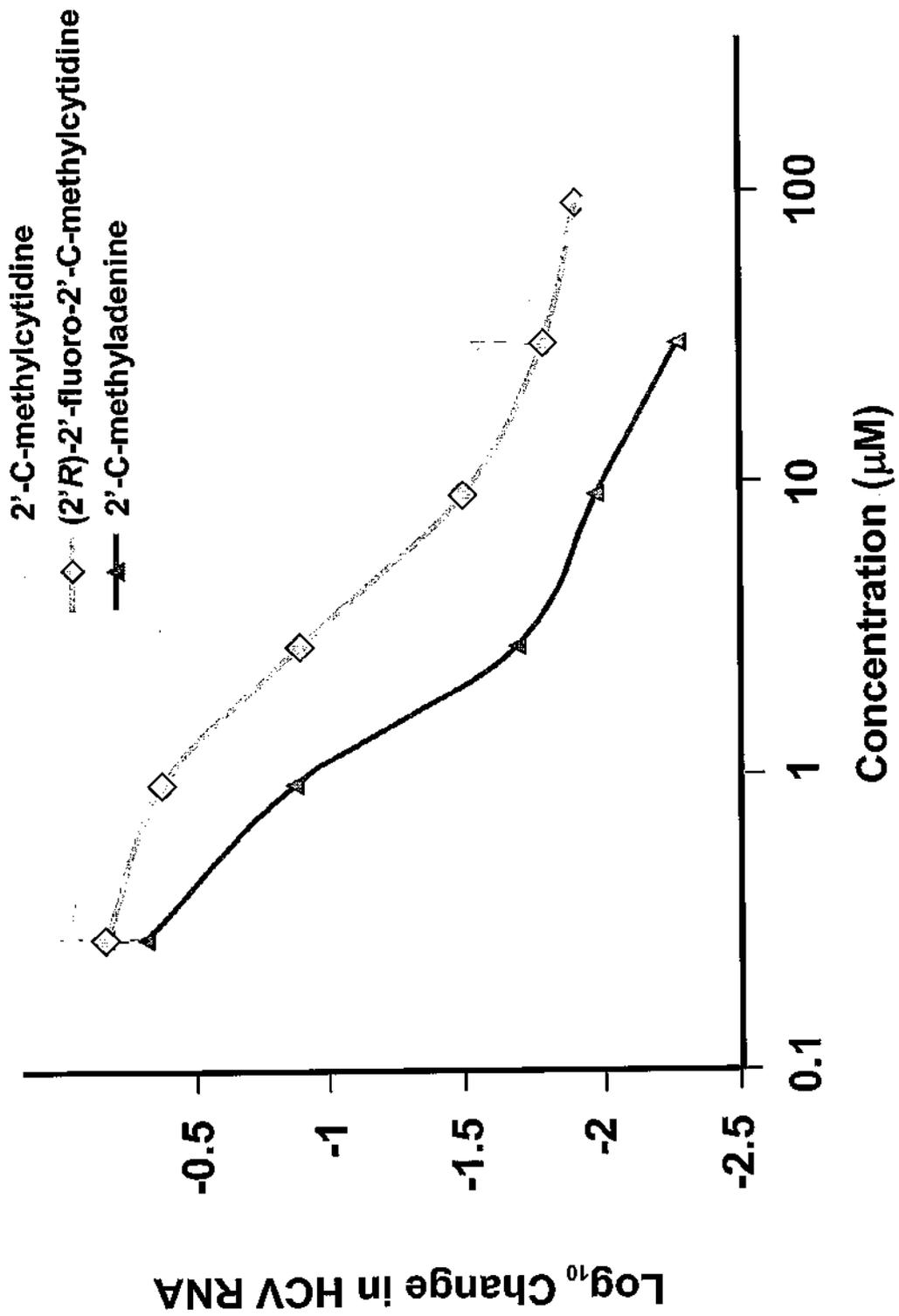


Figure 1A

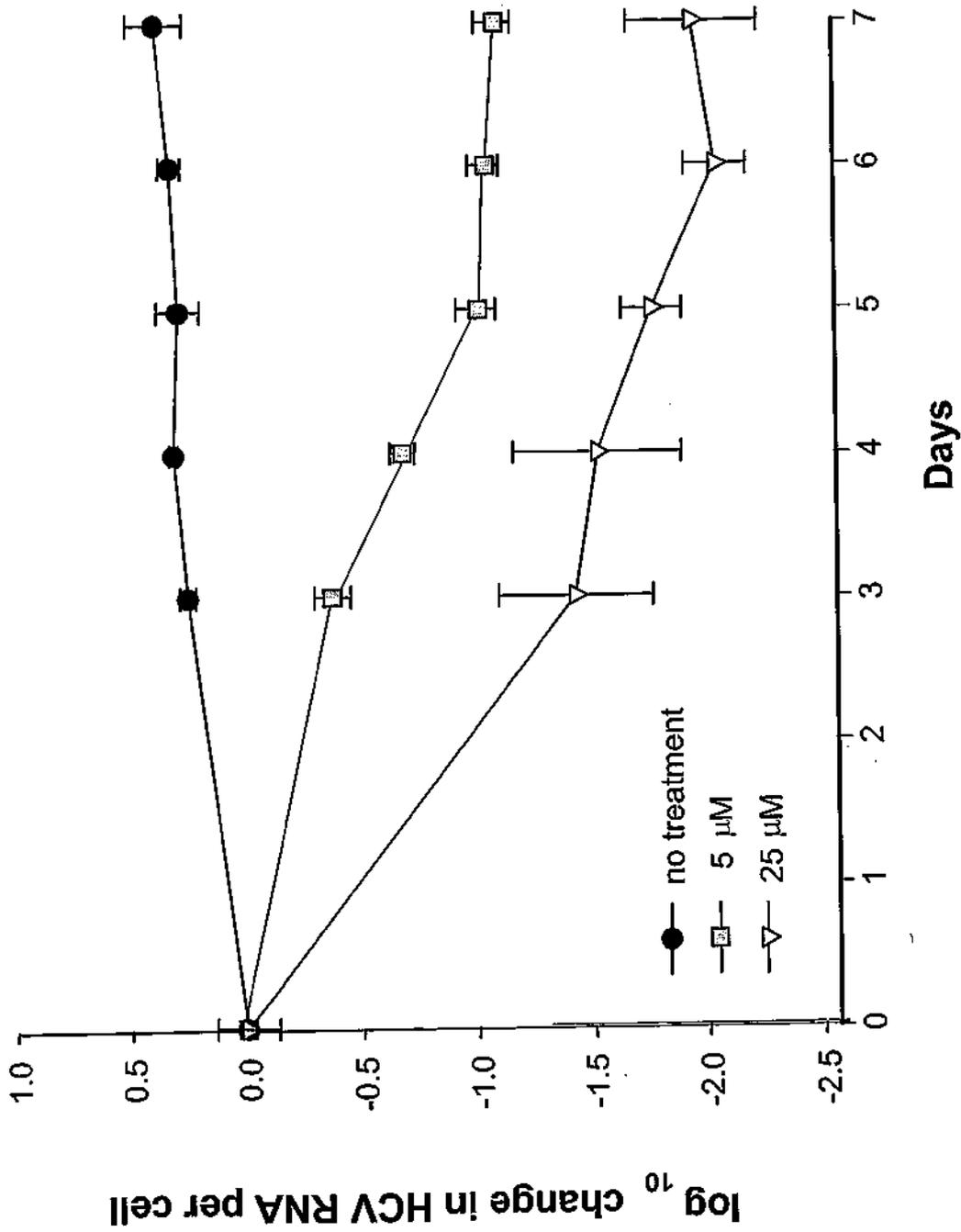


Figure 1B

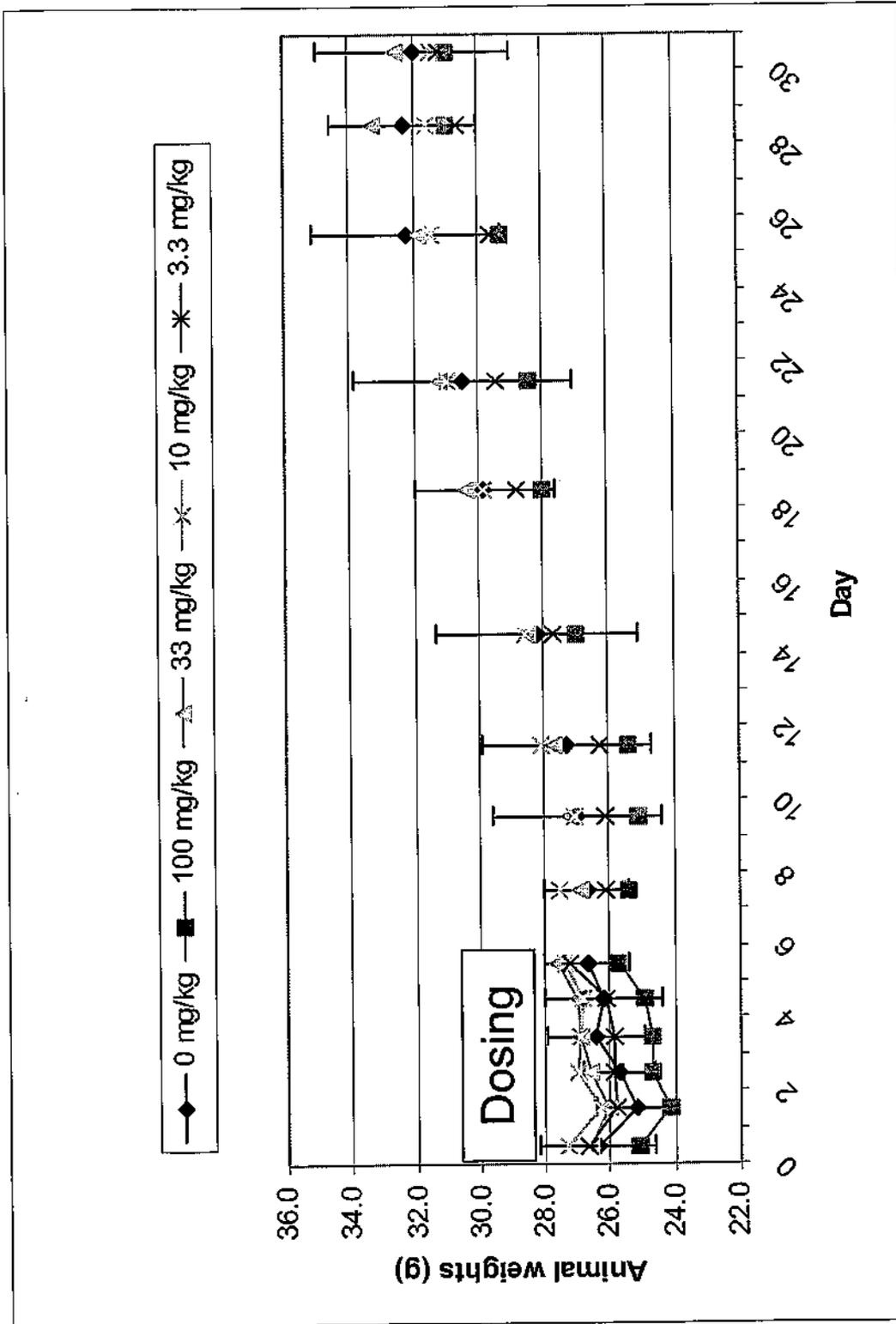


Figure 2

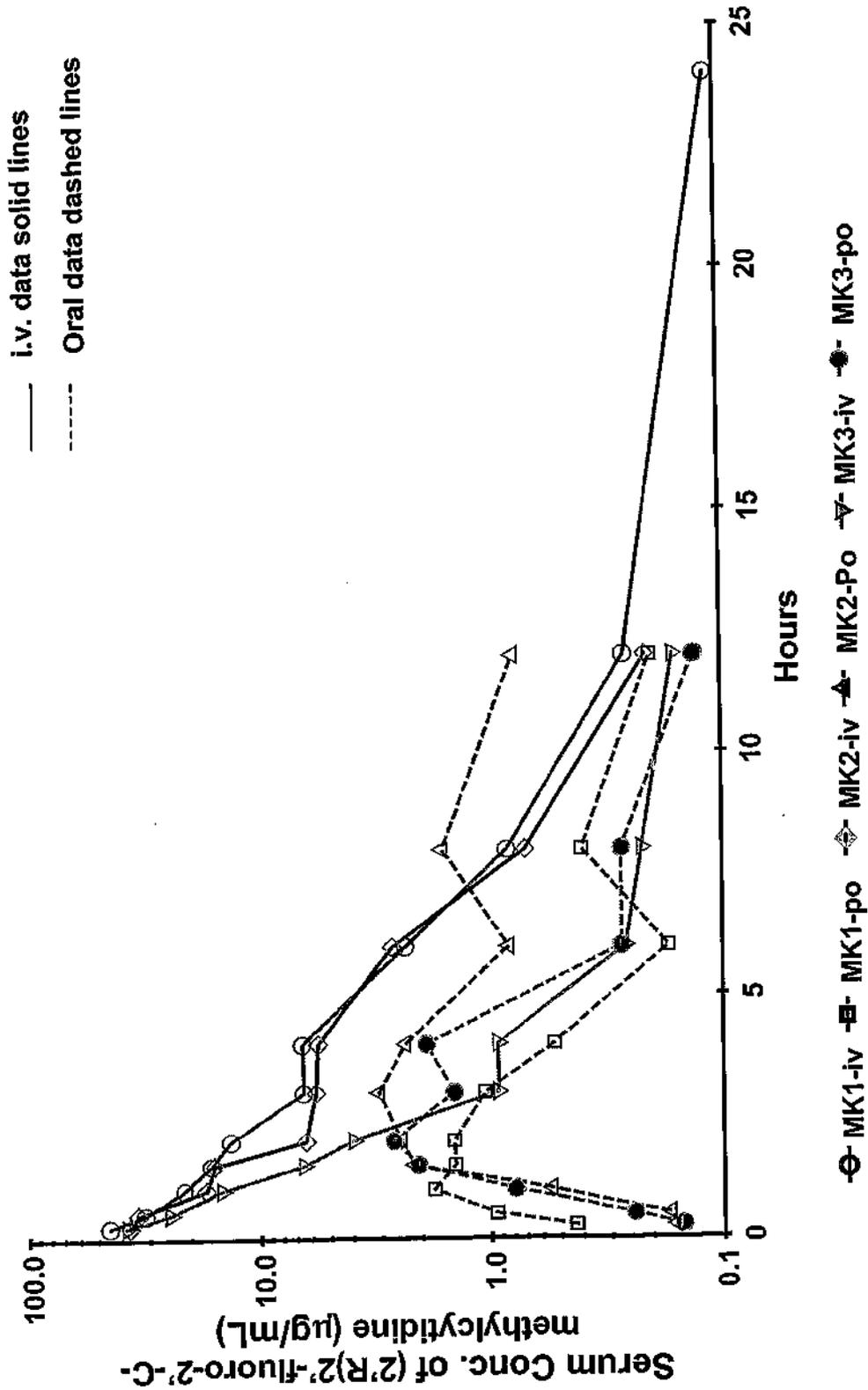


Figure 3

Characterization of the Metabolic Activation of Hepatitis C Virus Nucleoside Inhibitor β -D-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) and Identification of a Novel Active 5'-Triphosphate Species*

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β -D-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) is a potent inhibitor of hepatitis C virus (HCV) replication in the subgenomic HCV replicon system, and its corresponding 5'-triphosphate is a potent inhibitor of the HCV RNA polymerase *in vitro*. In this study the formation of PSI-6130-triphosphate was characterized in primary human hepatocytes. PSI-6130 and its 5'-phosphorylated derivatives were identified, and the intracellular concentrations were determined. In addition, the deaminated derivative of PSI-6130, β -D-2'-deoxy-2'-fluoro-2'-C-methyluridine (RO2433, PSI-6026) and its corresponding phosphorylated metabolites were identified in human hepatocytes after incubation with PSI-6130. The formation of the 5'-triphosphate (TP) of PSI-6130 (PSI-6130-TP) and RO2433 (RO2433-TP) increased with time and reached steady state levels at 48 h. The formation of both PSI-6130-TP and RO2433-TP demonstrated a linear relationship with the extracellular concentrations of PSI-6130 up to 100 μ M, suggesting a high capacity of human hepatocytes to generate the two triphosphates. The mean half-lives of PSI-6130-TP and RO2433-TP were 4.7 and 38 h, respectively. RO2433-TP also inhibited RNA synthesis by the native HCV replicase isolated from HCV replicon cells and the recombinant HCV polymerase NS5B with potencies comparable with those of PSI-6130-TP. Incorporation of RO2433-5'-monophosphate (MP) into nascent RNA by NS5B led to chain termination similar to that of PSI-6130-MP. These results demonstrate that PSI-6130 is metabolized to two pharmacologically active species in primary human hepatocytes.

Hepatitis C is a major health problem affecting ~170 million people worldwide of which around 3 million chronically infected patients reside within the United States (1). The current standard treatment for hepatitis C consisting of pegylated interferon- α and ribavirin only results in about a 50% sustained virological response in patients infected with genotype 1 hepa-

titis C virus (HCV),² the most predominant genotype in the United States and Europe (2–4). New treatment options with improved clinical efficacy and greater tolerability are urgently needed. Novel antiviral agents targeting essential processes of HCV replication as part of optimized combination regimens could achieve increased clinical efficacy and potentially improved adverse event profiles as well as shortened treatment duration as compared with the current standard of care.

HCV RNA replication is mediated by a membrane-associated multiprotein replication complex (5, 6). The HCV NS5B protein, the RNA-dependent RNA polymerase, is the catalytic subunit of the HCV replication complex and is responsible for the synthesis of the RNA progeny and, hence, is a prime target of anti-viral inhibition. Nucleoside analogs have been established as successful antiviral agents targeting the active site of DNA polymerases for the treatment of other viral diseases, including human immunodeficiency virus, hepatitis B virus, and herpes simplex virus (7). The majority of marketed antiviral nucleoside analogs need to be converted to the active 5'-triphosphate forms in the target cells. These nucleotide triphosphate analogs then serve as alternative substrates for the viral DNA polymerases and compete with the incorporation of the corresponding natural nucleotide triphosphates. Upon incorporation by the viral DNA polymerases, the lack of the 3'-hydroxyl group in the deoxyribose moiety leads to the termination of the nascent viral DNA (chain termination).

In the past few years a number of ribonucleoside analogs with 2'-C-methyl, 2'-O-methyl, or 4'-azido substituents on the ribose moiety have been reported to be inhibitors of HCV replication in the subgenomic replicon system (8–13). Prodrugs of two nucleoside analogs, 2'-C-methylcytidine (NM107) and 4'-azidocytidine (R1479), have successfully progressed into clinical development and shown efficacy in HCV-infected patients (14, 15). The corresponding nucleotide triphosphate analogs are substrates for HCV polymerase NS5B and inhibit RNA synthesis activity of HCV NS5B *in vitro*. The incorporation of the nucleotide analogs into nascent HCV RNA strongly reduces the efficiency of further RNA elongation by NS5B,

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² The abbreviations used are: HCV, hepatitis C virus; MP, 5'-monophosphate; DP, 5'-diphosphate; TP, 5'-triphosphate; HPLC, high performance liquid chromatography.

resulting in termination of the nascent RNA product. Therefore, these nucleoside analogs are non-obligatory chain terminators despite the presence of a 3'-hydroxyl group.

Recently, β -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) has been identified as a potent and selective inhibitor of HCV replication in the subgenomic replicon system with little or no cytotoxicity in various human cell lines or bone marrow precursor cells (16). The corresponding triphosphate of PSI-6130 is an inhibitor of HCV NS5B competitive with natural CTP (17). Conversion to the active 5'-triphosphate form by cellular kinases is an important part of the mechanism of action for nucleoside analogs. In this study we determined the metabolism of β -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine in primary human hepatocytes isolated from several donors. We show that β -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine was converted to β -D-2'-deoxy-2'-fluoro-2'-C-methyluridine 5'-triphosphate and β -D-2'-deoxy-2'-fluoro-2'-C-methyluridine 5'-triphosphate via deamination of the phosphorylated cytidylates. Furthermore, we determined the kinetics of the formation of the two active triphosphates and the potency of the two triphosphates against the native HCV replicase and NS5B as well as the molecular mechanism of action of the two triphosphates.

EXPERIMENTAL PROCEDURES

Compounds— β -D-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) was provided by Pharmasset, Inc. (18). A stock solution of 10 mM PSI-6130 was prepared in Dulbecco's phosphate-buffered saline and stored at -20°C . Tritium-labeled PSI-6130 was synthesized at Roche Palo Alto LLC. The tritiated compound was dissolved in 50% (v/v) ethanol at the concentration of 0.97 mCi/ml with a specific activity of 25.78 Ci/mmol. The stock solution was stored at -20°C . The phosphorylated derivatives of PSI-6130, namely PSI-6130-MP, -DP, and -TP, were provided by Pharmasset, Inc. β -D-2'-Deoxy-2'-fluoro-2'-C-methyluridine (RO2433) was synthesized at Roche Palo Alto LLC. RO2433-MP, -DP, and -TP were synthesized by TriLink BioTechnologies (San Diego, CA). Compound stock solutions were prepared in nuclease-free H_2O and stored at -20°C . 3'-dCTP was purchased from TriLink BioTechnologies.

Cell Culture of Primary Human Hepatocytes—Plated fresh human hepatocytes or hepatocyte suspensions were obtained either from CellZDirect, Inc. or from In Vitro Technologies, Inc. Fresh human hepatocytes obtained from each company were plated or cultured on 6-well collagen coated plates (BD Biosciences #356400) at 1.5 million cells per well using complete serum containing medium obtained from the respective companies. Cells were allowed to recover for at least 18 h before the addition of the compound. All incubations were carried out at 37°C in a humidified 5% CO_2 atmosphere.

To determine the time course of uptake and phosphorylation of PSI-6130, human primary hepatocytes were incubated with ^3H -labeled PSI-6130 at a final concentration of 2 μM and 10 $\mu\text{Ci}/\text{ml}$. The compound was added 72, 48, 24, 16, 6 and 1 h before cell harvesting. All time points and untreated cell controls were set up in duplicates.

To determine the dose response of the phosphorylation of PSI-6130, human primary hepatocytes were incubated with

^3H -labeled PSI-6130 at 0, 2, 10, 25, 50, 100, and 250 μM for 24 h. Final concentrations of PSI-6130 were achieved by supplementing ^3H -labeled PSI-6130 with non-radiolabeled PSI-6130. Duplicate cell samples were harvested after 24 h of incubation.

To determine the half-life of the triphosphates of PSI-6130 and RO2433, human primary hepatocytes were incubated for 24 h with ^3H -labeled PSI-6130 at 2 μM and 10 $\mu\text{Ci}/\text{ml}$. The cell monolayer was washed once with the cell culture medium without PSI-6130 and then incubated with fresh medium without PSI-6130 at 0-, 0.5-, 1-, 2-, 4-, 6-, 8-, 24-, 48-, and 72-h time points after the removal of PSI-6130. Duplicate cell samples were set up for each time point. The viable cell numbers of the untreated cell controls for each experiment were determined at the end of the experiment using the trypan blue exclusion method.

Preparation of Cell Extract for High Performance Liquid Chromatography (HPLC) Analysis—At the time of cell harvest the cell culture medium was aspirated, and the cells were washed once with cold phosphate-buffered saline. The cells were scraped into 1 ml of pre-chilled 60% (v/v) methanol and extracted in methanol for 24 h at -20°C . The extracted samples were then centrifuged at $10,000 \times g$ for 15 min to remove cell debris. The supernatant was transferred to new tubes and evaporated in a speed vacuum at room temperature. The pellets were stored at -80°C until analysis.

The dried pellets of cell extracts were dissolved in H_2O and filtered through a nanosep MF centrifugal device (Pall Life Sciences #ODM02C34). Before HPLC analysis, cell extract samples were spiked with unlabeled reference standards PSI-6130, RO2433, and their phosphorylated derivatives.

HPLC—The phosphorylated derivatives of PSI-6130 were separated by ion exchange HPLC with a Whatman Partisil 10 SAX (4.6×250 mm) column coupled to a radiometric detector (β -RAM, IN/US Systems, Inc.). The mobile phase gradient changed linearly from 0% buffer A (H_2O) to 100% buffer B (0.5 M KH_2PO_4 + 0.8 M KCl) between 4 and 8 min. 100% buffer B ran from 8 to 18 min and changed back to 100% A in 1 min. Buffer A ran until 25 min. The flow rate was 1 ml/min. A ratio of 5:1 Flo Scint IV or Ultima-FloTM AP (PerkinElmer Life Sciences) to column eluent was used for the detection of radiolabeled species in the β -RAM detector (IN/US Systems, Inc.).

The separation of PSI-6130 and RO2433 was performed by reverse phase chromatography with a Zorbax SB-C8 column (4.6×250 mm, 5 μm) coupled to a radiometric detector (β -RAM). The gradient changed linearly from 100% buffer A (0.01 M heptane sulfonic acid, sodium salt, 0.1% (v/v) acetic acid in water) to 10% buffer B (0.01 M heptane sulfonic acid sodium salt, 0.1% (v/v) acetic acid in 1:1 methanol water) between 0 and 3 min and then changed linearly from 10% buffer B to 95% buffer B between 3 and 18 min. 95% buffer B ran from 18 to 22 min and changed back to 100% A in 0.1 min. Buffer A ran until 25 min. The flow rate was 1 ml/min. PSI-6130 and its intercellular metabolites were identified by comparison of the retention times of the intracellular species in the radiochromatogram with the retention times of nonradioactive reference standards spiked in the cell extract samples and detected by UV absorption at 270 nm.

Metabolism and Mechanism of Action of β -D-2'-Deoxy-2'-fluoro-2'-C-methylcytidine

Acid Phosphatase Treatment of Cell Extracts—Hepatocyte cell extracts were incubated with acid phosphatase (Sigma #P-0157) at a final concentration of 0.05 mg/ml (23.9 units/ml) at 37 °C for 2.5 h to dephosphorylate any phosphorylated metabolites of PSI-6130. After digestion the samples were analyzed by reversed phase HPLC.

HCV Replicon Assay—The 2209-23 cell line containing a bicistronic HCV subgenomic replicon (genotype 1b, Con1 strain), which expresses a Renilla luciferase reporter gene as an index of HCV RNA replication, has been described before (9). The analysis of inhibition of HCV replication by nucleoside analogs and IC_{50} determinations were performed as described (12).

HCV Replicase Assay—The membrane-associated native HCV replication complexes were isolated from 2209-23 replicon cell lines as described (6). The inhibition of the RNA synthesis activity of the HCV replicases by PSI-6130-TP was determined as described (6) except that 5 μ l of cytoplasmic replicase complex (2.5×10^6 replicon cell equivalent) was added to a 20- μ l reaction for 60 min. The inhibition of the RNA synthesis activity of the HCV replicases by RO2433-TP was determined in reactions containing 6.25 μ l of cytoplasmic replicase complex (3.1×10^6 replicon cell equivalent), 50 mM HEPES, pH 7.5, 10 mM KCl, 10 mM dithiothreitol, 5 mM $MgCl_2$, 20 μ g/ml actinomycin D, 1 mM ATP, GTP, and CTP, 24 μ Ci of (0.4 μ M) [α - ^{33}P]UTP (PerkinElmer #NEG607H), 1 units/ μ l SUPERase. In (Ambion), 10 mM creatine phosphate, 200 μ g/ml creatine phosphokinase with or without the nucleotide triphosphate inhibitor in a final volume of 20 μ l for 90 min.

HCV Polymerase Assay—The inhibition potency of PSI-6130-TP on the RNA-dependent RNA polymerase activity of recombinant NS5B570-Con1 (genotype 1b, GenBankTM accession number AJ242654) was measured as the incorporation of radiolabeled nucleotide monophosphate into acid-insoluble RNA products as described (6) with the following modifications; IC_{50} determinations were carried out using 200 nM *in vitro* transcribed complementary internal ribosome entry site RNA template, 1 μ Ci of tritiated UTP (42 Ci/mmol), 500 μ M ATP, 500 μ M GTP, 1 μ M CTP, 1 \times TMDN buffer (40 mM Tris-HCl, pH 8.0, 4 mM $MgCl_2$, 4 mM dithiothreitol, 40 mM NaCl) and 200 nM NS5B570-Con1. The inhibition potency of RO2433-TP was determined as described above with the following modification of NTP concentrations: 1 μ Ci of tritiated CTP (39 Ci/mmol), 500 μ M ATP, 500 μ M GTP, 1 μ M UTP. The compound concentration at which the enzyme-catalyzed rate is reduced by 50% (IC_{50}) was calculated using equation,

$$Y = \% \text{ Min} + \frac{(\% \text{ Max} - \% \text{ Min})}{\left(1 + \frac{X}{(IC_{50})}\right)} \quad (\text{Eq. 1})$$

where Y corresponds to the relative enzyme activity, % Min is the residual relative activity at saturating compound concentration, % Max is the relative maximum enzymatic activity, and X corresponds to the compound concentration.

The apparent Michaelis constants ($K_{m(\text{app})}$) for UTP or CTP were measured using assay conditions above with the following modifications; $K_{m(\text{app})}$ for CTP was measured using 2 μ Ci of

tritiated UTP (0.93 μ M), 4.07 μ M unlabeled UTP, 50 μ M ATP, 50 μ M GTP, and 5 nM to 50 μ M CTP; $K_{m(\text{app})}$ for UTP was measured using 2 μ Ci of tritiated CTP (1.67 μ M), 3.33 μ M unlabeled CTP, 50 μ M ATP, 50 μ M GTP, and 5 nM to 50 μ M UTP. Apparent $K_{m(\text{app})}$ values were calculated by nonlinear fitting using Equation 2,

$$Y = \frac{(V_{\text{max}(\text{app})})X}{K_{m(\text{app})} + X} \quad (\text{Eq. 2})$$

where Y corresponds to the rate of RNA synthesis by NS5B, $V_{\text{max}(\text{app})}$ is the maximum rate of RNA synthesis at saturating substrate concentration, and X corresponds to CTP or UTP concentration.

K_i values were derived from the Cheng-Prusoff Equation (Equation 3) for competitive inhibition,

$$K_i = \frac{IC_{50}}{\left(1 + \frac{[NTP]}{K_{m(\text{app})}}\right)} \quad (\text{Eq. 3})$$

where [NTP] is the concentration of CTP or UTP, and $K_{m(\text{app})}$ is the apparent Michaelis constant for CTP or UTP. Mean K_i values were averaged from independent measurements at 0.2, 1, 5, and 25 μ M CTP or UTP concentrations in triplicate experiments.

Gel-based Nucleotide Incorporation Assay—The RNA template-directed nucleotide incorporation and extension of nucleotide triphosphates and nucleotide triphosphate analogs by HCV polymerase was performed with a 19-nucleotide RNA oligo (5'-AUGUAUAAUUAUUGUAGCC-3') under assay conditions as described (9). The incorporation and extension of CTP and CTP analogs were determined with 5'-end-radiolabeled GG primer and nucleotide triphosphates at the indicated concentrations. The incorporation and extension of UTP and UTP analogs were performed similarly with the same RNA oligo template, 5'-end-radiolabeled GGC primer and nucleotide triphosphates at the indicated concentrations. The radiolabeled RNA products were separated on a TBE-urea acrylamide gel and analyzed using phosphorimaging (GE Healthcare).

RESULTS

Metabolic Profile of PSI-6130—Cellular extracts from primary human hepatocytes incubated with tritium-labeled β -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) were resolved by ion exchange HPLC. PSI-6130 and metabolites derived from PSI-6130 were identified by comparing the retention times of radiolabeled species with the retention times of unlabeled reference compounds (Fig. 1A). As shown in Fig. 1B, PSI-6130 (3.0 min) and the 5'-phosphorylated derivatives PSI-6130-DP (13.2 min) and PSI-6130-TP (16.8 min) were identified in human hepatocytes incubated with PSI-6130. In addition, metabolites with retention times corresponding to those of the deaminated product of PSI-6130, β -D-2'-deoxy-2'-fluoro-2'-C-methyluridine (RO2433, 3.8 min) and its corresponding 5'-phosphates RO2433-DP (12.5 min) and RO2433-TP (14.8 min), were detected (Fig. 1B). The monophosphates of the cytidine and uridine analogs PSI-6130-MP and RO2433-MP were not separated sufficiently under the

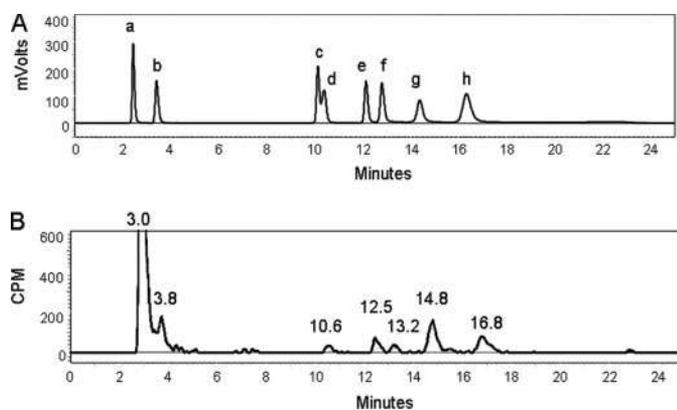


FIGURE 1. Ion exchange HPLC profile of an extract of primary human hepatocytes incubated with PSI-6130. A, HPLC separation and retention times of reference compounds PSI-6130 (a, 2.5 min), RO2433 (b, 3.4 min), RO2433-MP (c, 10.2 min), PSI-6130-MP (d, 10.4 min), RO2433-DP (e, 12.2 min), PSI-6130-DP (f, 12.8 min), RO2433-TP (g, 14.4 min), and PSI-6130-TP (h, 16.3 min). B, HPLC profile of an extract from hepatocytes incubated with ^3H -labeled PSI-6130 at $2\ \mu\text{M}$ for 24 h. The retention times in min of the intracellular species are indicated above the radioactive peaks. Intracellular PSI-6130 and its metabolites were identified by comparing the retention times of the radioactive peaks with those of the UV absorption peaks of reference compounds. There is a calibrated 0.3–0.4-min delay in the retentions times of radioactive trace compared with those of UV trace due to sample traveling from UV detector to radiometric detector.

chromatography conditions and, therefore, co-eluted as a single radioactive peak at 10.6 min. It has been reported that 2'-O-methylcytidine was extensively metabolized to CTP and UTP due to deamination coupled with demethylation of the 2'-substituent or base swapping after glycosidic bond cleavage (19). None of the intracellular metabolites of PSI-6130 was eluted with retention time corresponding to those of 2'-C-methyl-CTP, CTP, and UTP (data not shown). Therefore, there was no evidence for metabolism of PSI-6130 at the 2'-position or evidence for hydrolysis at the glycosidic bond. These data suggest that the primary routes of PSI-6130 metabolism in human hepatocytes were phosphorylation at the 5'-position and deamination at the base.

The hepatocyte extracts were also analyzed by reversed phase HPLC to identify unphosphorylated metabolites of PSI-6130. Two unphosphorylated species with retention times corresponding to PSI-6130 (20.3 min) and its uridine metabolite RO2433 (11.9 min) were observed, with PSI-6130 being the predominant intracellular species (Fig. 2B). There was no evidence of formation of uracil, uridine, cytosine, cytidine, or 2'-deoxycytidine (data not shown). These data agree well with the ion exchange HPLC analysis result and suggest the absence of metabolism at the 2' position and at the glycosidic bond. The phosphorylated metabolites, with the exception of PSI-6130-MP, were not resolved by reversed phase HPLC and co-eluted early as a single broad peak (Fig. 2, A, and B). Acid phosphatase treatment completely converted all the intracellular metabolites to PSI-6130 and RO2433. Therefore, all detected intracellular metabolites represent phosphorylated derivatives of PSI-6130 and RO2433. These results established that PSI-6130 could be phosphorylated to its pharmacologically active 5'-triphosphate analog and that PSI-6130 and/or its phosphates could be deaminated to the corresponding uridine analogs in primary human hepatocytes.

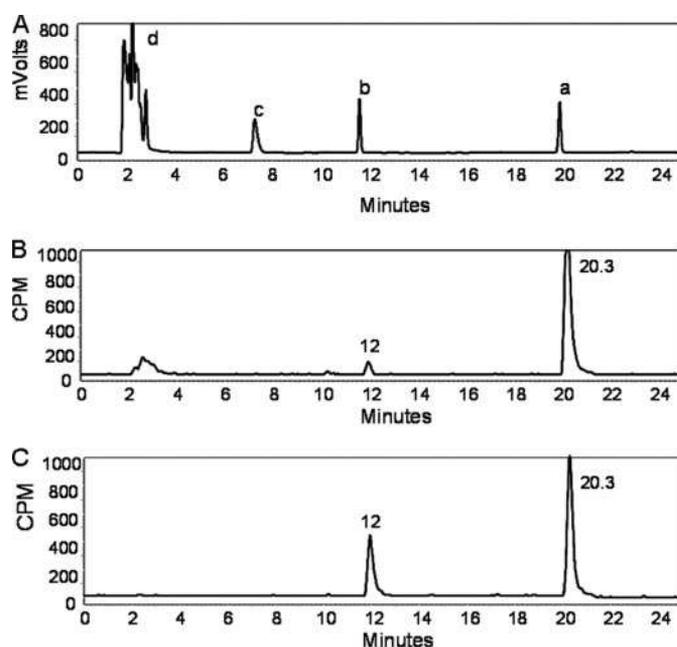


FIGURE 2. Identification of PSI-6130 and its metabolites by reversed phase HPLC and acid phosphatase treatment. A, reversed phase HPLC separation of standard compounds: PSI-6130 (a, 20 min), RO2433 (b, 11.7 min), PSI-6130-MP (c, 7.5 min), (d, PSI-6130-DP, PSI-6130-TP, RO2433-MP, RO2433-DP, and RO2433-TP). B, reversed phase HPLC profile of an extract from hepatocytes incubated with $25\ \mu\text{M}$ extracellular PSI-6130 for 24 h. C, reversed phase HPLC profile of the same extract after acid phosphatase treatment.

In Vitro Potency of RO2433 and RO2433-TP—As described above, incubation of human hepatocytes with PSI-6130 resulted in the formation of substantial concentrations of the triphosphate of its uridine analog, RO2433-TP. Next we determined whether the PSI-6130-derived uridine analog RO2433 could inhibit HCV replication targeting NS5B polymerase. Huh7 cells containing a subgenomic genotype 1b Con1 strain HCV replicon were incubated with RO2433 or PSI-6130 for 72 h, and dose-dependent inhibition of luciferase reporter activity was determined. RO2433 did not inhibit the HCV replication in the HCV subgenomic replicon system at concentrations up to $100\ \mu\text{M}$, whereas PSI-6130 inhibited HCV replication with a mean IC_{50} of $0.6\ \mu\text{M}$ under the same assay conditions (Table 1). The lack of potency in the replicon could be related to inefficient compound phosphorylation. To address whether the triphosphate of RO2433 directly inhibits the HCV RNA polymerase, the RNA synthesis activity of the native membrane-associated HCV replication complexes isolated from the same replicon cells was tested in the presence of RO2433-TP. RO2433-TP inhibited the RNA synthesis activity of HCV replicase with a mean IC_{50} of $1.19\ \mu\text{M}$, whereas PSI-6130-TP inhibited HCV replicase with a mean IC_{50} of $0.34\ \mu\text{M}$ (Table 1). RO2433-TP also inhibited the RNA synthesis activity of the recombinant HCV Con1 NS5B on a heteropolymeric RNA template derived from the 3'-end of the negative strand of the HCV genome with an IC_{50} of $0.52\ \mu\text{M}$ and K_i of $0.141\ \mu\text{M}$, as compared with an IC_{50} of $0.13\ \mu\text{M}$ and K_i of $0.023\ \mu\text{M}$ for PSI-6130-TP under the same assay conditions (Table 1). These results established that both RO2433-TP and PSI-6130-TP are intrinsically potent inhibitors of RNA synthesis by HCV polymerase.

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TABLE 1

Potency of PSI-6130 and RO2433 and their 5'-triphosphates

Values presented are the mean \pm S.D. from greater than three independent experiments. ND, not determined.

Compound	IC ₅₀		Replicase GT1b	K _i NS5B GT1b	K _m NS5B GT1b
	Replicon GT1b	NS5B GT1b			
PSI-6130	0.6 \pm 0.04	μ M ND	ND	μ M ND	μ M ND
RO2433	>100	ND	ND	ND	ND
PSI-6130-TP	ND	0.13 \pm 0.01	0.34 \pm 0.1	0.023 \pm 0.002	0.22 (CTP)
RO2433-TP	ND	0.52 \pm 0.11	1.19 \pm 0.09	0.141 \pm 0.03	0.37 (UTP)

To investigate the molecular mechanism of HCV polymerase inhibition by PSI-6130-TP and RO2433-TP, we measured their incorporation and chain-termination properties. The incorporation of nucleotide and nucleotide analogs by HCV polymerase NS5B was determined in a gel-based assay using a short RNA template (Fig. 3A). Incorporation of CMP, PSI-6130-MP, and 3'-dCMP was initiated from a ³³P-labeled GG primer (Fig. 3A). Incorporated CMP (Fig. 3B, lane 6) could be further extended in the presence of the next nucleotide UTP (Fig. 3B, lanes 7–10). PSI-6130-TP and 3'-dCTP could also serve as substrates for HCV NS5B and were incorporated into the nascent RNA product (Fig. 3B, lane 11 and 16). After incorporation of PSI-6130-MP or 3'-dCMP, further extension in the presence of the next nucleotide UTP was completed blocked even when UTP was present at concentrations up to 1 mM (Fig. 3B, lane 12–15 and lanes 16–20, respectively). Incorporation of UMP, RO2433-MP, and 3'-dUMP was initiated from a ³³P-labeled GGC primer using the same RNA template (Fig. 3A). Incorporated UMP (Fig. 3C, lane 6) could be further extended in the presence of the next nucleotide ATP (Fig. 3C, lanes 7–10). Full-length RNA product was observed in the presence of UTP and ATP but absence of CTP (Fig. 3C, lanes 7–10), possibly due to misincorporation by NS5B through G-U wobble base-pairing. HCV NS5B was also able to incorporate RO2433-MP (Fig. 3C, lane 11) and 3'-dUMP (Fig. 3C, lane 16) but was unable to further extend the incorporated RO2433-MP and 3'-dUMP in the presence of the next nucleotide ATP (Fig. 3C, lanes 12–15 and lanes 17–20, respectively). The control samples with GG primer and UTP-only as well as GGC primer and ATP-only did not lead to further extension of the primers, suggesting the incorporation of PSI-6130-MP and RO2433-MP was base-specific. Taken together, these results demonstrate that PSI-6130-TP and RO2433-TP serve as alternative substrates for HCV NS5B and act as functional chain terminators once incorporated into nascent RNA. While this manuscript was under preparation it was reported that the incorporation of PSI-6130-MP into the nascent RNA by HCV polymerase led to chain termination, in agreement with our observations (17).

Kinetics of Phosphorylation of PSI-6130 in Primary Human Hepatocytes—To determine the steady state level of the two triphosphates in hepatocytes after exposure to PSI-6130, primary human hepatocytes from 4 different donors were incubated with 2 μ M PSI-6130 for up to 72 h. The uptake of PSI-6130 by human hepatocytes was fast, and total intracellular activity reached steady state levels at 1 h of PSI-6130 incubation, the earliest time point in this study (Table 2). PSI-6130-TP was detectable in hepatocytes from all 4 donors at 6 h after PSI-6130 incubation and increased with time to reach steady state levels

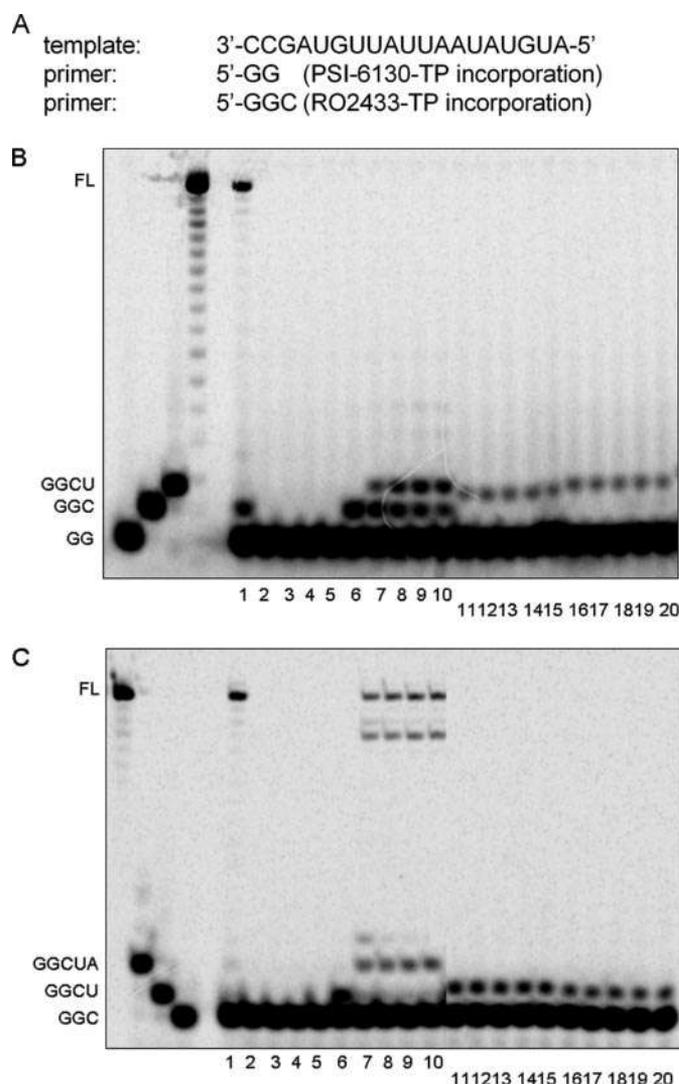


FIGURE 3. PSI-6130-TP and RO2433-TP are substrates of HCV polymerase and chain-terminators. A, sequence of the RNA template and primers. B, the incorporation of CTP and CTP analogs was initiated with GG primer according as described under "Experimental Procedures." The nucleotide triphosphates included in the reactions were as follows: 10 μ M CTP, ATP, and UTP (lane 1); 2, 10, 100, and 1000 μ M UTP (lanes 2–5); 10 μ M CTP without UTP (lane 6) or with 2, 10, 100, and 1000 μ M UTP (lanes 7–10); 10 μ M PSI-6130-TP without UTP (lane 11) or with 2, 10, 100, and 1000 μ M UTP (lanes 12–15); 10 μ M 3'-dCTP without UTP (lane 16) or with 2, 10, 100, and 1000 μ M UTP (lanes 17–20). FL, full-length. C, incorporation of UTP and UTP analogs initiated with GGC primer. The nucleotide triphosphates included in the reactions were as follows: 100 μ M CTP, ATP, and UTP (lane 1); 20, 50, and 200 μ M ATP (lanes 2–5); 100 μ M UTP without ATP (lane 6) or with 2, 20, 50, and 200 μ M ATP (lanes 7–10); 100 μ M RO2433-TP without ATP (lane 11) or with 20, 50, and 200 μ M ATP (lanes 12–15); 100 μ M 3'-dUTP without ATP (lane 16) or with 20, 50, and 200 μ M ATP (lanes 17–20).

TABLE 2**Time course of uptake and phosphorylation of PSI-6130**

Levels of total intracellular species, PSI-6130-TP, and RO2433-TP were determined in hepatocytes incubated with $2 \mu\text{M}$ PSI-6130 for the indicated lengths of time. Data shown are mean \pm S.D. derived from four independent experiments using hepatocytes from four donors. BQL, below the quantification limit.

Time	Total intracellular species	PSI-6130-TP	RO2433-TP
<i>h</i>	<i>pmol/million cells</i>	<i>pmol/million cells</i>	<i>pmol/million cells</i>
1	30.4 ± 18.8	BQL	BQL
6	32.9 ± 16.8	0.6 ± 0.5	0.3 ± 0.01^a
16	34.4 ± 20.8	0.7 ± 0.6	0.6 ± 0.3
24	36.4 ± 21.3	1.1 ± 0.7	1.3 ± 1.1
48	31.9 ± 17.1	1.3 ± 0.6	2.0 ± 1.1
72	34.6 ± 15.4	1.0 ± 0.4	2.0 ± 1.3

^a Mean \pm S.D. derived from 2 donors as RO2433-TP was only detectable in 2 out of 4 donors at the 6-h time point.

at 24–48 h. The formation of the triphosphate of the uridine metabolite, RO2433-TP, demonstrated a delayed time course relative to that of PSI-6130-TP. RO2433-TP was detectable in hepatocytes from only 2 of 4 donors at 6 h and in hepatocytes of all 4 donors at 16 h. RO2433-TP formation reached steady state levels at 48–72 h. RO2433-TP concentrations were lower than those of PSI-6130-TP at time points earlier than 16 h but surpassed those of PSI-6130-TP at 24 h and beyond. The mean steady state level concentrations of PSI-6130-TP and RO2433-TP after incubation with $2 \mu\text{M}$ PSI-6130 were 1.3 ± 0.6 and 2.0 ± 1.1 pmol/ 10^6 cells at 48 h, respectively. Unchanged PSI-6130 was the major intracellular species at all time points tested after incubation of human hepatocytes with radiolabeled PSI-6130 (Fig. 4).

To determine whether increasing the exposure of hepatocytes to PSI-6130 will lead to increased uptake of PSI-6130 and formation of PSI-6130-TP and RO2433-TP, primary human hepatocytes from 3 different donors were incubated with PSI-6130 at different concentrations up to a maximal concentration of $250 \mu\text{M}$ for 24 h. Total intracellular species, determined by total intracellular radioactivity in the cell extracts, increased linearly with the extracellular PSI-6130 up to $250 \mu\text{M}$ (Table 3). The mean total intracellular species at $250 \mu\text{M}$ extracellular PSI-6130 reached 3591 pmol/ 10^6 cells, with the unphosphorylated PSI-6130 being the predominant species (data not shown). The formation of both PSI-6130-TP and RO2433-TP increased linearly with the extracellular PSI-6130 concentrations up to $100 \mu\text{M}$ (Fig. 5). The concentrations of PSI-6130-TP and RO2433-TP at $100 \mu\text{M}$ extracellular PSI-6130 were 34.3 and 71.2 pmol/ 10^6 cells, respectively. At $250 \mu\text{M}$ extracellular PSI-6130, PSI-6130-TP and RO2433-TP levels were slightly increased over the levels obtained at $100 \mu\text{M}$ extracellular PSI-6130, but the relationship was no longer linear. PSI-6130-TP and RO2433-TP were the major intracellular phosphorylated species at all extracellular PSI-6130 concentrations (Table 3), suggesting a high capacity of the hepatocytes to convert the parent compound to the triphosphate forms and no accumulation of either mono- or diphosphates. At all concentrations of extracellular PSI-6130, RO2433-TP levels were higher than those of PSI-6130-TP.

Intracellular Stability of PSI-6130-TP and RO2433-TP—Primary human hepatocytes from 4 different donors were incubated for 24 h with $2 \mu\text{M}$ PSI-6130, at which point extracellular PSI-6130 was removed, and the concentrations of intracellular

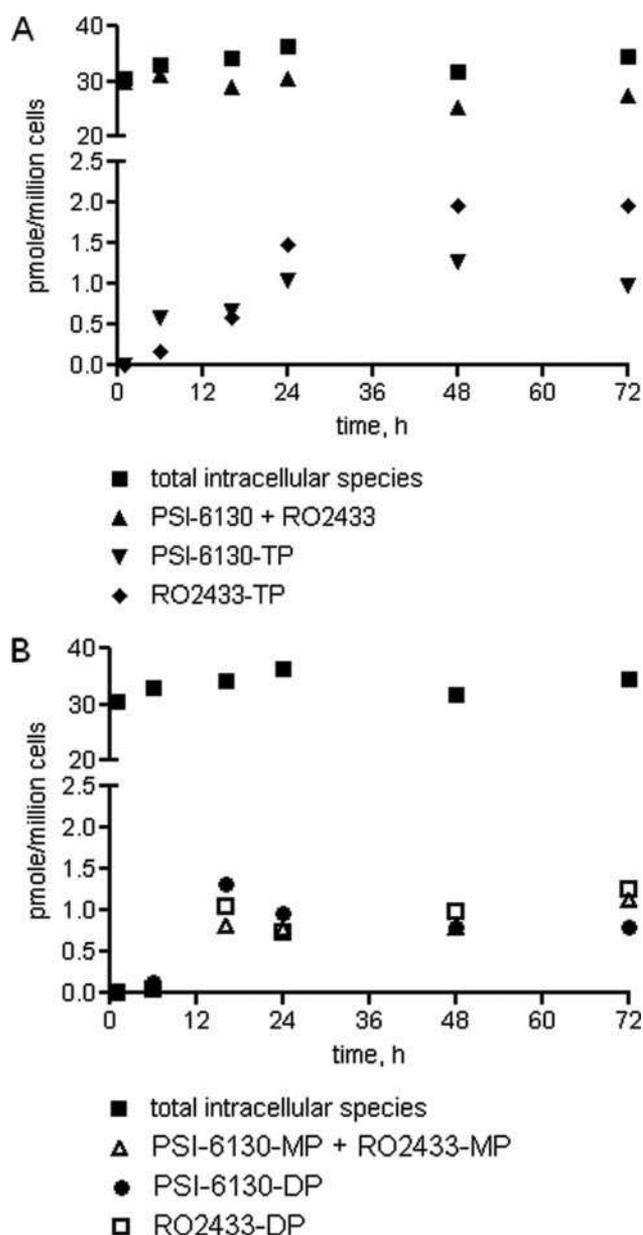


FIGURE 4. Time course of uptake and metabolism of PSI-6130. A, time course of uptake of PSI-6130 and formation of PSI-6130-TP and RO2433-TP. B, time course of formation of PSI-6130-MP + RO2433-MP, PSI-6130-DP, and RO2433-TP. The data show mean values from four experiments performed with primary hepatocytes from four donors.

PSI-6130-TP and RO2433-TP were quantified at different time points up to 72 h (see “Experimental Procedures”).

The PSI-6130-TP level remained constant up to 1 h after the removal of extracellular PSI-6130. Thereafter, PSI-6130-TP decreased after a single-phase exponential decay kinetics pattern with a mean half-life of 4.7 ± 0.6 h (Fig. 6, Table 4). The PSI-6130-TP level was below quantification limit at 48 h after the removal of extracellular PSI-6130. The RO2433-TP level increased slightly to reach steady state level at 1–2 h after extracellular PSI-6130 removal and remained at steady state level for up to 6 h before decreasing toward base-line levels. RO2433-TP decreased with a mean half-life of 38.1 ± 16.1 h (Fig. 6, Table 4). The levels of unphosphorylated species PSI-6130 and RO2433 decreased rapidly after removal of extracellular RO1656 (data

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TABLE 3

Dose response of uptake and phosphorylation of PSI-6130

Levels of total intracellular species, PSI-6130-TP, and RO2433-TP were determined in hepatocytes incubated with 2, 10, 25, 50, 100, or 250 μ M PSI-6130 for 24 h. Data shown are the mean \pm S.D. of values of three experiments using hepatocytes from three donors. BQL, below the quantification limit.

Extracellular PSI-6130	Total intracellular species	PSI-6130-MP + RO2433-MP	PSI-6130-DP	RO2433-DP	PSI-6130-TP	RO2433-TP
μ M	pmol/million cells	pmol/million cells	pmol/million cells	pmol/million cells	pmol/million cells	pmol/million cells
2	34.5 \pm 9.8	0.3 \pm 0.3	0.3 \pm 0.4	0.9 \pm 0.7	1.4 \pm 1.3	2.4 \pm 1.5
10	165.5 \pm 38.2	1.1 \pm 1.0	2.4 \pm 2.7	4.8 \pm 2.7	5.6 \pm 5.8	9.6 \pm 6.4
25	439.1 \pm 150.4	5.0 \pm 5.9	3.4 \pm 4.3	9.5 \pm 4.9	11.3 \pm 13.0	21.7 \pm 13.4
50	847.9 \pm 327.7	8.2 \pm 8.2	8.2 \pm 7.3	20.3 \pm 12.2	18.7 \pm 17.7	34.9 \pm 17.2
100	1529.9 \pm 413.1	3.4 \pm 5.9	13.4 \pm 15.4	34.7 \pm 25.0	34.3 \pm 36.3	71.2 \pm 14.7
250	3591.3 \pm 1102.5	BQL	19.6 \pm 19.6	40.9 \pm 13.2	40.4 \pm 35.4	84.0 \pm 28.7

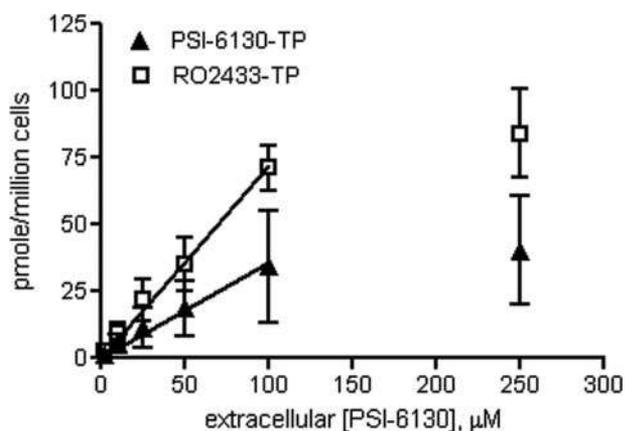


FIGURE 5. Dose response of the formation of PSI-6130-TP and RO2433-TP. Primary human hepatocytes were incubated with PSI-6130 for 24 h at 2, 10, 25, 50, 100, 250 μ M concentrations. The data plotted represent the mean values and S.D. of experiments with hepatocytes from three donors.

not shown). Unphosphorylated PSI-6130 and RO2433 were reduced by more than half within 0.5 h after extracellular PSI-6130 removal, suggesting rapid equilibration of uncharged nucleoside analogs across the hepatocyte cell membrane.

DISCUSSION

The antiviral activity of a nucleoside inhibitor is the combined outcome of uptake of the nucleoside into the host cells, conversion of the nucleoside to the active triphosphate, intracellular stability of the triphosphate, and the ability of the triphosphate form to interfere with the RNA synthesis activity of the viral polymerase. In this study we performed experiments to address each of the steps involved in the mechanism of action of β -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine.

The analysis of radiolabeled PSI-6130 incubated with primary human hepatocytes demonstrated the conversion of PSI-6130 into its 5'-phosphorylated derivatives (mono-, di-, and triphosphate) and to the uridine analog R2433 and its corresponding 5'-phosphorylated derivatives. Unmodified PSI-6130 remained the major intracellular species at all time points studied. These results suggest that PSI-6130 is only subjected to intracellular 5'-phosphorylation and base deamination, with no evidence for metabolism at other positions. In contrast, another cytidine analog, 2'-O-methylcytidine, demonstrated intracellular conversion with significant efficiency to CTP and UTP instead of 2'-O-methyl-CTP due to deamination combined with extensive demethylation at the 2' position or base swapping after deglycosylation (8, 19). The intracellular instability of 2'-O-methylcytidine most likely accounts for the poor potency

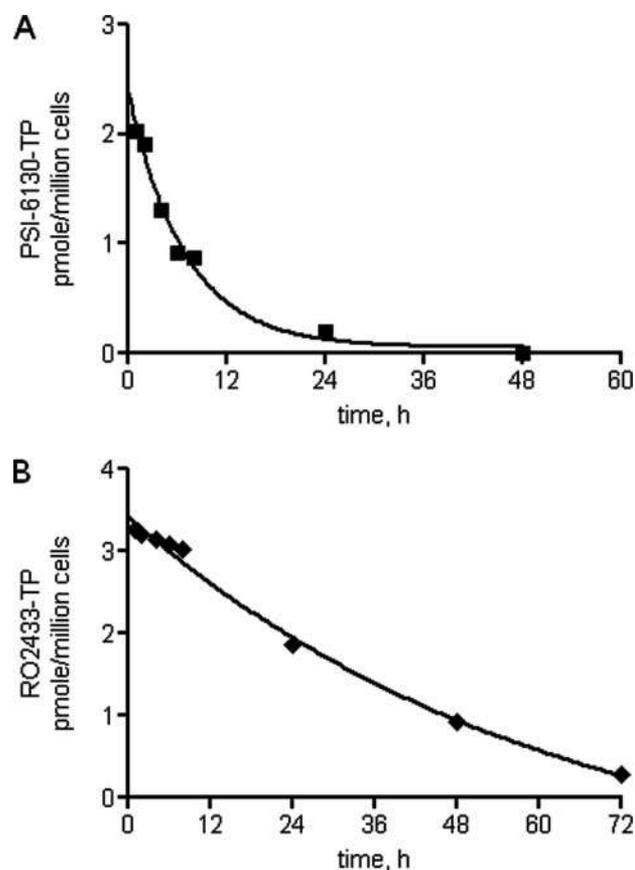


FIGURE 6. Intracellular stability of PSI-6130-TP and RO2433-TP. Primary human hepatocytes were incubated with PSI-6130 at 2 μ M for 24 h, at which point extracellular PSI-6130 was removed, and PSI-6130-TP and RO2433-TP levels were determined at 1, 2, 4, 6, 8, 24, 48, and 72 h after extracellular PSI-6130 removal. Representative kinetic profiles of PSI-6130-TP (A) and RO2433-TP (B) elimination in hepatocytes from one donor are shown.

TABLE 4

Intracellular half-life of PSI-6130-TP and RO2433-TP

The half-life ($t_{1/2}$) values were calculated by nonlinear fitting of intracellular triphosphate concentrations to a single phase exponential decay equation. $t_{1/2}$ was defined as the time needed for the triphosphates to be reduced to 50% that of the highest level of the triphosphates after extracellular parent compound removal. Data shown are the mean \pm S.D. of values of four independent experiments using hepatocytes from four donors.

$t_{1/2}$, h (mean \pm S.D. ($n = 4$))	
PSI-6130-TP	RO2433-TP
4.7 \pm 0.6	38 \pm 16

of this cytidine analog in the HCV replicon system (8, 19). Importantly, both triphosphates of PSI-6130 and RO2433 were potent inhibitors of the RNA synthesis activity of the native HCV replicase and recombinant NS5B (Table 1), suggesting

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that the antiviral potency of PSI-6130 is not compromised by intracellular deamination.

The formation of the active triphosphates PSI-6130-TP and RO2433-TP increased with time and reached steady levels at 48 h after exposure to parent PSI-6130. The mean steady state intracellular concentrations of PSI-6130-TP and RO2433-TP at 2 μ M extracellular PSI-6130 were 1.3 and 2.0 pmol/ 10^6 cells, respectively (Table 2). These values are equivalent to about 0.43 μ M for PSI-6130-TP and 0.67 μ M for RO2433-TP, based on a 3- μ l volume of normal human liver parenchymal cells as determined by the stereological method (20). These steady state concentrations are above the K_i values of PSI-6130-TP (0.023 μ M) and RO2433-TP (0.141 μ M) determined for HCV NS5B polymerase and are in good agreement with the observed submicromolar IC_{50} of PSI-6130 in the HCV replicon system (Table 1).

Among the phosphorylated species of PSI-6130 and RO2433, the rank of the phosphate levels from highest to lowest intracellular concentration was: triphosphates > diphosphates > monophosphates at all time points of compound incubation and all extracellular concentrations (Fig. 4, Table 3). This suggests that the first cellular activation step to monophosphate is the rate-limiting step among the three phosphorylation steps. Recently, it has been reported that the human cellular kinases responsible for the sequential three-step phosphorylation of PSI-6130 to triphosphate are 2'-deoxycytidine kinase, uridine/cytidine monophosphate kinase, and uridine/cytidine diphosphate kinase (17). In that study the efficiency of PSI-6130 as a substrate for 2'-deoxycytidine kinase was reported to be almost 2 orders of magnitude lower than that of PSI-6130-MP and PSI-6130-DP for their respective kinases. Therefore, the results of the reported substrate efficiencies of the three kinases involved in PSI-6130-TP formation agree well with relative formation of PSI-6130 phosphates in human hepatocytes. Importantly, the intracellular concentrations of PSI-6130-TP and RO2433-TP demonstrated an excellent linear relationship with the extracellular concentration of PSI-6130 up to 100 μ M, demonstrating a high capacity of human hepatocytes to form the biologically active triphosphates from PSI-6130.

Despite the intrinsic potency of RO2433-TP against HCV polymerase, RO2433 was not active in the HCV replicon system at concentrations up to 100 μ M. RO2433 was either not phosphorylated in the replicon cells or could not penetrate the cell membrane. However, RO2433, when formed intracellularly from radiolabeled PSI-6130, dissociated rapidly across the cell membrane with a half-life faster than 30 min. Therefore, RO2433 is most likely not efficiently phosphorylated to form RO2433-MP. Similarly, the uridine analog of the HCV replication inhibitor R1479 (4'-azidocytidine) was inactive in the replicon system. However, when delivered as a monophosphate prodrug, 4'-azidouridine could be converted into a potent inhibitor of HCV replication, demonstrating that a block of monophosphate formation resulted in lack of antiviral activity of 4'-azidouridine (21). Assuming a likely block of RO2433 phosphorylation to its monophosphate, RO2433-MP in human hepatocytes was most likely formed through the deamination of PSI-6130-MP by the cellular dCMP deaminase and subsequently further phosphorylated to RO2433-DP and -TP by uridine/cytidine monophosphate kinase and possibly nucleoside

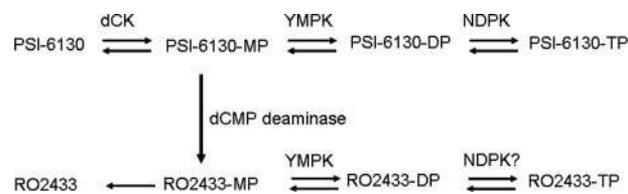


FIGURE 7. **Proposed metabolic pathway of PSI-6130.** Enzymes involved in the phosphorylation and deamination catalysis: deoxycytidine kinase (*dCK*), uridine/cytidine monophosphate kinase (*YMPK*), nucleoside diphosphate kinase (*NDPK*), deoxycytidylate deaminase (*dCMP deaminase*).

diphosphate kinase (22–24). The proposed metabolic pathway for PSI-6130 is illustrated in Fig. 7.

Using a primer-directed nucleotide incorporation assay mediated by HCV NS5B, we demonstrated that the incorporation of both PSI-6130-MP and RO2433-MP resulted in the complete blockage of the next nucleotide incorporation similar to that of the obligatory chain terminator 3'-dCMP and 3'-dUMP (Fig. 3, B and C). Therefore, the 2'-C-methyl-2'-Fluoro motif resulted in functional chain terminators on the respective uridine and cytidine analogs. It has been proposed that the chain termination activity of 2'-C-methyl nucleotide analogs is related to a steric clash of the 2'-methyl group with the ribose of the next incoming nucleotide substrate based on modeling of the NS5B initiation complex from bacteriophage Φ 6 RNA-dependent RNA polymerase and NS5B crystal structures (10, 25). Similar steric hindrance could occur with PSI-6130-TP and RO2433-TP after incorporation due to the presence of the 2'-C-methyl group.

PSI-6130 is a potent and highly selective nucleoside inhibitor of HCV replication targeting NS5B polymerase in the HCV replicon system. Here we demonstrated that PSI-6130 was converted to two pharmacologically active triphosphate species, PSI-6130-TP and its uridine analog RO2433-TP, in primary human hepatocytes. It is worth noting that RO2433-TP demonstrated greater intracellular stability ($t_{1/2} = 38$ h) as compared with PSI-6130-TP ($t_{1/2} = 4.7$ h). PSI-6130 has recently entered clinical development for the treatment of HCV infected patients. The longer intracellular half-life of RO2433-TP may have pharmacologic relevance for maintaining more constant concentrations of the antiviral triphosphate over the dosing period in clinical studies.

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Certain phosphoramidate derivatives of dideoxy uridine (ddU) are active against HIV and successfully by-pass thymidine kinase

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Abstract

As part of our effort to deliver masked phosphates inside living cells we have discovered that certain phosphate triester derivatives of the inactive nucleoside analogue, dideoxy uridine (ddU) are inhibitors of HIV replication at μM levels. Moreover, we note that certain phosphoramidate derivatives retain their activity in thymidine kinase-deficient cells, which indicates that they do indeed act by intracellular release of the free nucleotide, and that they successfully by-pass the nucleoside kinase. The increased structural freedom in drug design which this allows may have implications for dealing with the emergence of resistance and may stimulate the discovery of improved therapeutic agents.

Key words: Nucleoside; Nucleotide; Anti-HIV

1. Introduction

Although nucleoside analogues, such as 3'-azidothymidine (AZT, **1**, Fig. 1) continue to dominate anti-HIV drug therapy they have a number of major limitations, such as their inherent toxicity, a dependence on kinase-mediated activation to generate the bio-active (tri)phosphate forms, and the emergence of resistance [1–2].

We [3–6] and others [7–9] have pursued a masked phosphate approach in an attempt to improve on the therapeutic potential of the parent nucleoside analogues. In this approach, inactive phosphate derivatives of the nucleoside analogue are designed to penetrate the cell membrane and liberate the bio-active nucleotides intracellularly. Masking of the phosphate group is necessary on account of the extremely poor membrane penetration by the polar (charged) free nucleotide. One mechanism by which masked phosphates may lead to enhanced selectivity of action arises from what we have termed 'kinase bypass' [3]. Thus, the complete dependence of administered (anti-HIV) nucleoside analogues on host nucleoside kinase-mediated activation places constraints upon the structures of nucleoside analogues which might be active. Nucleoside analogues which fall outside these strict constraints will be inactive, even if their 5'-triphosphates (the bio-active form) are potent and selective inhibitors of a viral target, such as reverse transcriptase (RT). Several such cases are known. Dideoxythymidine, and 3'-*O*-methylthymidine are examples of nucleoside

analogues which are inactive against HIV, whilst their triphosphates are exceptionally potent inhibitors of HIV RT [10]; the inactivity of the nucleoside being attributed to poor phosphorylation by host kinases. If the masked phosphate strategy were able to deliver nucleotides intracellularly, the nucleoside kinase would be by-passed and the structural constraints such host enzymes impose would be obviated. In this way, wider structural variation of the nucleoside analogue would be permitted, and more specific (less toxic) inhibitors of viral function may arise. We have recently reported on the success of this 'kinase by-pass' strategy with several highly modified 3'-substituted nucleosides [11–12].

We now report the success of this approach with the simple nucleoside analogue dideoxy uridine (ddU, **2**). This is essentially inactive against HIV, but judicious phosphorylation leads to the introduction of a significant, selective antiviral effect. Moreover, this effect is retained in thymidine kinase-deficient cells, indicating a successful by-pass of this enzyme, and strongly supporting the suggested intracellular delivery of free nucleotides by this strategy. Whilst other researchers have recently reported the failure of the by-pass approach with phosphoramidates derived from ddu [13], we herein clearly demonstrate the success of this strategy with our previously reported aryloxy phosphoramidates [6].

2. Materials and methods

2.1. Chemistry

General synthetic procedures were similar to those we have described [5]. All nucleotides were pure by high-field multi-nuclear NMR and reverse phase High Performance Liquid Chromatography (HPLC):

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ACS system, 50 + 250 mm × 4.6 mm, Spherisorb ODS2 5 μ column, gradient elution using 5% acetonitrile in water (A), and 5% water in acetonitrile (B), with 20% B for 0–10 min, then a linear gradient to 80% B at 30 min, with a flow rate of 1 ml/min.

2.1.1. 2',3'-dideoxy uridine (ddU, 2).

(a) 5'-Trityl uridine. Trityl chloride (4.1 g, 14.7 mmol) was added to a solution of uridine (3 g, 12.3 mmol) in pyridine (50 ml) and the reaction mixture heated at 50°C for 24 h. The solvent was removed under high vacuum and the residue purified by chromatography on silica using 5% methanol in chloroform as eluant. Pooling and evaporation of appropriate fractions, followed by recrystallisation from ethanol gave the product as a white solid (5.32 g, 89%). $\delta_{\text{H}}(\text{d}_c\text{-DMSO})$ 3.3(2H, dd, H5', J = 10.8, 4.5 Hz), 4.0(1H, m, H3'), 4.15(2H, m, H2', H4'), 5.2(1H, d, OH, J = 4.8 Hz), 5.35(1H, d, H5, J = 8.1 Hz), 5.6(1H, d, OH, J = 3.7 Hz), 5.8(1H, d, H1', J = 3.1 Hz), 7.2–7.5(15H, m, Ar), 7.8(1H, d, H6, J = 8.1 Hz), 11.2(1H, s, NH). $\delta_{\text{C}}(\text{d}_c\text{-DMSO})$ 63.24(C5'), 69.56(C3'), 73.44(C2'), 82.36(C4'), 86.45(C1'), 88.97(CPh.), 101.47(C5); 127.18, 127.00, 128.32(Ar), 140.62(C6), 143.43(Ar), 150.52(C4), 163.07(C2).

(b) 5'-Trityl-2',3'-thiocarbonyl uridine. A solution of 5'-trityl uridine (1 g, 2.05 mmol) and thiocarbonyldiimidazole (400 mg, 2.24 mmol) in THF (20 ml) was stirred at ambient temperature for 17 h. After evaporation of the solvent under reduced pressure the residue was purified by chromatography on silica using 5% methanol in chloroform as eluant. Pooling and evaporation of appropriate fractions gave the product as a white solid (990 mg, 91%). $\delta_{\text{H}}(\text{CDCl}_3)$ 3.5(2H, m, H5'), 4.5(1H, m, H4'), 5.5(1H, m, H2'); 5.6–5.9(2H, m, H3', H5), 7.2–7.6(16H, m, Ar, H6), 9.5(1H, s, NH).

(c) 2',3'-Dideoxy-2',3'-dideoxy uridine (d4U). A solution of thiocarbonate (850 mg, 1.61 mmol) in triethylphosphite (10 ml) was heated at 150°C for 30 min. Excess triethylphosphite was evaporated under high vacuum and the residue purified by chromatography on silica using 5% methanol in chloroform as eluant. Pooling and evaporation of appropriate fractions gave the product and triethylphosphite (ratio = 60/40 by NMR). This mixture was dissolved in acetic acid and left for 17 h with stirring at ambient temperature. The solid obtained after evaporation of the solvent was purified by chromatography on silica using 8% methanol in chloroform as eluant. Pooling and evaporation of appropriate fractions gave the product (240 mg, 71%). $\delta_{\text{H}}(\text{CDCl}_3, \text{CD}_3\text{OD})$ 3.7(2H, dd, H5', J = 12.3, 3.0 Hz), 4.85(1H, m, H4'), 5.6(1H, d, H5, J = 8.1 Hz), 5.8(1H, m, H2'), 6.3(1H, m, H3'), 6.9(1H, m, H1'), 7.7(1H, d, H6, J = 8.1 Hz). $\delta_{\text{C}}(\text{CDCl}_3, \text{CD}_3\text{OD})$ 62.64(C5'), 87.42(C4'), 89.86(C1'), 101.95(C5), 125.81(C2'), 134.71(C3'), 141.29(C6), 151.01(C4), 164.25(C2).

(d) 2',3'-Dideoxy uridine (2, ddU). D4U (240 mg, 1.14 mmol) was dissolved in methanol (10 ml) and ethanol (10 ml) and 10% palladium on charcoal (200 mg) was added. This mixture was shaken under a hydrogen atmosphere for 4 h. The catalyst was removed by filtration through celite and the residue purified by chromatography on silica using 8% methanol in chloroform as eluant. Pooling and evaporation of appropriate fractions gave the product as a white solid (200 mg, 83%). $\delta_{\text{H}}(\text{CDCl}_3, \text{CD}_3\text{OD})$ 1.9–2.1(3H, m, H2', H3'), 2.4(1H, m, H2'), 3.8(2H, dd, H5', J = 12.2, 2.7 Hz), 4.15(1H, m, H4'), 5.7(1H, d, H5, J = 8.1 Hz), 6.0(1H, H1', dd, J = 6.6, 3.3 Hz) 7.9(1H, d, H6, J = 8.1 Hz); FAB MS *m/e* 213.0880(MH⁺, 72%, C₁₀H₁₃N₂O₅ requires 213.0875), 149(32), 137(93), 129(40), 113(68), 101(100).

2.1.2. 2',3'-Dideoxy uridine 5'-bis[2,2,2-trichloroethyl] phosphate (3a). DdU (2) (0.08 g, 0.38 mmol) and *N*-methyl imidazole (0.155 g, 1.89 mmol) were mixed in THF (10 ml) and bis(2,2,2-trichloroethyl) phosphorochloridate (0.186 g, 0.49 mmol) was added slowly at ambient temperature. After 1 h the solvent was evaporated and the residue dissolved in CHCl₃ (30 ml) washed with HCl (1 M; 10 ml), sodium bicarbonate solution (15 ml) and water (15 ml). The organic phase was dried (MgSO₄) and evaporated under reduced pressure. The crude product was purified by chromatography on silica eluting with 4% methanol in chloroform. Pooling and evaporation of appropriate fractions under reduced pressure gave the product (0.20 g, 95%). $\delta_{\text{H}}(\text{CDCl}_3)$ 2.1(3H, m, H2', H3'), 2.45(1H, m, H2'), 4.4(2H, m, H5'), 4.55(1H, m, H4'), 4.7(4H, m, CH₂OP), 5.75(1H, d, H5, J = 8 Hz), 6.1(1H, m, H1'), 7.6(1H, d, H6, J = 8 Hz), 9.4(1H, s, NH); FAB MS *m/e* 552.8792(MH⁺, 10%, C₁₅H₁₆N₂O₅PCl₃ requires 552.8826); HPLC RT = 32.6 min.

2.1.3. 2',3'-Dideoxy uridine 5'-(ethyl methoxyalaninyl)phosphate (3b). DdU (2) (0.05 g, 0.23 mmol) and *N*-methyl imidazole (0.09 g,

1.18 mmol) were mixed in THF (10 ml) and ethyl methoxyalaninyl phosphorochloridate (0.11 g, 0.47 mmol) was added slowly at ambient temperature. After 4 h the solvent was evaporated and the crude product purified entirely as described for (3a) above, except that a second chromatographic column was necessary, using an eluant of 15% methanol in diethyl ether, in order to obtain pure (3b) (0.051 g, 54%). $\delta_{\text{H}}(\text{CDCl}_3)$ 1.3(6H, m, Ala-Me, POCC), 2.0(3H, m, H2', H3'), 2.4(1H, m, H2'), 3.70, 3.71(3H, s, OMe), 3.8–4.25(7H, m, Ala-CH, Ala-NH, H4', H5', POC), 5.7(1H, d, H5, J = 8 Hz), 6.05(1H, m, H1'), 7.68, 7.73(1H, d, H6, J = 8 Hz), 9.5(1H, bs, NH); FAB MS *m/e* 406.1392 (MH⁺, 10%, C₁₅H₂₂N₂O₅P requires 406.1379), 294(100), 94(7); HPLC RT = 29.9, 30.3 min.

2.1.4. 2',3'-Dideoxy uridine 5'-(phenyl methoxyalaninyl)phosphate (3c). This was prepared by a procedure which was entirely analogous to (3a) above, except that the reaction was stirred for 17 h, and 4% methanol in chloroform was used as chromatographic eluant. Thus, from 92 mg of ddU was isolated 178 mg (90%) of (3c). $\delta_{\text{H}}(\text{CDCl}_3)$ 1.32, 1.33(3H, d, Ala-Me, J = 7 Hz), 1.8–2.1(3H, m, H2', H3'), 2.3(1H, m, H2'), 3.66, 3.67(3H, s, OMe), 4.0(1H, m, Ala-CH), 4.1–4.4(4H, m, H4', H5', Ala-NH), 5.60, 5.65(1H, d, H5, J = 9 Hz), 6.0(1H, m, H1'), 7.25(5H, m, Ph), 7.60, 7.61(1H, d, H6, J = 8 Hz), 10.0(1H, bs, NH); FAB MS *m/e* 454.1397 (MH⁺, 13%, C₁₉H₂₂N₂O₅P requires 454.1379), 342(35), 307(14), 200(15), 154(100), 136(79); HPLC RT = 34.4, 34.6 min.

2.2. Virology

2.2.1. Antiviral assays. The anti-HIV-1 activities and toxicities of compounds were assessed in two cell lines [14]. C8166 (a normal T-cell transformed by co-cultivation with leukaemia lymphocytes harbouring HTLV-1) were infected with the III-B strain of HIV-1. Secondly, JM, a semi-mature T-cell line derived from a patient with lymphoblastic leukaemia, were infected with HIV-1 strains GB8 or III-B. JM cells are relatively resistant to the antiviral effects of AZT and a number of its derivatives.

Cells were grown in RPMI 1640 with 10% calf serum. 4 × 10⁴ cells per microtiter plate well were mixed with 5-fold dilutions of compound prior to addition of 10 CCID₅₀ units of virus and incubated for 5–7 days. Formation of syncytia was examined from 2 days post-infection. Culture fluid was collected at 5–7 days and gp120 antigen production measured by ELISA. Cell viability of infected cells and cytotoxicity to uninfected cell controls were assessed by the MTT-Formazan method [15].

2.2.2. gp120 antigen assay. A microtiter antigen capture ELISA was developed [16] using a lectin (GNA) from *Galanthus nivalis* (Vector Labs., Peterborough, UK) and anti-HIV antibodies in human serum. The plates were coated with lectin (0.5 μ g), and after blocking with 10% calf serum, dilutions of virus supernatants in 0.25% Empigen solution (Albright and Wilson Ltd., Whitehaven, UK) were added to the wells and incubated at 4°C for 12–16 h. Bound antigen was detected using human anti-HIV-1 antibodies, and anti-human IgG antibodies coupled to horseradish peroxidase.

3. Results and discussion

Dideoxy uridine (ddU, 2) was prepared via three independent routes, all involving a final hydrogenation of the dideoxy dideoxy compound d4U. Thus, 5'-silylation of uridine, followed by thiocarbonate formation, elimination with triethyl phosphite, and deprotection with fluoride, gave 2',3'-dideoxy 2',3'-dideoxy uridine (d4U) [17] in an overall yield of 50%. Alternatively, a 5'-trityl protecting group could be used in this synthesis to give d4U, again via the 2',3'-thiocarbonate, in an overall yield of 58%. Alternatively, by the procedures of Horwitz [18] 2'-deoxyuridine could be converted to its 3',5'-dimethylate, which gave the 3',5'-oxetane, and which could be converted to d4U on treatment with strong base. The

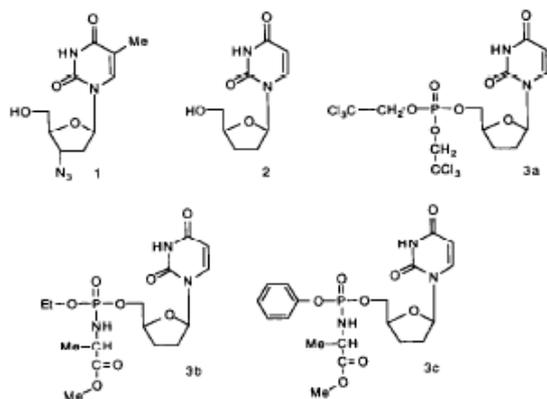


Fig. 1. The structures of potential anti-HIV nucleoside and nucleotide analogues.

overall yield by this route was 29%. Thus, in terms of yield the best route to d4U was via the 5'-trityl protected 2',3'-thiocarbonate.

The didehydro compound d4U generated by either of these routes was hydrogenated to give ddU (**2**) in 83% yield, and the structure and purity of this compound was confirmed by ^1H , ^{13}C NMR, mass spectrometry and HPLC.

The nucleoside analogue was then subjected to 5'-phosphorylation with a range of phosphates. A labile phosphate blocking group is clearly a pre-requisite for the masked phosphate approach, in order to facilitate (intracellular) release of the free nucleotides. Thus, simple dialkyl phosphate derivatives of AZT are extremely resistant to phosphate hydrolysis, and they display no antiviral effect [3]. On the other hand bis(trihaloethyl) phosphate derivatives of zidovudine and of 2',3'-dideoxycytidine (ddC) show significant lability towards hydrolysis [19], and are potent inhibitors of viral proliferation [5]. We have also noted that one of these phosphate masking groups, the bis(2,2,2-trichloroethyl) phosphate moiety is successful in the kinase by-pass activation of certain inactive 3'-modified nucleosides [11–12]. We therefore chose this phosphate as the first blocking group for ddU.

Thus, ddU was allowed to react with bis(2,2,2-trichloroethyl) phosphorochloridate in tetrahydrofuran containing *N*-methyl imidazole at room temperature to give (**3a**) in good yield. This was fully characterised by heteronuclear NMR, FAB mass spectrometry, and HPLC, all data being consistent with its structure and purity. We have also noted that certain phosphoramidate derivatives of AZT are potent and selective inhibitors of HIV [4]. Thus, ethyl methoxyalaninyl phosphorochloridate was prepared by methods we have reported [4] and was allowed to react with ddU to give the target compound (**3b**) in moderate yield. This compound was recently re-

ported by another group, following our earlier phosphoramidate strategy [13]. However, we have found that aryloxy phosphoramidates are especially potent phosphate blocking groups for AZT, and appear to release the free nucleotides within cells, on the basis of data in thymidine kinase-deficient cells [6,20]. Thus, phenyl methoxyalaninyl phosphorochloridate was similarly prepared [20] and was allowed to react with ddU to give (**3c**) in good yield. Full carbon-13 and (where appropriate) phosphorus-31 NMR data for the nucleoside (**2**) and the phosphates (**3a-c**) are given in Table 1.

The parent nucleoside (**2**) and the corresponding masked phosphates (**3a-c**) were tested for their ability to inhibit the replication of HIV-1 in C8166 cells, and in thymidine kinase-deficient [JM] cells, data being presented in Table 2. As expected, the parent nucleoside (**2**) is active only at the highest concentrations tested, and is essentially inactive in JM cells. The bis[trichloroethyl] phosphate (**3a**) is approximately 5–10 times more active in each assay. On the other hand, the simple phosphoramidate (**3b**) is devoid of antiviral activity in this assay, as has been recently noted by other researchers in a different assay [13]. However, the aryloxy phosphoramidate (**3c**) is a potent agent, being approximately 50-times more active than the parent nucleoside analogue. This confirms the importance of data derived from assays in kinase deficient cells for the interpretation of the activities of blocked nucleotides. As we have noted [20] only

Table 1
Carbon-13 and phosphorus-31 NMR data for compounds (**2**) and (**3a-c**)

		2	3a	3b	3c
Base	C2	164.3	163.4	163.9	163.7
	C4	150.5	150.6	150.7	150.4
	C5	101.4	102.6	102.1	101.9, 102.0
	C6	140.8	139.7	140.0	139.7
	Sugar	C1'	89.3	86.6	86.5, 86.3
	C2'	24.6	25.5	25.3, 25.4	25.0, 25.2
	C3'	32.5	32.2	32.5, 32.6	32.0, 32.1
	C4'	81.8	78.6 ^a	79.4 ^b	78.8 ^b
	C5'	62.6	69.6	66.5, 66.8	66.9, 67.0
P-OR	POC	–	77.3 ^a	66.0	–
	POCC	–	94.7 ¹⁰	16.2, 16.3	–
P-OAr	C1''	–	–	–	150.5
	C2''	–	–	–	119.8, 119.9 ⁵
	C3''	–	–	–	129.6, 129.7
	C4''	–	–	–	125.0
P-NHR	PNC	–	–	50.0, 50.1	50.0, 50.2
	C=O	–	–	174.4, 174.3 ^b	173.8, 174.0 ^b
	OMe	–	–	52.6	52.4
	CHMe	–	–	21.1, 21.0 ⁵	20.6, 20.7
δP	–	–3.6	7.2(d)	3.2	

^aMultiplet. All spectra were recorded in CDCl_3 , except for (**2**) which were recorded in CDCl_3 , plus CD_3OD . Data are presented as δ in ppm. All spectra were recorded using proton decoupling. In the case of carbon data phosphorus coupling constants in Hz are superscripted. Many carbon peaks for (**3b-c**) display diastereomeric splitting arising from mixed stereochemistry at the phosphate centre.

Table 2
Anti-HIV1 activity of nucleoside and nucleotide analogues

Compound	C8166		JM	
	ED ₅₀	CC ₅₀	ED ₅₀	CC ₅₀
2	200	> 1,000	1,000	> 1,000
3a	40	500	80	400
3b	> 1,000	> 1,000	400	400
3c	4	400	20	500

The antiviral activity and cytotoxicity of test compounds in two different cell lines. ED₅₀ represents the concentration of compound (in μM) that decreases viral antigen production in infected cells to 50% of control. CC₅₀ represents the concentration of compound (in μM) which causes 50% cytotoxicity to uninfected cells.

such data will allow a clear understanding of the likely mechanism of action of blocked nucleotides, and discriminate between the release of nucleosides and nucleotides.

In conclusion, we report the antiviral activity of certain masked phosphate derivatives of the inactive nucleoside ddU. We note that aryloxy phosphoramidates are particularly efficacious, and attribute the introduction of activity to kinase by-pass. Finally we stress the importance of data in kinase-deficient cells for the clear interpretation of results on blocked phosphates.

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D15

Aryloxy Phosphoramidate Triesters as Pro-Tides

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Abstract: We herein describe the development of aryloxy phosphoramidate triesters as an effective pro-tide motif for the intracellular delivery of charged bio-active antiviral nucleoside monophosphates. The review covers the discovery of such aryl phosphoramidates, their mechanism of action and structure-activity relationships. The application of this strategy to a range of antiviral nucleosides is highlighted.

Keywords: Nucleotide, Pro-drug, Phosphoramidate.

SCOPE OF THIS WORK

We will describe the discovery, *in vitro* evaluation, Structure-Activity Relationships (SARs) and Mechanism of Action (MoA) of phosphoramidate triesters of a range of antiviral nucleosides. We describe these compounds as triesters to emphasise the fact that all of the charges on the phosphate nucleus are blocked and to distinguish our compounds from the phosphoramidate diesters described by Wagner and Coworkers [1]. These latter compounds have been well reviewed [1,2], operated by a quite separate mechanism, and displayed distinct SARs to those compounds we will describe here. Therefore we will not include them below. We will thus describe the development of fully blocked phosphoramidates, culminating in our lead series, the aryloxy phosphoramidates.

ALKYL AND HALOALKYL PHOSPHATE TRIESTERS

Early work from our laboratories indicated that simple alkyl triesters of the antiviral agent araA (vidarabine) and the anti-neoplastic agent araC (cytarabine), of general formulas 1 and 2, (Fig. 1) respectively displayed significant biological activity in tissue culture [3,4]. However, analogous dialkyl phosphate triesters of AZT (3) were devoid of significant anti-HIV activity, in marked contrast to the parent nucleoside analogue [5]. Similarly, whilst haloalkyl phosphate triesters of araA and araC (4, 5) had enhanced biological activity [6], the corresponding AZT derivatives (6) and also those of 2',3'-dideoxycytidine (ddC) (7) were in general poorly active [7].

Thus, although the bis(trifluoroethyl) analogue (6) was active at 0.4 μ M, and thus >200 times more active than compound (3), it was still 100-fold less potent than AZT itself [7]. Attempts to boost the potency of these haloalkyl phosphate triesters by changing the degree of halogenation were in general not successful [8].

ALKYLOXY PHOSPHORAMIDATES

The original rationale for preparing phosphoramidate-based pro-tides was the possibility that HIV aspartate protease [9] might cleave a suitable oligo-peptide from the phosphate moiety of a blocked nucleotide phosphoramidate. Simple model mono-amino acyl analogues were prepared and evaluated in the first instance and were of sufficient interest to pursue in their own right. Thus, a series of simple alkyloxy phosphoramidates of AZT were prepared with a small family of methyl esterified aminoacids (8) [10]. By comparison to earlier dialkyl phosphates of AZT (3, 6) the alkyloxy phosphoramidates (8) showed significant anti-HIV activity. A notable dependence of the antiviral activity on the aminoacid side-chain began to emerge; with alanine being most efficacious, and with leucine and, particularly, isoleucine being less active [10,11]. By contrast, the alkyl phosphate chain could be varied from C₁ to C₆ with no significant change in activity [11].

In a subsequent study [12], α -aminoacids were compared to their β,γ derivatives etc (9). Anti-HIV activity was maximal for the parent α system (glycine) and diminished with increasing alkyl spacer length, being 10-fold less active for n=3 as compared to n=1 [12].

Given the earlier improvements in antiviral activity noted for the haloalkyl phosphate parents, we wondered whether haloalkyl phosphoramidates might also be more potent. Therefore, a small series of compounds (10) was prepared [13]. For each of the aminoacids glycine, alanine and valine, the alkyl chain either was ethyl, trifluoroethyl or trichloroethyl. However, by contrast to earlier observations, we herein noted no enhancement in antiviral potency compared with the haloalkyl compounds, with one striking exception. The trichloroethyl alanine compound (10, X=Cl, R=Me) was active at 0.08 μ M and thus 50 times more potent than either the ethyl or trifluoroethyl analogues. Interestingly, this enhancement was only seen for the alanine series, and not for the glycine and valine systems [13]. Thus, alanine emerged as a preferred aminoacid, although the mechanistic origins of this preference were, and still largely remain, unknown. Much of the preceding literature from our labs and others has utilized alanine as the empirical aminoacid of choice, although as we will note below there are other aminoacids, which may usefully substitute for it.

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PHOSPHORODIAMIDATES

Given the promising activity of alkyl phosphoramidates, particularly those related to alanine, we wondered whether diamidates might also be efficacious. Thus, several methyl esterified amino acyl phosphorodiamidates (11) were prepared and tested [14]. Non-amino acyl phosphorodiamidates derived from simple primary and secondary amines were also prepared. Structure-activity relationships were noted that indicated a strong preference for aminoacids such as phenylalanine [14]. Thus a different aminoacid SAR emerged for these diamidates as compared to the earlier alkyl phosphoramidates. It is intriguing to note that this preference for aromatic side-chains was also seen by Wagner for the rather un-related phosphoramidate diesters [1]. However, in general the diamidates appeared to offer no biological advantage over the amidates, and the chemical yields of the diamidates were significantly lower; hence they were not further pursued.

LACTYL DERIVED SYSTEMS

In an effort to establish the importance of the bridging aminoacid nitrogen atom for the biological activity of the phosphoramidates a small family of isosteric O-linked analogues derived from lactic and glycolic acid was prepared (12) [15]. In each case, lengthening of the alkyl phosphate chain (R^2) leads to a reduction in potency. It was also notable that glycolyl systems ($R^2=H$) were more active than lactyl ($R^2=Me$) by a factor of ca. 20. This is in contrast to the earlier work on phosphoramidates noted above where alanine was preferred over glycine [10,11]. A brief hydrolytic stability study was undertaken on compounds 12, which revealed liberation of polar compounds and traces of AZT in biological media, but not in DMSO/water. Thus, enzyme-mediated activation was possible. However, since the anti-HIV activity of even the most active compound in the series was significantly (>10-fold) lower than AZT itself, these compounds were not further pursued.

DIARYL PHOSPHATES OF AZT

One of our major breakthroughs in phosphoramidate pro-drug research was made in 1992, when we noted the efficacy of aryloxy phosphates and phosphoramidates [16]. Thus, diaryl phosphates (13, Fig. 2) were prepared from AZT using simple phosphorochloridate chemistry. For the first time, the anti-HIV activity of these phosphate derivatives of AZT exceeded that of the parent nucleoside in some cases. Thus, the bis (*p*-nitrophenyl) phosphate was ca. 3-fold more potent than AZT vs. HIV-1 in C8166 cells, with an EC_{50} of 3nM [16]. Moreover, whilst AZT was almost inactive (EC_{50} 100 μ M) in the JM cell line, the substituted diaryl phosphate was 10-times more active (EC_{50} 10 μ M). At the time, it was considered that JM was AZT - insensitive due to poor phosphorylation [17]. It later emerged that an AZT-efflux pump was the source of this poor AZT sensitivity [18]. However, the conclusion remains valid that the diaryl phosphate was *more* able to retain activity in the JM cell line, and that this may imply a (small) degree of intracellular phosphate delivery. The nitro group was implicated as vital to this activity, as the parent diphenyl phosphate was ca. 100-fold less active (C8166 cells). The electron-withdrawing power of the *p*-nitro groups and putative enhancements in

aryl leaving group ability were suggested as the major driving force of this SAR [16].

Thus, a series of analogues of (13) were prepared, with various alternative para substituents (CN, SMe, CF_3 , I, OMe, H) [19]. A very clear correlation emerged between electron-withdrawing power of the para substituent and antiviral potency; the nitro and cyano substituted compounds being the most potent, the parent phenyl substituted compound intermediate in activity and the methoxy analogue least active, being 500-fold less active than the nitro compound. The effect of location of the electron withdrawing nitro group on the aryl rings was also briefly pursued, with symmetrical bis-ortho nitro and bis-meta nitro analogues being prepared [20]. In a study of both HIV-1 and HIV-2 in several cell lines it was found that the location of the nitro group had little effect on activity. However, for the first time we were able to assess the activity of the phosphate pro-drugs in the 'true' kinase-deficient cell line CEM-TK⁻. This was a clear but disappointing result, with all of the diaryl phosphates losing almost all their activity, alongside AZT, in the TK⁻ cell line. This most likely implied poor intracellular phosphate delivery and that the diaryl phosphates were acting largely, if not entirely, as AZT pro-drugs, not as AZTMP pro-drugs as intended [20]. However, the earlier work using JM cells on phosphoramidates [16] had indicated that aryloxy phosphoramidates may offer a chance for true phosphate delivery, and this became the main focus of our work.

ARYLOXY PHOSPHORAMIDATES OF AZT

Thus, a series of aryloxy phosphoramidates of AZT was prepared (14) with various *p*-aryl substituents and several aminoacids [21]. Compounds were only studied in the AZT-resistant JM cell line to probe potential (implied) AZTMP release, and the alanine phosphoramidate emerged as strikingly effective. In HIV-1 infected JM all cultures, AZT was inhibitory at 100 μ M, whilst the phenyl methoxy alaninyl phosphoramidate (14, $R=Me$, $Ar=Ph$) was active at 0.8 μ M. This was taken as the first evidence of a successful nucleotide delivery. As had been noted by us previously in other series there was a marked preference for alanine over leucine (10-fold) and glycine (>100-fold). Moreover, whilst electron-withdrawing aryl substitution had been noted to be very effective in the diaryl systems [19], it was detrimental here. Para fluoro substitution had a slight adventitious effect, but not significantly so, whilst para-nitro substitution led to a 100-fold loss of activity. In a subsequent study [22] the range of aryl substituents was extended and compounds studied in true TK⁺ and TK⁻ cell lines. None of the phosphoramidates retained the high (2-4 nM) potency of AZT in TK competent cell lines (CEM and MT-4) against either HIV-1 or HIV-2 [22]. However, whilst AZT lost all of its activity in the TK⁻ deficient cell line CEM/TK⁻, most of the phosphoramidates retained antiviral activity, thus being ca >10-35-fold more active than AZT in this assay. Again, alanine emerged as an important component, with the glycine analogue being inactive in HIV-infected CEM/TK⁻ all cultures. In this assay, leucine and phenylalanine were as effective as alanine, although they were less so in CEM/TK⁺ assays. Thus, the parent phenyl methoxy alaninyl phosphoramidate emerged as an important lead compound [22].

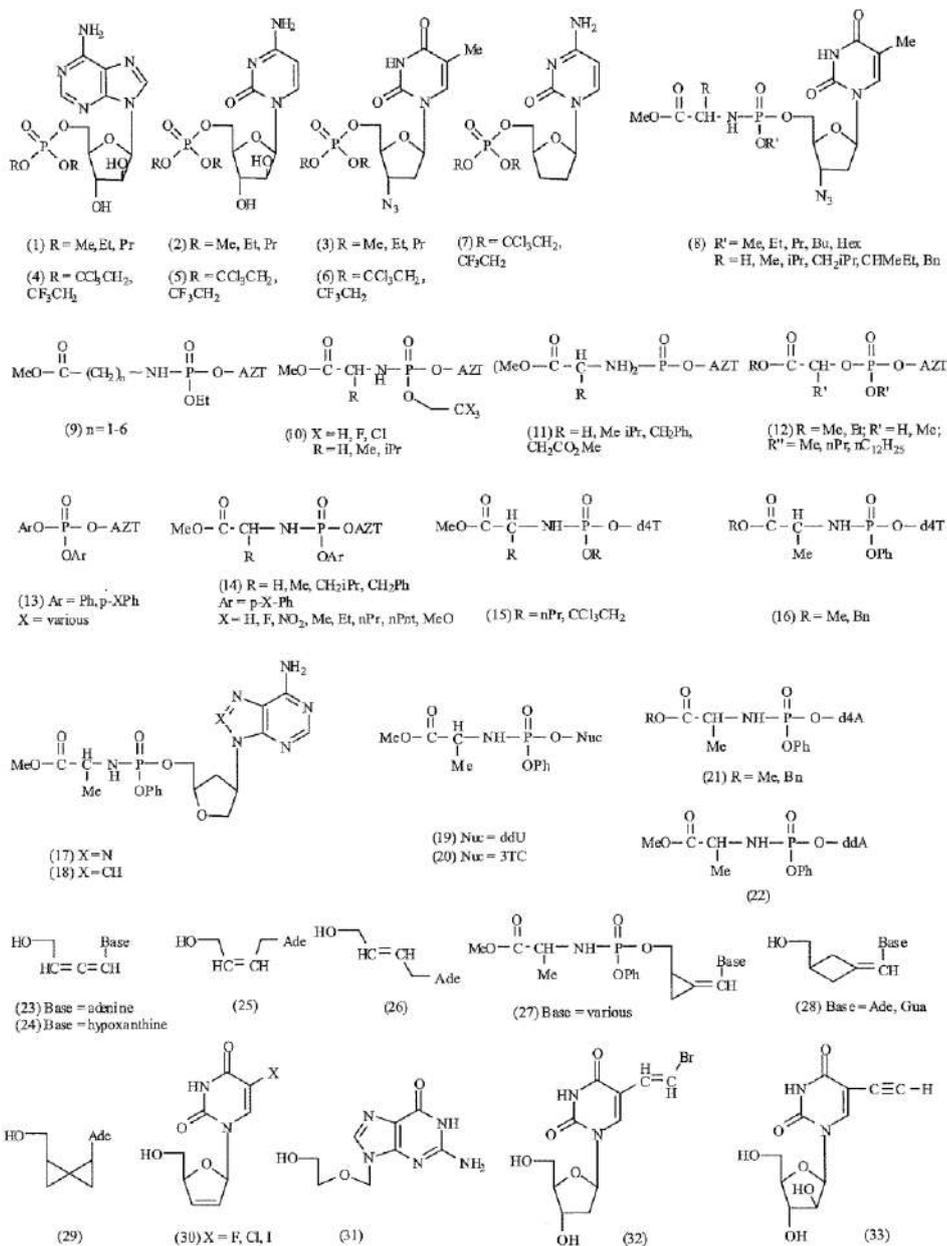


Fig. (1). Structures of some nucleosides and nucleotides. All nucleotides are 5'-linked.

APPLICATION TO OTHER NUCLEOSIDES

As with other research groups reported in this compilation, we had sought to find a universal phosphate delivery motif that could be applied to a range of nucleosides. Indeed as early as 1993 we suggested that the phenyl aryl phosphoramidate approach might be successful on a range of nucleosides (ddC, d4T) and phosphonates (PMEA) [22]. This has subsequently been confirmed to be the case with extensive application of the technology by others and us.

Stavudine (d4T) was an early application of ours [23]. This was a rational choice based on the known kinetics of phosphorylation of d4T. Thus, whilst the 2nd phosphorylation (AZTMP to AZTDP) but not the first phosphorylation (AZT to AZTMP) is regarded as rate limiting for AZT, the first step (d4T to d4TMP) is thought in general to be the slow step for d4T [24]. Thus, an intracellular (mono) nucleotide delivery should have maximal impact for d4T and similar nucleosides. In the first instance (halo)alkoxy phosphoramidates of d4T (15) were prepared [23] and found to retain activity in d4T-resistant JM cells. The activity was dependent on the haloalkyl group; the parent propyl system being poorly active. Subsequent studies in HIV-infected CEM/TK⁻ cell cultures [25] revealed the aryloxy phosphoramidates of d4T (16) to be highly effective and, notably, to retain their full activity in CEM/TK⁻ cells. In this study the benzyl ester emerged as slightly more potent than the parent methyl compound, being almost 10-times more active than d4T in CEM/TK⁺ assays and thus ca 300-500 fold more active than d4T, in CEM/TK⁻ assays. Extensive studies followed on these promising d4T derivatives [26,27] which we will discuss later.

In 1994 Franchetti and coworkers [28] applied the aryl phosphoramidate technology to 8-aza-isoddA (17) and isoddA (18). Very significant boosts in the antiviral potency of the parent nucleosides were noted; >25-fold for (17) and 350-800 fold for (18). This was important work, which demonstrated the power of the aryloxy phosphoramidate approach to greatly improve the biological profiles of poorly active nucleosides. Thus, (18) was transformed from 32 μ M activity versus HIV-2, to 40nM activity, on phosphoramidate formation. Subsequent analysis of these compounds by the Montpellier team [29] leads to the clear conclusion that they function as efficient intracellular phosphate delivery forms.

To a large extent this could be regarded as an example of what in 1990 we termed 'kinase bypass', wherein an inactive, or moderately active and poorly phosphorylated nucleoside could be 'activated' or potentiated by suitable pro-tide modification [5,30]. A further example of this has emerged in our labs on application of the technology to ddU [31]. Thus, whilst dideoxyuridine (ddU) is almost inactive (EC₅₀ 200 μ M) vs. HIV-1 in C8166 cells, the phosphoramidate (19) was noted to be active at low μ M levels and to retain potency in the AZT-resistant JM cell line. This activity was specific to the aryloxy phosphoramidate both with our lab [31] and the Montpellier group [32] noting poor activity for the alkoxy phosphoramidates.

Given the success of the phosphoramidate approach by the Franchetti lab when applied to iso nucleosides [28], we were interested to pursue other sugar modifications. Therefore, we applied the approach to 2',3'-dideoxy-3'-thiacytidine (3TC) [33]. In fact, compound 20 was found to be less effective than 3-TC in deoxycytidine (dCyd) kinase competent HIV-1 and -2 infected cell assays, but assay in dCK deficient cells indicated far less of an impact on potency for the phosphoramidate than the parent CEM cells 3TC (ca. 20-fold vs 2000-fold). Interestingly, both compounds were equally effective versus hepatitis B virus in hepatoma G2 cells indicating efficient pro-tide activation in these cells but not in the CEM cells used for the HIV assay [33]. This was amongst the first indications that the (relative) efficacy of phosphoramidates might be cell-line dependent.

One of the most remarkable demonstrations of the effectiveness of the aryloxy phosphoramidate approach came from our application of the technology to the dideoxydihydro purine d4A [34]. Compounds of the type 21 were found to be exquisitely potent inhibitors of HIV-1 and 2. Both the methyl and benzyl esters displayed EC₅₀ values of ca. 6-18nM thus being 1000-4000 times more potent than the parent nucleoside analogue d4A. Although the phosphoramidates (21) are more cytotoxic than d4A (ca. 30-fold), their extraordinary potency enhancements still leave them with enhanced selectivities (50-150 fold) [34], and they are taken as a good example of nucleoside 'kinase' (in this case adenosine) bypass. Subsequent application of the technology to dideoxyadenosine ddA (22) revealed a similar outcome; a >100-fold potency boost, with some increase in cytotoxicity [35].

The Detroit-based lab a Jiri Zemlicka has pioneered the synthesis of highly modified nucleosides with alkene, alkyne, alkene, methylenecyclopropane, methylenecyclobutane and spiro-pentane modifications and successfully applied our phosphoramidate technology. Indeed, they have recently reviewed these efforts [36].

Thus, phenyl methoxy alaninyl phosphoramidates of the anti-HIV active adenallene (23) and the inactive hypoxallene (24) were prepared [37].

A 10-20 fold boost in anti-HIV potency was noted on phosphoramidate formation from (23). Alkenyl adenic nucleosides such as (25) and (26) were similarly studied [38,39]. In these cases, both the Z (25) and E (26) nucleosides were inactive, whilst the phosphoramidate of (25) was active in the 1-10 μ M range and non-toxic; the isomeric phosphoramidate (26) remained inactive. The hypoxanthine analogue of (25) was also poorly active [38,39].

A study of methylenecyclopropane nucleoside phosphoramidates (27) was conducted by the Zemlicka group [40-43]. Besides these active Z-isomers, the inactive E-series were also phosphorylated and compounds evaluated against a very wide range of viruses (HCMV, HSV-1, HSV-2, HHV-6, EBV, VZV, HBV, HIV-1 and HIV-2). Amongst the conclusions were the following:

- The Z-adenine compound is a potent inhibitor of a variety of viruses, but is cytotoxic.

- The Z-guanine analogue is active against HCMV, HBV, EBV and VZV and is non-cytotoxic.
- The Z-diaminopurine is highly active against HBV and HIV-1, with lower activity against other viruses and is non-cytotoxic. This compound emerged as the best candidate for further development. Again, whilst the E-isomers of the parent nucleosides were either inactive or very poorly active, their phosphoramidates emerged as potent and selective antivirals, particularly the adenine compound.

The Zemlicka group has also applied the phosphoramidate technology to methylene cyclobutane (28) [44] and spiro-pentane (29) [45] nucleosides with varying degrees of efficacy.

Further pursuing the kinase bypass approach we prepared some inactive novel d4T derivatives with 5-halo substituents in place of the 5-methyl group (30) and converted them to their phosphoramidates [46]. Whilst all compounds (30) were poorly active/inactive at >10-50 μ M, the phosphoramidates were all active. The phenyl methoxy alaninyl phosphoramidate of the 5-chloro compound (30, X = Cl) was active at sub μ M concentrations, being >100-fold more potent than its nucleoside parent, and was also non-cytotoxic [46].

Inspired by Zemlicka, who had shown that phosphoramidate formation could broaden the spectrum of activity of nucleoside analogues, we sought its application to a variety of nucleosides with different therapeutic targets. Thus, the anti-herpetic agents acyclovir (31), BVDU (32) and netivudine (33) were all converted to their phosphoramidates [47-49].

In general terms, the approach failed for acyclovir (31), where the phosphoramidate was significantly less active than the parent versus HSV-2, and slightly more active versus HCMV [47]. Similarly, with BVDU (32) we noted a reduction in anti-VZV activity (5-25 fold) for the phosphoramidates, as well as very poor activity in TK-assays [48]. This was taken as evidence of a low degree of kinase bypass resulting from inefficient pro-tide activation in this case or, alternatively, fact dephosphorylation of the

released nucleoside monophosphate. It is therefore surprising to note the apparent efficacy of these phosphoramidates of BVDU in anti-cancer assays reported by the NewBiotics Group [50]. It may be that the necessary pro-tide activating enzymes (see below) are absent in the cell lines used in our antiviral assays, but present in the (tumour) cell lines used by NewBiotics, or that the apparent anti-cancer activity of BVDU phosphoramidates does not arise via monophosphate release. However, the apparent clinical progression of these agents [51] would suggest that the anti-cancer arena may well be a fruitful area for future phosphoramidate studies.

Finally, our application of the technology to the potent anti-VZV agent netivudine (33) [49] was again disappointing, with little activity being noted.

Thus, in conclusion others and we have demonstrated that the phenol methoxyalaninyl group may significantly enhance the potency, selectivity and activity of spectrum of a range of nucleosides and by-pass their dependence on nucleoside-kinase mediated activation. The approach is very successful for the Zemlicka agents with highly modified sugar regions, and for ddA and d4A. It is also effective for d4T and AZT in nucleoside kinase deficient cells. However, it is clearly dependent on the nature of the parent nucleoside and the cell line/target studied. The example of BVDU highlights this final point [48,50].

APPLICATION TO ACYCLIC NUCLEOSIDE PHOSPHONATES

Until recently all of the published work on phosphoramidate pro-tides was on nucleoside analogues, as noted above. However, recently there have been a few reports of application of the technology to phosphonates, and in particular acyclic nucleoside phosphonates (ANPs). The Gilead group who has been active in the commercialization of ANPs have reported [52] that aryloxy phosphoramidates (34, Fig. 2) of PMPA (tenofovir) are highly active anti-retrovirals. We reached the same conclusion for PMPA and the closely related PMEA [53]. Boosts in antiviral potency of 30-100 fold were noted for PMPA and PMEA, with the usual preference for alanine as the amino acid component.

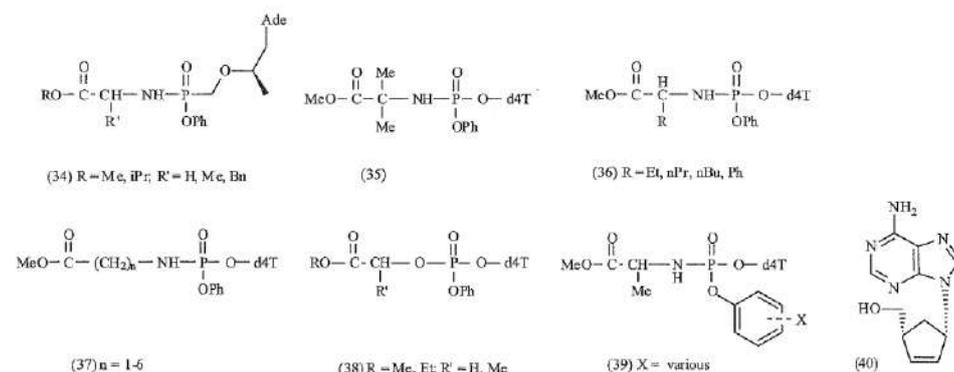


Fig. (2). Structures of some nucleosides.

Interestingly, we noted a significant preference for L-alanine over D-alanine (5-60 fold), whereas Gilead observed a preference for one phosphonate diastereoisomer over the other, observations we will further discuss later under 'stereochemistry' matters. Gilead commenced clinical trials on their amide GS7340 in 2002, most notably progressing with one phosphate diastereoisomer, afforded by an efficient large-scale synthesis and isomer separation [54].

D4T Aryloxy Phosphoramidate SARs

We have conducted fairly extensive structure-activity relationship studies of various regions of the phosphoramidate unit when attached to d4T, with over 250 analogues prepared to date [26,27]. These will be discussed by the respective region of the phosphoramidate:

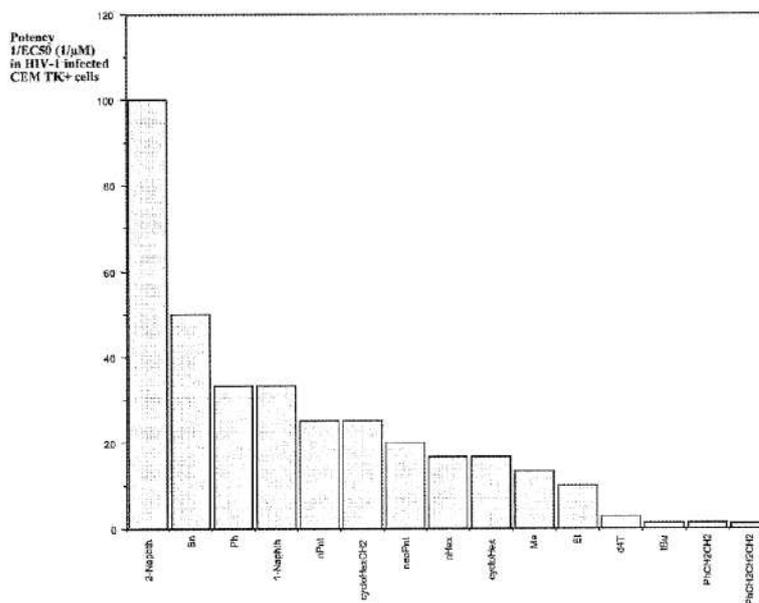
Ester Region

Some early work on AZT alkyloxy phosphoramidates revealed the importance of the carboxyl ester region for anti-HIV activity [55], which was subsequently confirmed for d4T aryloxy analogues [56]. Thus, a range of primary, secondary, tertiary, alkyl, benzyl and linear and branched esters related to (16) were prepared and evaluated against HIV-1 and -2 in thymidine kinase - competent and -deficient cell lines. Data are presented in Graph 1 as plot of potency ($1/EC_{50}$) for a range of esters, versus HIV-1 in CEM TK⁺ cells, with d4T as control. A number of esters lead to potent activity, comparable with, or slightly more potent than, the methyl parent with compound. The benzyl, and

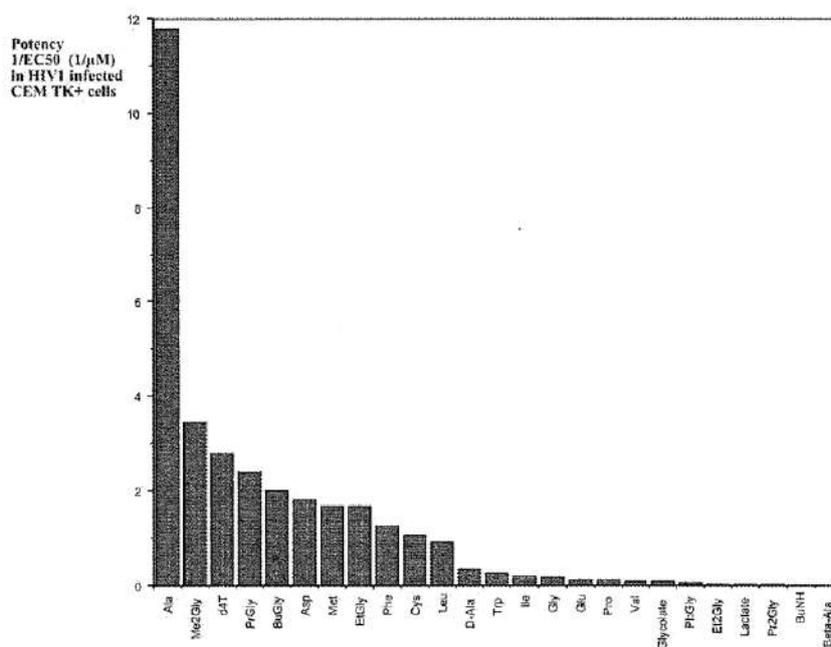
naphthyl esters in particular were noted to be highly potent. The t-butyl ester analogue on the other hand was >10-times less potent than the methyl ester parent compound. As we will note below under Mechanism of Action studies, this correlates well with the poor esterase susceptibility of this particular ester. We later conducted a QSAR analysis using calculated physical properties (TSARTM) for 15 esters related to (16), which showed a good degree of correlation between predicted and measured activity, with a clear dependence on the shape and electronic distribution of the ester [57]. In particular, there emerged a strong dependence on the directional component of the ester group lipophilicity (the 'lipole moment'), indicating that electron withdrawing groups in the ester, but removed from the ester bond, should boost potency.

Amino Acid

As noted above, alanine had arisen as the amino acid of choice based on limited studies with alkyloxy phosphoramidates of AZT. We now compared 13 amino acids related to (16, R=Me) [58]. Very clear SARs emerged from this study as presented in Graph 2, along with other highly amino-modified systems will be described later. Thus, versus HIV-1 in CEM TK⁺ cells, alanine remained the most effective amino acid. However, several other amino acids led to potencies, which were not significantly reduced, notably the un-natural, achiral α,α -dimethylglycine compound (35), which was slightly more potent than the alanine compound in CEM / TK⁺ cells. In fact, all of the phosphoramidates retained full potency in the nucleoside



Graph 1. The effect of ester changes on antiviral potency.



Graph 2. The effect of amino acid changes on antiviral potency.

kinase deficient CEM cell assay, indicating action via intracellular d4TMP release. However, some aminoacids were less effective; proline in particular, leads to a compound 20-100 times less potent than (16); whether this relates to the importance of a free aminoacid NH, or steric or conformational issues relating particularly to proline is not entirely clear. However, valine and isoleucine were also poorly effective, indicating some steric restriction issues related to the side-chain. On the contrary, however, glycine is a striking example; the simple loss of the alanine methyl group resulting in a ca 60-70 fold reduction in potency.

The potency of (35) was an important discovery, since for the first time it indicated that 'un-natural' (or less common) aminoacids could be utilized in the phosphoramidate approach. Indeed, we subsequently studied the α,α -diethyl- and -dipropyl analogues of (35), but found that these were poorly active (≥ 100 -fold less potent than the dimethyl parent) [59]. On the other hand, considerable tolerance was allowed for un-natural, non-alkyl glycines, of the type (36). Thus, whilst α -ethylglycine was 10-fold less effective than alanine, the *n*-propyl and *n*-butyl analogues showed no subsequent losses in potency [59].

These compounds are therefore all substantially more potent than the 'natural' glycine system. Replacement of the side-chain in (36) by a phenyl did however lead to a further

loss of activity, to yield a material similar in potency to the glycine compound [58,59].

Amino Acid Stereochemistry

Given the importance of the aminoacid side-chain it became interesting to probe its stereochemical requirements. Thus, we prepared the isomeric, D- compound related to (16, R=Me) and found it to be 20-30 fold less effective than the L-parent [60]. Despite this reduction in potency, the D-compound did retain full potency in TK-deficient CEM assays, indicating its functioning entirely as a d4TMP delivery form, with little or no free d4T release. It is further interesting to note that the D-alanine compound is of similar (slightly higher) potency as the glycine analogue [58,60]. This implies that a methyl group on the L-face of the aminoacid (as in L-alanine and α,α -dimethylglycine) contributes about a log in potency to the baseline of glycine, that the D-face-methyl group of D-alanine cannot substitute, and that the Pro-D-methyl group of α,α -dimethylglycine is neither advantageous nor detrimental to potency.

Amino Acid Replacement

In this section we briefly describe some large-scale aminoacid changes, which have a significant (largely negative) impact on potency. Thus, replacement of the aminoacid in (16) by a family of non-aminoacyl simple *n*-

alkylamines (C3 to C12) leads to a complete removal of activity [61]. The same result was observed for simple alkylamine analogues of the AZT phosphoramidates (14) [61]. Thus, an aminoacid appears a pre-requisite for a successful aryloxy phosphoramidate approach. This is in marked contrast to recent data from the Montpellier group who has prepared phosphoramidate-SATE hybrids of AZT, and found them to be highly potent and not to depend on an aminoacid type structure for potency [62]. As noted by Peyrottes *et al.* in this Compilation, this indicates a different mechanism of action for SATE-phosphoramidates as compared to aryloxy phosphoramidates (see below).

We had previously noted that chain extended aminoacid-related aryloxy phosphoramidates of type (9) were poorly effective [12]. It was of interest to extend this study to analogous beta- and other aminoacids, when applied to aryloxy derivatives of d4T. Thus, compounds (37) were prepared and found to be very poorly active; all extended compounds were ca 40-times less potent than the glycine parent compound [63]. Interestingly the β -alanine compound (37, n=2) was thus ca 2500 times less potent than its structural isomer, the alanine lead compound (16, R=Me). It is striking therefore, that the beta- and further extended aminoacids are no more effective as aryloxy phosphoramidate motifs than simple n-alkylamines [61,63] (Graph 2) and that the substantial potency benefit offered by aminoacids such as alanine does not extend to their beta- and longer isomers. We will discuss this SAR further below under Mechanism of Action.

Finally in this section, we note that the bridging aminoacid nitrogen atom is vital for the antiviral potency, since its replacement by a bridging oxygen atom, as in (12), but now for aryloxy phosphoramidates of d4T (38), leads to a very significant loss (ca.600-fold) of activity [64].

Aryl Substitution

We had previously noted the effect of aryl substitution on aryloxy phosphoramidates of AZT (14) [21,22]. We now extended this study to d4T with a variety of substituents ranging from electron donating to electron withdrawing (39) [65]. It was notable that strongly electron withdrawing groups (p-CN, p-NO₂) lead to slight reductions in potency, whilst several substituents, such as the p-COOMe and, particularly p-Cl groups showed significant potency boosts. The p-chloro compound emerges as a new lead structure, being 14-times more potent than the phenyl parent compound, with an EC₅₀ versus HIV-1 in wild-type nucleoside kinase competent CEM cells of 5nM, and being fully active in the CEM/TK⁻ assays. The chloro compound was also the most lipophilic studied, and logP measurements indicated a correlation (r=0.9) between measured logP and antiviral activity. However, this may be an artefact of the small sample size, and logP may not in itself correlate with activity in wider compound series. In general, this study concluded that lipophilic substituents which were mildly electron-withdrawing (σ 0.15 - 0.48) were preferred [65]. Indeed, a subsequent more rigorous QSAR analysis of 21 compounds of this type confirmed lipophilicity as an important factor, with steric and electronic factors of more secondary importance [66].

Uckan *et al.* also noted the efficacy of compounds of the type (39), and in particular with a para-bromo substituent

[67-69]. This group later went on to suggest these agents as virucidals [70,71].

Phosphate Stereochemistry

One of the notable structural features of all of the phosphoramidate triesters, excluding some phosphorodiamidates [14], is the presence of a chiral centre at the phosphate. Due to the chirality of the nucleoside all of the compounds prepared are thus isolated as a pair of diastereoisomers. In this regard the phosphoramidate approach is similar to the cycloSAL approach of Meier reported in this compilation. As early as 1990 we had started to partly separate the diastereoisomers [10]. Thus, some alkoxy phosphoramidates of AZT (8) were partly separated by flash silica chromatography and fractions enriched in the more lipophilic ('fast') and less lipophilic ('slow') isomer were separately evaluated. We found a small, ca 3-fold, difference in potency, with the 'fast' isomer being less potent. A subsequent study on some mixed haloalkyl triesters of the type (6) indicated a 10-fold difference, with the 'fast' isomer being more potent [8]. In neither case was any link made between the absolute chirality at the phosphorus atom and the relative lipophilicity/potency; such work was only to emerge later. Thus, working with alanine phosphoramidates of PMEA (34), researchers at Gilead found a 10-fold difference in potency, with the S-phosphate isomer being more potent [52]. Their recent disclosure of a large scale synthesis and purification of the most active isomer clears the way towards clinical evaluation of single isomers of phosphoramidate triesters [54].

MECHANISM OF ACTION STUDIES

The putative first activation step for these phosphoramidates is esterase mediated carboxyl ester cleavage. In an effort to model this in a predictive sense, we exposed various esters of type (16) to pig liver esterase, and followed the P-31 NMR signal [56]. The parent compound always shows two closely spaced signals, due to the phosphate diastereoisomers. Upon esterase treatment these signals collapsed to give a downfield singlet (δ_p ca 8.2ppm). This was characterised as the amino acyl phosphate monoester (Fig. 3, A). Whilst the rate of cleavage of various esters did not readily correlate with antiviral activity, we did note that esterase lability was a necessary (but not sufficient) condition for high biological potency; the t-butyl ester in particular was not hydrolysed, and that phosphoramidate was the least potent assayed in cell culture [56].

As noted above, subsequent studies on compounds with chain-extended aminoacids of type (37) [63] showed the importance of the α -aminoacid for activity. Indeed, the esterase study on these compounds, each of which had a methyl ester, showed that they were all well processed. However, only the α -compound, derived from glycine, proceeded to form the intermediate of type A (Fig. 3). The beta- and longer homologues were processed only to the carboxylate B (Fig. 3). It appears that for entropic, or steric, reasons the loss of the phenyl group from these systems does not proceed under the conditions of the assay, diesters of type A do not arise, and the antiviral activity is poor. We surmise that an alpha aminoacid (or similar) is required for neighboring group assistance to displace the phenyl group,

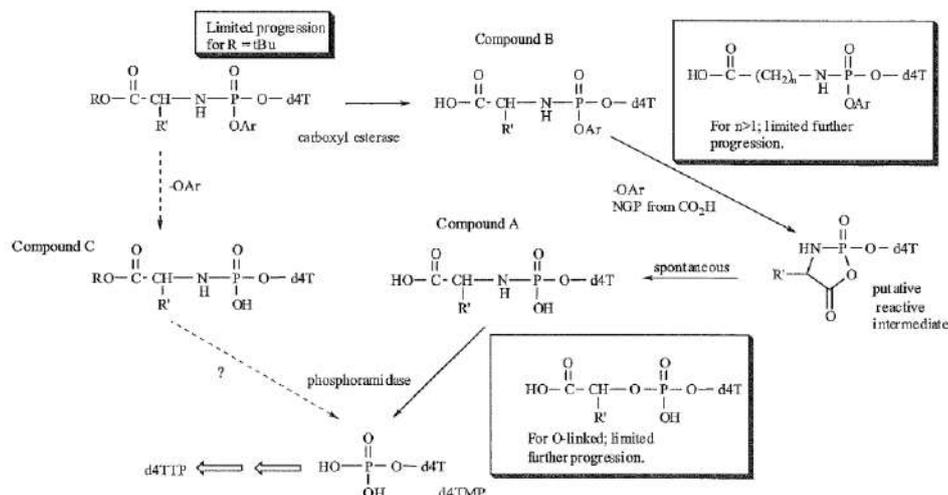


Fig. (3). Proposed mechanism of activation of aryloxy phosphoramidates of d4T. The pathway via compound A is considered to predominate, unless the aryl groups bears a strongly electron withdrawing group and esterase is limiting. The putative reactive intermediate between B and A has not been observed, but is surmised.

and that in its absence phenyl loss does not take place, and moreover, that in the absence of such a displacement the phenyl group is not lost *in vitro*, and thus the antiviral action is limited.

Similar mechanistic conclusions were derived by the Montpellier group [72] on the isoddA compounds of Franchetti (18) using an elegant online ISRP-cleaning HPLC method they had earlier reported for POM esters [73]. However, in contrast to our work, under some conditions this group noted an alternative pathway involving loss of the aryl group prior to ester hydrolysis, to give compounds of the type C (Fig. 3) [72]. We believe this to be specific to compounds with strong electron withdrawing aryl substituents (e.g. nitro) and/or under conditions of reduced esterase activity, and may be of limited *in vitro* significance for un-substituted phenyl systems.

We confirmed our general view using radio labelled compound (16, R=Me) incubated with a range of cell lines used in the antiviral assays and then studied by HPLC [74]. In these studies high levels of metabolite A were noted, which varied with the cell line, but in general corresponded with high anti-retroviral activity. Thus, compound 'A' was suggested as an important intracellular depot form for the free nucleotide [74]. This study was subsequently extended to include a wide range of compounds of the general type (36), with varying aminoacids and esters, and also chain extended compounds (37), and AZT analogues (14) [75]. Again, the generation of intermediates of the general type 'A' was seen as a necessary condition for high antiviral potency. For the first time, this study rationalised many of the SARs noted previously, such as the strong aminoacid preferences we had seen [58]. For example, valine had been noted to be a poorly effective aminoacid, and its phosphoramidate was now of to be poorly processed by

carboxyl esterase, human serum, and CEM cell extracts to the type 'A' compound [75].

In this study, the final step in conversion of metabolites 'A' to the free nucleotide was also studied, namely the cleavage of the aminoacid moiety. This represents a phosphoramidase activity [EC 3.9.1.1] as originally defined by Dixon and Webb [76]. However, a variety of phosphoramidase preparations have been reported from a range of sources, and "pure" enzyme has proved elusive [77-81]. Using a rat liver cytosolic preparation we were able to partially purify a fraction with the ability to hydrolyse compounds of type A to generate d4TMP. The fraction was distinct from creatine kinase, alkaline phosphatase and phosphodiesterase on the basis of its lack of inhibition by known reagents (enzyme inhibitions), and may truly be described as a phosphoramidase [81]. It appears to have a molecular weight in the range of 50-100KDa, has a pH optimum of 7.4, and is inhibited by the phosphoramidase inhibitor iodobenzene [82].

Lastly, when exposed to carboxyl esterase hydrolysis the poorly active lactate and glycolate compounds (38) [64] gave a rapid hydrolysis to give amino acyl intermediates related to 'A' (but O-bridged). Thus, we surmise that such lactates are poorly effective as antivirals due to poor onward processing of this intermediate by phosphodiesterase under the conditions of the assay, and that such analogues are not substrates for the putative phosphoramidase that hydrolyses 'A'.

CONCLUSIONS

The aryloxy phosphoramidate approach has emerged, along with SATE, CycloSAL, aryl phosphoramidate diester and others [1,2] as a viable method for the intracellular

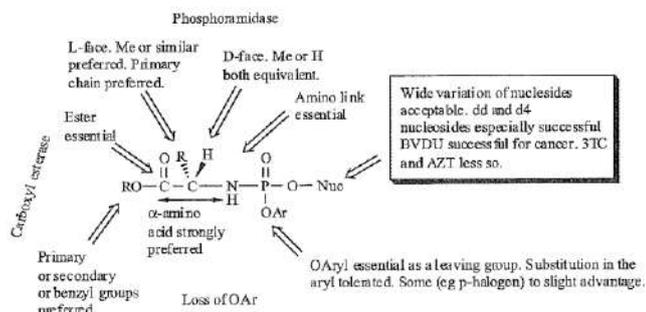


Fig. (4). Preferred moieties for a successful phosphoramidate triester outcome. Possible major origins of key SARs are highlighted with the appropriate activation step.

delivery of free monophosphates of a range of nucleoside analogues. The approach works poorly for AZT, where the second phosphorylation is rate limiting [24] but well for d4T and a range of dd and d4 nucleosides. D4A is a particularly dramatic example with >1,000 fold boosts in potency on phosphoramidate formation. Indeed, recent work from our labs in collaboration with researchers at GlaxoSmithKline on the carbocyclic L-d4A (40) has shown potency enhancements of almost 10,000 on phosphoramidate formation [83]. We believe that this has reached the levels that we originally described over 10 years ago as 'kinase bypass' [5], wherein an 'inactive' nucleoside is activated by phosphate pro-drug formation. This suggests the prudence of pro-tide synthesis on a range of nucleoside analogues, and particularly not just those selected as active in initial screens; the inactivity of other structures may simply correspond to poor initial phosphorylation, which may now be by-passed with pro-tides. The activity of the phosphoramidates of the highly modified Zemlicka nucleosides highlights this point well [36].

In terms of SARs, it has emerged that an alpha amino acid is essential for this approach, by contrast to phosphoramidate-SATE hybrids of the Montpellier group. Alanine remains a good choice of amino acid, although the achiral α,α -dimethylglycine is a good alternative. The ester and aryl moieties can be varied considerably, provided the ester can be cleaved by esterase, and the aryl is a reasonable leaving group. A P-N bond is also essential for activity, with lactate isosteres being poorly active. This appears to correlate with putative phosphoramidase mediated cleavage of the key amino acyl intermediate (A, Fig. 3). Summary SARs and their mechanistic origins are collected together in (Fig. 4).

Finally, the issue of phosphate stereochemistry is worth considering. All of the current syntheses of phosphoramidate triesters currently give mixtures, often approximately 1:1, of the phosphate diastereoisomers. These can be separated by chromatography, and often have 5-10 fold potency differences. Whilst it may be possible for mixtures to be progressed towards the clinic, such progression faces substantial regulatory hurdles, and separation and clinical evaluation of pure isomers seem more likely. The recent example of Gilead's GS-7340 supports this view [54]. On the kilo scale, Gilead is using moving bed chromatography

to separate the unwanted diastereomer. On a smaller scale, we have reported the novel use of molecular imprinted polymers (MIPs), which may be a useful tool in the research lab in this regard [84].

As *in vivo* data begin to emerge on aryloxy phosphoramidates [85] and with clinical evaluation proceeding, the next few years will confirm whether or not they have a role to play in future drug development for viruses and beyond.

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Selective Intracellular Activation of a Novel Prodrug of the Human Immunodeficiency Virus Reverse Transcriptase Inhibitor Tenofovir Leads to Preferential Distribution and Accumulation in Lymphatic Tissue

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An isopropylalaninyl monoamidate phenyl monoester prodrug of tenofovir (GS 7340) was prepared, and its in vitro antiviral activity, metabolism, and pharmacokinetics in dogs were determined. The 50% effective concentration (EC₅₀) of GS 7340 against human immunodeficiency virus type 1 in MT-2 cells was 0.005 μM compared to an EC₅₀ of 5 μM for the parent drug, tenofovir. The (*L*)-alaninyl analog (GS 7340) was >1,000-fold more active than the (*D*)-alaninyl analog. GS 7340 has a half-life of 90 min in human plasma at 37°C and a half-life of 28.3 min in an MT-2 cell extract at 37°C. The antiviral activity (>10× the EC₅₀) and the metabolic stability in MT-2 cell extracts (>35×) and plasma (>2.5×) were also sensitive to the stereochemistry at the phosphorus. After a single oral dose of GS 7340 (10 mg-eq/kg tenofovir) to male beagle dogs, the plasma bioavailability of tenofovir compared to an intravenous dose of tenofovir was 17%. The total intracellular concentration of all tenofovir species in isolated peripheral blood mononuclear cells at 24 h was 63 μg-eq/ml compared to 0.2 μg-eq/ml in plasma. A radiolabeled distribution study with dogs resulted in an increased distribution of tenofovir to tissues of lymphatic origin compared to the commercially available prodrug tenofovir DF (Viread).

Highly active antiretroviral therapy (HAART) for the treatment of human immunodeficiency virus is effective in reducing plasma viral loads below current assay detection limits and is responsible for significant reductions in AIDS-related mortality in the United States (13). Combinations of protease and reverse transcriptase inhibitors are extremely potent at blocking de novo infection; however, they have no effect on latently infected cells. The half-lives of these latent cellular reservoirs were originally estimated to be >3 years, leading to the conclusion that it may not be possible to eradicate human immunodeficiency virus (HIV) from an infected individual by using current HAART (2). It has subsequently been shown that even in patients who have undetectable plasma viremia (<50 copies/ml), low-level replication is ongoing (11, 15, 36), resulting in repopulation of latent reservoirs and thus accounting for the long apparent half-lives observed (12, 22, 23, 35). The failure of HAART to completely shut down virus replication in vivo is a function of both the intrinsic potency of the drug regimen and its distribution to the cellular sites of virus replication. The lymphatic tissues and the peripheral blood mononuclear cells (PBMCs) are the primary sites of virus replication and potential virus latency (9, 19). A drug targeting strategy that selectively enhances active drug concentrations in these tissues without excessive systemic exposure is conceptually attractive and would potentially lead to a more effective HAART with fewer potential side effects.

Tenofovir, {9-[(*R*)-2-(phosphonomethoxy)propyl]adenine} (PMPA) (Fig. 1) is a nucleotide analog that inhibits HIV reverse transcriptase and shows potent in vitro and in vivo activity against HIV (3, 7) but has low oral bioavailability in preclinical models (6). An oral prodrug of tenofovir, tenofovir disoproxil fumarate (tenofovir DF; Viread) (Fig. 1), is indicated in combination with other antiretrovirals for the treatment of HIV infection. The long intracellular half-life (~50 h) of the active diphosphate metabolite of tenofovir in resting PBMCs (26) allows this drug to be administered once daily. The prodrug tenofovir DF was designed to undergo rapid metabolism to the parent drug, tenofovir, in the systemic circulation after oral administration. Interestingly, in preclinical studies with dogs, the intracellular levels of tenofovir in PBMCs were fivefold higher after oral administration of tenofovir DF than after an equivalent subcutaneous exposure of tenofovir. Correspondingly, in human clinical trials, the change in HIV virus load was threefold higher after oral administration of tenofovir DF than after an equivalent exposure of intravenously (i.v.) administered tenofovir (5). The “enhanced” anti-HIV activity observed in patients with the oral prodrug relative to the intravenously administered parent drug may be attributable to an increase in the intracellular concentration of tenofovir, which is likely the result of better intracellular distribution of the oral prodrug.

These results led us to explore a new class of orally administered tenofovir prodrugs designed to circulate systemically as the prodrug and to undergo selective conversion to tenofovir inside cells. In this report, we describe the in vitro and in vivo characterization of GS 7340, an isopropylalaninyl monoami-

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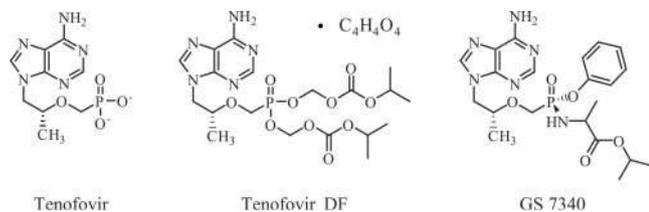


FIG. 1. Structure.

date phenyl monoester prodrug of tenofovir (Fig. 1). This molecule demonstrates extremely potent *in vitro* activity and selective targeting to lymphoreticular tissues and PBMCs *in vitro* and *in vivo*.

MATERIALS AND METHODS

Chemicals. The synthesis of tenofovir and tenofovir DF was described previously (1, 31). The monoamidate prodrugs of tenofovir were prepared by using a modified procedure from the literature (32). Detailed procedures and identification will be published elsewhere. The radiolabeled analogs [^{14}C]tenofovir DF (specific activity, 42 mCi/mmol) and [^{14}C]GS 7340 (specific activity, 53 mCi/mmol) were obtained from Moravex Biochemicals (Brea, Calif.). The radiochemicals were verified by high-performance liquid chromatography (HPLC) before use and were estimated to be >98% pure. All other chemicals and solvents were obtained from commercial sources.

***In vitro* antiviral activity and cytotoxicity.** Triplicate serial dilutions of the test compounds were incubated in 96-well plates with MT-2 cells (20,000 cells/well) infected with HIV-1 IIIb at a multiplicity of infection of 0.01. After 5 days at 37°C, the virus-induced cytopathic effect was determined by using a colorimetric cell viability assay based on the metabolic conversion of 2,3-bis-(methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) as previously described in the literature (34). The concentration of each compound that inhibited the virus-induced cytopathic effect by 50% (EC_{50}) was estimated from the inhibition plots. To determine the compound cytotoxicity, uninfected MT-2 cells in 96-well plates (20,000 cells/well) were incubated with appropriate serial dilutions of tested compounds for 5 days, followed by the XTT-based cell viability assay. Cell growth was expressed as a percentage of the signal relative to untreated control. The concentration of each drug that reduced the cell growth by 50% was estimated from the inhibition plots.

***In vitro* metabolism studies.** MT-2 cell extract was prepared from MT-2 cells according to a previously published procedure (21). Extract (80 μl) was transferred into a screw-cap centrifuge tube and incubated at 37°C for 5 min. Test compounds were dissolved in HEPES buffer (0.2 mg/ml) containing 0.010 M HEPES, 0.05 M potassium chloride, 0.005 M magnesium chloride, and 0.005 M DL-dithiothreitol, and 20 μl was added to the MT-2 cell extract. Aliquots (each, 20 μl) were taken at specified times and mixed with 60 μl of methanol containing 0.015 mg/ml of 2-hydroxymethylnaphthalene (internal standard). The mixture was centrifuged at 15,000 $\times g$ for 5 min, and the supernatant was analyzed by HPLC. The same procedure was employed for the human plasma (pooled from George King Biomedical Systems, Inc.), except that test compounds were dissolved in Tris-buffered saline containing 0.05 M Tris, 0.0027 M KCl, and 0.138 M NaCl (pH 7.5).

The reverse-phase gradient HPLC method used to analyze samples from the MT-2 cell extract and plasma metabolism studies employed a 4.6- by 250-mm, 5- μm particle size Zorbax $\text{R}_x\text{-C}_8$ column (MAC-MOD Analytical, Inc.; Chadds Ford, Pa.) with UV detection at 260 nm. The mobile phase was varied from 50 mM potassium phosphate (pH 6.0)/ CH_3CN (95:5) to 50 mM potassium phosphate (pH 6.0)/ CH_3CN (50:50) over 30 min at a flow rate of 1.0 ml/min.

Human whole blood was incubated for 1 h at 37°C separately with [^{14}C]radiolabeled GS 7340, tenofovir DF, and tenofovir at a concentration of 5 μg -eq tenofovir per ml (17.4 μM). The blood was subjected to treatment with the Ficoll-Paque sodium diatrizoate solution (described below). The treatment resulted in the formation of multiple layers containing different cell types. The bottom layer contained mostly erythrocytes (RBCs) aggregated by Ficoll-Paque. The PBMC layer was washed and extracted with 70% methanol. Aliquots of the plasma and RBC layers (0.5 ml) were also extracted. Radioactivity in all layers was measured by oxidation/scintillation counting and by a comparison with radioactivity from the standard solutions. All extracts were reconstituted in water

and analyzed by HPLC with radiometric flow detection (8). The experiment was repeated, incubating with [^{14}C]GS 7340 at 0.7, 2.3, 6.9, and 20.8 μM .

MT-2 cells (10^7) were incubated in a standard cell culture medium with 10 μM of [^{14}C]GS 7340 at 37°C for 24 h. At specified time points, an aliquot of the cell suspension was taken, and cells were counted, washed three times with ice-cold phosphate-buffered saline (PBS), and extracted with 70% methanol. The supernatants were analyzed using HPLC with radiometric flow detection (8).

Isolation of CD4^+ T cells and monocytes from whole blood. Whole human blood was incubated for 1 h at 37°C with 17.4 μM [^{14}C]GS 7340. PBMCs were obtained by density gradient centrifugation over Ficoll-Paque. CD4^+ T helper (Th) cells or monocytes were isolated from PBMCs by depletion of non-Th cells and nonmonocytes, respectively. The non-Th cells were indirectly magnetically labeled using a cocktail of hapten-conjugated CD8, CD11B, CD16, CD19, CD36, and CD56 antibodies and paramagnetic beads coupled to an anti-hapten monoclonal antibody (Miltenyi Biotec, Inc., Auburn, CA). For depletion of nonmonocytes, the T cells, NK cells, B cells, dendritic cells, and basophils from PBMCs were indirectly magnetically labeled using a cocktail of hapten-conjugated CD3, CD7, CD19, CD45RA, CD56 and anti-immunoglobulin antibodies and paramagnetic beads coupled to an anti-hapten monoclonal antibody. The magnetically labeled cells were depleted by retention on an extraction column in the magnetic field. The eluted respective cell types (CD4 or monocytes) were lysed and analyzed for tenofovir metabolites by radiochromatography (8).

***In vivo* administration and sample collection.** The in-life phase was conducted in accordance with the recommendations of the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication 86-23) and was approved by the Institutional Animal Care and Use Committee at Stanford Research Institute (Menlo Park, CA). Male beagle dogs (four to six/group; body weight, 10 ± 2 kg) were used for the studies. Prodrugs were formulated as solutions in 50 mM citric acid and administered as a single dose by oral gavage. For PBMCs, blood samples were collected at 0 (predose), 2, 8, and 24 h postdose. For plasma, blood samples were collected at 0 (predose), 5, 15, and 30 min and 1, 2, 3, 4, 6, 8, 12, and 24 h postdose. Blood (1.0 ml) was processed immediately for plasma by centrifugation at 2,000 rpm for 10 min. Plasma samples were frozen and maintained at -70°C until analyzed.

PBMC preparation. Whole blood (8 ml) drawn at specified time points was mixed in equal proportion with PBS, layered onto 4 ml of Ficoll-Paque solution (Pharmacia Biotech), and centrifuged at $400 \times g$ for 40 min. The PBMC layer was removed and washed once with PBS. The formed PBMC pellet was reconstituted in 0.5 ml of PBS, and cells were resuspended and counted with a hemocytometer. The number of cells multiplied by the mean single-cell volume was used to calculate intracellular concentrations. A reported value of 200 femtoliters was used as the resting PBMC volume (28).

Determination of tenofovir and GS 7340 and GS 7339 in plasma and PBMCs. The concentration of tenofovir in dog plasma samples was determined by derivatizing tenofovir with chloroacetaldehyde to yield a highly fluorescent N^1, N^6 -ethenoadenine derivative (18). Plasma (100 μl) was mixed with 200 μl of 0.1% trifluoroacetic acid in acetonitrile to precipitate proteins. Samples were then evaporated to dryness under reduced pressure at room temperature. Dried samples were reconstituted in 200 μl of derivatization cocktail (0.34% chloroacetaldehyde in 100 mM sodium acetate, pH 4.5), vortexed, and centrifuged. The supernatant was then transferred to a clean screw-cap tube and incubated at 95°C for 40 min. Derivatized samples were then evaporated to dryness and reconstituted in 100 μl of water for HPLC analysis. Conversion of intact prodrug to tenofovir during the analysis procedures was determined to be <10% with prodrug standards.

Ribonucleotides present in the PBMC extracts were removed by selective oxidation using a modified procedure of Tanaka et al. (33). PBMC extracts were mixed 1:2 with methanol and evaporated to dryness under reduced pressure. The dried samples were derivatized with chloroacetaldehyde as described above for the plasma assay, mixed with 20 μl of 1 M rhamnose and 30 μl of 0.1 M sodium periodate, and incubated at 37°C for 5 min. Following incubation, 40 μl of 4 M methylamine and 20 μl of 0.5 M inosine were added, and samples were further incubated at 37°C for 30 min. Samples were then evaporated to dryness under reduced pressure and reconstituted in water for HPLC analysis. Independently, it was demonstrated that the chloroacetaldehyde derivatization and periodate oxidation resulted in <6% conversion of the mono- and diphosphate metabolites of tenofovir to the N^1, N^6 -ethenoadenine derivative of tenofovir.

The HPLC system comprised a P4000 solvent delivery system with AS3000 autoinjector and F2000 fluorescence detector (Thermo Separation, San Jose, CA). The column was an Inertsil ODS-2 column (4.6 by 150 mm). The mobile phases were as follows: A, 5% acetonitrile in 25 mM potassium phosphate buffer with 5 mM tetrabutyl ammonium bromide, pH 6.0; B, 60% acetonitrile in 25 mM potassium phosphate buffer with 5 mM tetrabutyl ammonium bromide, pH 6.0.

TABLE 1. In vitro anti-HIV-1 activity (EC_{50}), cytotoxicity (CC_{50}), and in vitro metabolic stabilities of tenofovir and tenofovir prodrugs

Prodrug	EC_{50}^a (μ M)	CC_{50}^a (μ M) ^b	Selectivity index ^b	$t_{1/2}$ (min) ^b	
				MT-2 cell extract	Human plasma
Tenofovir	5.0 \pm 2.6	6,000 \pm 2,700	1,250	NR	NR
Tenofovir DF	0.05 \pm 0.03	50 \pm 28	1,000	70.7 \pm 10.1	0.41 \pm 0.20
GS 7171	0.01 \pm 0.005	95 \pm 37	9,500	ND	ND
GS 7339	0.06 \pm 0.04	>100	>1,700	>1,000	231 \pm 72
GS 7340	0.005 \pm 0.002	40 \pm 29	8,000	28.3 \pm 7.4	90 \pm 12
GS 7485 (D-Ala)	10 \pm 4	ND	ND	>700	>200
GS 7160	10 \pm 2.5	ND	ND	ND	ND

^a The data represent means \pm SD from two to four independent determinations.

^b NR, not reactive; ND, not determined.

The flow rate was 2 ml/min, and the column temperature was maintained at 35°C by a column oven. The gradient profile was 90% A/10% B for 10 min for tenofovir, followed by 65% A/35% B for 10 min for GS 7340. Detection was by fluorescence with excitation at 236 nm and emission at 420 nm, and the injection volume was 10 μ l. Data was acquired and stored by an Artemis data acquisition system (Beckman, Palo Alto, CA).

Pharmacokinetic calculations. C_{max} and T_{max} were observed values. Tenofovir exposures were expressed as areas under tenofovir concentration curves from zero to 24 h (AUC_{0-24}). The AUC values were calculated using the trapezoidal rule. All other pharmacokinetic parameters, including the elimination half-life, were calculated by noncompartmental methods using WinNonlin (Pharsight Corp., Mountain View, CA).

Tissue distribution study. Two male beagle dogs (each, 10 to 12 kg) were dosed orally (10 mg-eq tenofovir/kg; 30 to 35 μ Ci/kg) by gavage with [¹⁴C]GS 7340 or [¹⁴C]tenofovir DF. Dosing solutions of GS 7340 and tenofovir DF were prepared 1 h prior to dosing by dissolving necessary quantities of GS 7340 (fumarate salt) or tenofovir DF and dried [¹⁴C]GS 7340 or [¹⁴C]tenofovir disoproxil (radiolabeled free base) in 50 mM citric acid (pH 2.2). Urine, as well as cage rinse and feces, was collected at 24 h after administration. Plasma samples were obtained at 0, 0.0833, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 24 h postdose. Additionally, blood samples were collected at 0, 2, 8, and 24 h postdose and processed for PBMCs as described above. At 24 h after the dose, the animals were sacrificed, and tissues were removed for further analysis. Plasma, peripheral blood mononuclear cells, whole blood, bile, cerebrospinal fluid, and urine were analyzed for total radioactivity by oxidation. Feces were homogenized in water (10% [wt/wt]), and aliquots of homogenate were processed by oxidation for counting. The brain, liver, spleen, heart, lungs, kidneys, uterus, stomach, small intestines, stomach contents, small intestine contents, and large intestine contents were homogenized separately in water (20% [wt/wt]), and aliquots of each homogenate were oxidized and counted.

RESULTS

In vitro anti-HIV activity. GS 7340 was synthesized from (*R*)-PMPA and (*L*)-isopropyl alanine ester in a nonstereospecific synthesis, resulting in the formation of equal amounts of two stereoisomers at phosphorus. These two diastereomers, GS 7339 and GS 7340, were subsequently separated by chromatography. To assess the ability of these prodrugs to cross the cellular membrane and undergo intracellular metabolism to tenofovir, their in vitro activities were measured against HIV-1 in MT-2 cells. The antiviral activities for the diastereomeric mixture (GS 7171), the individual diastereomers (GS 7339 and GS 7340), the diastereomeric mixture of D-alaninyl analog (GS 7485), and the alaninyl monoamidate metabolite (GS 7160) are shown in Table 1. Compared to tenofovir, the individual isomers, GS 7339 and GS 7340, were 83- and 1,000-fold more active, respectively, whereas the D-isopropyl alaninyl analog (GS 7485) and the metabolite (GS 7160) showed activity similar to tenofovir. The enhanced activities of the L-alaninyl prodrugs compared to those of tenofovir are a result of greater

cellular permeability and rapid conversion to tenofovir inside the MT-2 cells. The dramatically reduced activity (1,000 fold) of the D-alaninyl analog (GS 7485), relative to the L-alaninyl analog (GS 7171) demonstrates a strong metabolic preference inside the MT-2 cells for the L-amino acid (see below). The 12-fold-greater activity of GS 7340 compared to that of GS 7339 further suggests that intracellular metabolism is also sensitive to stereochemistry at the phosphorus. The greater selectivity index (\sim 10 \times) for GS 7340 than tenofovir DF may reflect the kinetics of cell loading; GS 7340 results in a higher initial intracellular concentration of tenofovir, which may differentially affect antiviral potency and cytotoxicity.

In vitro metabolism and accumulation in PBMCs. The antiviral activities in tissue culture were determined in buffer containing 5% heat-inactivated fetal calf serum, a medium in which the prodrugs in this study were stable. To enhance the in vivo accumulation of tenofovir inside cells, a prodrug must exhibit greater stability in plasma relative to the intracellular environment. Table 1 lists the in vitro half-lives of the monoamidate prodrugs and tenofovir DF in MT-2 cell extract and human plasma. GS 7340 was metabolized 3-fold faster in MT-2 cell extract than in plasma, whereas tenofovir DF, a prodrug designed to release tenofovir into systemic circulation, was metabolized 170-fold faster in plasma. As inferred from the antiviral activity, GS 7339 and the D-amino acid analogs (GS 7485) are more stable in MT-2 cell extract than plasma.

The putative first step in the conversion of the GS 7340 to tenofovir is the hydrolysis of the amino acid ester, which is sensitive to stereochemistry at both the amino acid and phosphorus. The metabolites observed from the degradation of GS 7340 were identified as the alaninyl amidate (GS 7160) in MT-2 cell extract and the isopropyl alaninyl amidate (GS 7161) in plasma (Fig. 2) by coinjection of authentic samples prepared independently. In the MT-2 cell extract, the alaninyl amidate metabolite was slowly hydrolyzed to tenofovir. No evidence of further conversion to the mono- or diphosphate metabolites was observed in the MT-2 cell extracts.

The metabolism of GS 7340 was further explored by incubating [¹⁴C]GS 7340 in fresh whole human blood. After a 1-h incubation at 37°C, plasma, PBMCs, and RBCs were separated and total radioactivity was analyzed by HPLC with radiometric detection. The results are shown in Table 2, along with those of tenofovir and tenofovir DF. The total recovered radioactivity in PBMCs after a 1-h incubation with GS 7340 was 9.3-fold and 38-fold higher than with tenofovir DF or tenofovir, respec-

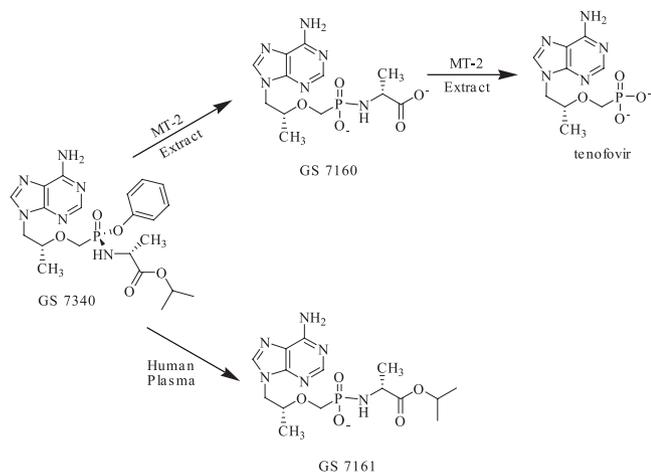


FIG. 2. Routes of metabolism.

tively. The greater radioactivity in RBCs after incubation with GS 7340 or tenofovir DF than with tenofovir can be accounted for by the decreased permeability of the latter across RBC membrane. The HPLC radiochromatograms depicting the product distribution in PBMCs, plasma, and RBCs after incubation of GS 7340 in whole blood are shown in Fig. 3. In plasma, the major species present was intact GS 7340 (84%), with minor amounts of GS 7161 (13%), GS 7160 (2%), and tenofovir (1%). In RBCs, the major species observed was intact GS 7340 (57%). In PBMCs, there was no intact GS 7340 detected, and the major species present were tenofovir (45%), tenofovir monophosphate (16%), tenofovir diphosphate (21%), and GS 7160 (18%). The phosphorylation of tenofovir in PMBCs to the mono- and diphosphate species after 1-h incubation in whole blood was not saturable over a 30-fold concentration range (Fig. 4). Additionally, in MT-2 cells incubated with 10 μ M GS 7340, the formation of the diphosphate metabolite was linear out to 24 h (Fig. 5.) The concentration of the diphosphate species inside the MT-2 cells at 24 h exceeded the initial extracellular concentration of GS 7340 by 250 fold. These results are consistent with the data generated in MT-2 extract and isolated plasma; however, unlike the MT-2 extract, conversion to the active diphosphate metabolite was readily observed with intact MT-2 cells and PBMCs.

The whole-blood experiments were extended to determine the distribution of tenofovir into CD4⁺ T lymphocyte and monocyte fractions isolated from the PBMCs, following incubation with radiolabeled GS 7340. The results in Table 3 show

TABLE 2. Distribution of all tenofovir-related species after incubation of ¹⁴C-labeled drug in human whole blood^a

Drug	Total amt recovered ^b (μ g-eq)		
	Plasma	PBMC	RBC
GS 7340	43.0 \pm 7.8	1.25 \pm 0.22	12.6 \pm 3.02
Tenofovir DF	48.10 \pm 8.7	0.133 \pm 0.029	10.5 \pm 5.17
Tenofovir	55.70 \pm 4.5	0.033 \pm 0.014	3.72 \pm 1.86

^a A total of 10 μ g-eq/ml of ¹⁴C-labeled compound was incubated for 1 h at 37°C in human blood, followed by Ficoll-Paque separation of the blood fractions.
^b Mean \pm SD, n = 3.

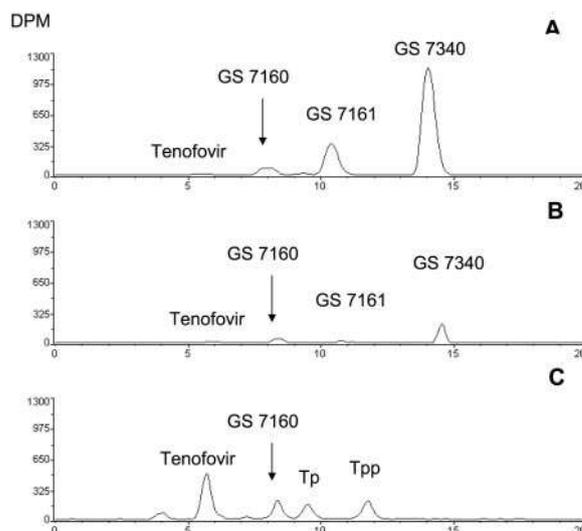


FIG. 3. Radiochromatograms labeled with ¹⁴C from plasma (A), aggregated red blood cells (B), and PBMCs (C), obtained after incubation of 17.4 μ M GS 7340 with whole human blood for 1 h at 37°C.

that the concentration of total GS 7340 metabolites in CD4⁺ cells was approximately 70% of that in the entire PBMC fraction. The concentration of total GS 7340 metabolites in monocytes was approximately half of that in CD4⁺ cells. There are no major differences in the relative concentration of phosphorylated tenofovir species in CD4⁺ cells or monocytes.

In vivo pharmacokinetics in samples from dogs. The diastereomeric mixture, GS 7171, was administered as an oral solution in 50 mM citric acid (pH 2.3) at a dose of 10 mg-eq/kg (of tenofovir) to five male beagle dogs. Figure 6 shows the resulting plasma levels of tenofovir and the individual diastereomers (GS 7339 and GS 7340) from 0 to 24 h after administration. Elimination of both diastereomers (GS 7340 and GS 7339) was rapid relative to tenofovir in plasma. Consistent with the faster metabolism observed in vitro (Table 1), GS 7340 was cleared more quickly from plasma than GS 7339. To measure the intracellular levels of tenofovir after oral administration of GS 7171, PBMCs were isolated from dog blood samples at 0, 2, 8, and 24 h postadministration. After cells were counted, the concentration of tenofovir in the PBMCs was analyzed by a

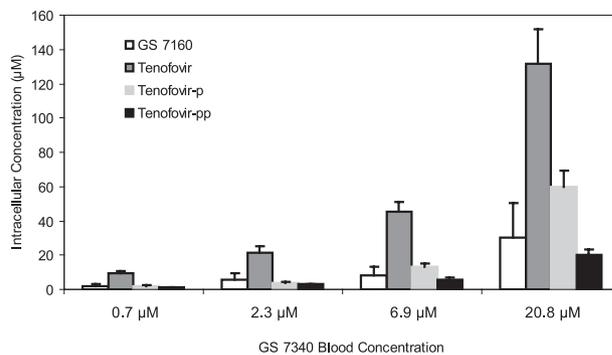


FIG. 4. Metabolism of [¹⁴C]GS 7340 in PMBCs after 1-h incubation in whole human blood at 37°C (mean \pm SD; n = 3 samples of whole blood).

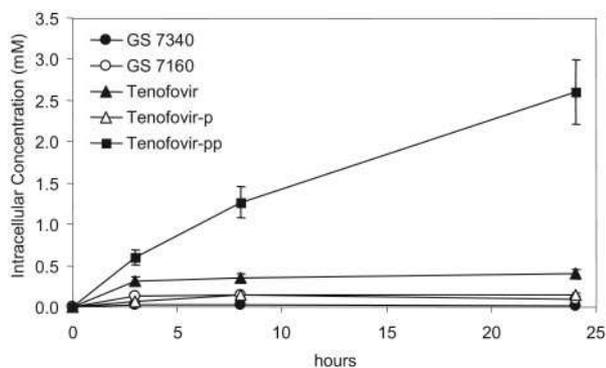


FIG. 5. Formation of tenofovir and metabolites in MT-2 cells during a 24-h incubation with 10 μ M [14 C]GS 7340 (mean \pm SD; $n = 3$ samples of MT-2 cells).

precolumn fluorescent derivatization reverse-phase HPLC method (18). Tenofovir concentration in PBMCs increased rapidly within 2 h to levels that greatly exceeded plasma levels (Fig. 6). The ratio of the total tenofovir AUC from 0 to 24 h in PBMCs to that in plasma was >90 after a single dose of GS 7171. GS 7339 and GS 7340 were also administered separately to two sets of five dogs (10 mg-eq/kg of tenofovir). A bar graph comparing the plasma and PBMC AUC₀₋₂₄ values for tenofovir after administration of GS 7340, GS 7339, GS 7171, and tenofovir DF is shown in Fig. 7. Oral administration of GS 7340, the more rapidly cleared isomer, resulted in a >34 -fold increase in the AUC₀₋₂₄ in PBMCs relative to tenofovir DF and a 6-fold increase relative to GS 7339. In the case of tenofovir DF, the AUC₀₋₂₄ ratio of tenofovir in PBMCs to plasma was 4.7. Based on these data and in vitro metabolism studies, the more rapidly cleared isomer GS 7340 was chosen as a lead prodrug for further preclinical development.

The concentration of tenofovir in PBMCs determined by the fluorescent derivatization method does not include the tenofovir mono- or diphosphate species. As part of the radiolabeled distribution study (see below), PBMCs were collected at 2, 8, and 24 h, and total radioactivity was determined. The concentration of total tenofovir metabolites at 24 h was 63 μ g-eq/ml. The calculated $t_{1/2}$ value in PBMCs was ~ 25 h, which is consistent with the intracellular half-life observed in vitro with resting lymphocytes (26).

The absolute bioavailabilities of tenofovir after oral administration of GS 7171, GS 7339, and GS 7340 were calculated by

TABLE 3. Concentration of metabolites in CD4⁺ lymphocytes and monocytes isolated from whole human blood after incubation with 17 μ M 14 C-labeled GS 7340 for 1 h

Cell type	Intracellular concn ^a (μ M)				Total concn (μ M)
	Tenofovir	Tenofovir-P	Tenofovir-PP	GS 7340	
CD4 ⁺ ^b	218 \pm 31	122 \pm 8	157 \pm 15	0	703 \pm 36
Monocytes ^b	138 \pm 55	89 \pm 8	73 \pm 15	0	364 \pm 24
PBMCs ^c	ND ^d	ND	ND	ND	921 \pm 64

^a Values are means \pm SD ($n = 2$ samples of whole blood).

^b Calculated based on cell volumes of 0.2 pl/cell for T lymphocytes and 0.4 pl/cell for monocytes (4).

^c Based on average volume of 0.2 pl/cell.

^d ND, not determined.

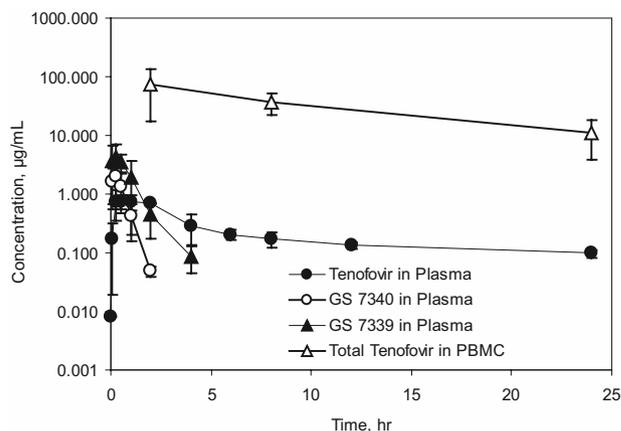


FIG. 6. Mean concentration versus time profiles for tenofovir, GS 7340, and GS 7339 in plasma and tenofovir in PBMCs after oral administration of GS 7171 (10 mg-eq/kg tenofovir) to dogs (mean \pm SD for samples from four dogs).

comparing the resulting plasma tenofovir AUC₀₋₂₄ to that observed after the i.v. administration of tenofovir itself. Data are shown in Table 4. The bioavailabilities were 20, 13, and 17%, respectively. The oral bioavailability of the prodrug (as prodrug) was $>70\%$ at the 20-mg/kg dose and was calculated by comparing the plasma levels of GS 7340 after oral administration to those after an i.v. bolus administration of GS 7340 (data not shown).

Tissue distribution of GS 7340 after oral administration in dogs. The results of a tissue distribution study in dogs following oral administration of [14 C]GS 7340 or [14 C]tenofovir DF are shown in Table 5. Two dogs per group received a single oral dose (10 mg-eq/kg; 35 μ Ci/kg), and tissues were harvested at 24 h postadministration. Except for the kidney and liver, radioactivity was generally higher in all tissues after administration of GS 7340 than with tenofovir DF. In the lymph nodes, concentrations of radioactivity were 5- to 15-fold higher after administration of GS 7340. In lung, ileum, spleen, bone marrow, and muscle, concentrations were consistently higher than with tenofovir DF. The increased concentrations of radioactivity in the bile for GS 7340 suggest that there is a hepatic

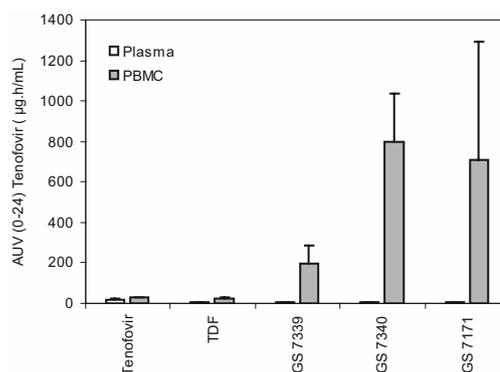


FIG. 7. Tenofovir in PBMCs and plasma (AUC_{0-24 h}) after subcutaneous administration of tenofovir and after oral administration of tenofovir DF, GS 7339, GS 7340, and GS 7171 (10 mg-eq/kg) to dogs (mean \pm SD, $n = 5$ dogs).

TABLE 4. Pharmacokinetics of tenofovir in plasma after oral administration of GS 7340, GS 7339, and GS 7171 in fasted dogs^a

Drug (route)	AUC ₀₋₂₄ (μg · h/ml)	C _{max} (μg/ml)	T _{max} (h)	t _{1/2} (h)	%F ^d
GS 7340 (p.o.) ^b	7.88 (2.24)	0.82 (0.17)	1.0 (0.0)	23.2 (12.9)	16.9 (4.8)
GS 7339 (p.o.) ^b	6.14 (2.06)	0.94 (0.21)	1.25 (0.5)	10.4 (2.2)	13.1 (4.4)
GS 7171 (p.o.) ^c	8.17 (1.2)	0.91 (0.21)	0.94 (0.79)	20 (4)	19.7 (2.8)

^a Data are means ± SD for groups of five dogs. p.o., oral.

^b Dose as tenofovir (mg-eq/kg) = 10.85.

^c Dose as tenofovir (mg-eq/kg) = 9.64.

^d %F calculated from AUC_{0-∞} = 4.30 μg-h/ml for a 1-mg/kg dose of intravenous tenofovir.

clearance mechanism for either the intact prodrug or the metabolites.

DISCUSSION

Although there is still much debate regarding the ultimate reservoir for HIV, the evidence for ongoing HIV replication during HAART in patients who have undetectable viremia is compelling. Using an ultrasensitive viral RNA assay, Ramratnam et al. have shown that low-level viral bursts can be detected in samples from stable, long-term patients on HAART (22). The number of bursts observed over a 6-month period was directly correlated to the terminal viral elimination half-life from plasma. These data suggest that the long apparent half-lives (>44 mo) reported for “latent cells” (10) may be an overestimation of the true latency half-life, since new latent cells are being formed as a result of low-level virus replication. More-potent HAART therapies or therapies that better target the sites of ongoing HIV replication are required to completely halt virus replication. Currently, the intrinsic potencies of potential drugs are optimized against HIV in tissue culture systems with PBMCs or immortalized cell lines to replicate the virus. These assays are very useful for optimizing chemical structure during the initial phases of drug design; however, they do not provide any guidance into the pharmacokinetics, intracellular half-life, or distribution of a drug in vivo. Intracellular half-life and distribution are particularly important because a significant fraction of memory CD4⁺ T cells exists outside of lymphoid tissue (24). Additionally, suboptimal drug concentrations in replication competent compartments will select and amplify resistant virus (14, 25).

Tenofovir belongs to a class of nucleotide analogs that have prolonged intracellular half-lives (20). The long intracellular half-life of tenofovir is a result of rapid metabolism within the cell to the nucleotide diphosphate and its limited efflux from cells. The phosphonate moiety of tenofovir is recognized by cellular kinases as a monophosphate, thereby bypassing the initial phosphorylation step, which can be rate limiting for nucleoside analogs (25). Because tenofovir is a dianion at physiological pH, it has low cellular permeability, which is reflected in its in vitro HIV-activity (EC₅₀ = 5 μM). However, in vivo, the long intracellular half-life of tenofovir results in a potent and durable anti-HIV effect in both preclinical models and clinical studies (30). The amidate prodrug GS 7340 was designed to overcome the permeability limitations of tenofovir by masking the dianion with a neutral promoiety and increasing the plasma stability of the prodrug relative to its intracellular stability. The in vitro anti-HIV potency of GS 7340 (EC₅₀ = 0.005 μM) is comparable to the most potent protease inhibi-

tors and reflects the greater cell permeation of GS 7340 compared to tenofovir. In addition to favorable potency and a long intracellular half-life, the in vivo administration of GS 7340 results in an enhanced distribution to lymphatic tissue compared to tenofovir DF. The concentration of tenofovir inside PBMCs at 24 h was >100 fold the concentration of tenofovir in plasma at the same time point, and the ratio of AUC₀₋₂₄ for tenofovir in PBMCs versus plasma was >150 (Fig. 7). With tenofovir DF, the PBMC/plasma ratio for AUC_{0-24 h} was 5. Since the HPLC assay only measured tenofovir inside PBMCs and not the mono- or diphosphate species, these differences should be considered the minimum ratios. Using total reactivity at 24 h to compare the PBMC-to-plasma concentration, the ratio for GS 7340 was 316, suggesting that the majority of the tenofovir species inside cells exists as mono- or diphosphate.

In our drug optimization studies, we used PBMCs as a surrogate marker for distribution to lymphatic tissues, since the exchange of lymphocytes between blood and lymph is rapid and only a small percentage of the total lymphocytes reside in the blood compartment. The increased concentration of radioactivity in the lymph nodes, ileum, lung, bone marrow, and thymus after administration of GS 7340 relative to tenofovir DF suggests that the amidate is preferentially metabolized to tenofovir and accumulates in tissues that contain high concentrations of lymphatic cells. Although we do not have detailed cellular distribution data for these tissues, the fact that tenofovir is present in tissues that have all been implicated as possible HIV reservoirs is encouraging. It must be noted, however, that the expanded distribution and the higher intracellular levels of tenofovir after administration of GS 7340 open the possibility of safety issues not observed with tenofovir DF.

Other in vitro studies have been published using an amidate prodrug approach to mask the monophosphates of zidovudine (AZT) and stavudine (d4T). The monoamidate prodrugs of the monophosphate of AZT exhibit minimal or no enhancement in in vitro anti-HIV activity (16, 17). The modest in vitro effects seen with these AZT prodrugs have been explained in part by the lability of the AZT monophosphate bond, which can readily hydrolyze back to AZT. Selected amidate prodrugs of d4T monophosphate do show significant increases in in vitro activity relative to that of d4T itself (up to 100 fold), suggesting that the subsequent phosphorylation is rapid compared to the hydrolysis of the nucleoside phosphate bond (29). The principal advantage of this approach with nucleosides is the ability to deliver the nucleoside monophosphate into the cell, thereby removing a potential rate-limiting step in the formation of the nucleoside triphosphate. The half-life of the nucleoside in cells is unchanged.

TABLE 5. Tissue distribution of ¹⁴C-labeled tenofovir DF and ¹⁴C-labeled GS 7340 in dogs following an oral dose of tenofovir of 10 mg-eq/kg^a

Tissue or fluid	Tenofovir DF		GS 7340		Tissue concn ratio of GS 7340 to tenofovir DF
	% Dose	Concn (μg-eq/g) ^b	% Dose	Concn (μg-eq/g) ^b	
Liver	12.40	38.3 ± 14.3	16.45	52.9 ± 4.7	1.4
Kidney	4.58	87.9 ± 38.4	3.78	80.2 ± 3.4	0.9
Lungs	0.03	0.53 ± 0.06	0.34	4.34 ± 0.15	8.2
Iliac lymph nodes	<0.01	0.51 ± 0.14	0.01	5.42 ± 0.86	10.6
Axillary lymph nodes	<0.01	0.37 ± 0.25	0.01	5.54 ± 0.42	14.8
Inguinal lymph nodes	<0.01	0.28 ± 0.28	<0.01	4.12 ± 0.44	15.0
Mesenteric lymph nodes	<0.01	1.20 ± 0.64	0.04	6.88 ± 0.78	5.7
Thyroid gland	<0.01	0.30 ± 0.20	<0.01	4.78 ± 1.31	15.8
Pituitary gland	<0.01	0.23 ± 0.07	<0.01	1.80 ± 0.13	7.8
Salivary gland (left + right)	<0.01	0.45 ± 0.1	0.03	5.54 ± 0.10	12.3
Adrenal gland	<0.01	1.9 ± 0.79	<0.01	3.47 ± 0.13	1.8
Spleen	<0.01	0.63 ± 0.11	0.17	8.13 ± 0.19	12.8
Pancreas	<0.01	0.57 ± 0.62	0.01	3.51 ± 0.57	6.2
Prostate	<0.01	0.24 ± 0.03	<0.01	2.14 ± 0.38	9.1
Testes (left + right)	0.02	1.95 ± 0.84	0.02	1.99 ± 0.74	1.0
Skeletal muscle	<0.01	0.11 ± 0.00	0.01	1.12 ± 0.17	10.1
Heart	0.03	0.46 ± 0.17	0.15	1.97 ± 0.03	4.3
Femoral bone	<0.01	0.08 ± 0.03	<0.01	0.28 ± 0.05	3.5
Bone marrow	<0.01	0.2 ± 0.08	<0.01	2.05 ± 0.92	10.2
Skin	<0.01	0.13 ± 0.06	<0.01	0.95 ± 0.17	7.2
Abdominal fat	<0.01	0.16 ± 0.01	<0.01	0.90 ± 0.07	5.8
Eye (left + right)	<0.01	0.06 ± 0.03	<0.01	0.24 ± 0.00	3.7
Brain	<0.01	<LOD	<0.01	<LOD	ND
Cerebrospinal fluid	<0.01	<LOD	<0.01	<LOD	ND
Spinal cord	<0.01	<LOD	<0.01	0.04 ± 0.00	ND
Stomach	0.11	1.93 ± 1.03	0.26	2.68 ± 0.27	1.4
Jejunum	1.34	3.01 ± 1.37	0.79	4.16 ± 0.73	1.4
Duodenum	0.49	4.96 ± 0.54	0.44	8.77 ± 1.13	1.8
Ileum	0.01	0.50 ± 0.19	0.16	4.61 ± 1.91	9.2
Large intestine	1.63	2.57 ± 0.33	2.65	47.2 ± 42.2	7.9
Gall bladder	<0.01	3.58 ± 1.99	0.04	25.0 ± 4.7	7.0
Bile	<0.01	9.63 ± 9.42	0.22	40.5 ± 4.9	4.2
Feces	40.96	ND	0.19	ND	NA
Total GI tract contents	5.61	ND	21.64	ND	NA
Urine	23.72	ND	14.73	91.9 ± 34.0	NA
Plasma at 24 h	<0.01	0.20 ± 0.09	<0.01	0.20 ± 0.02	1.0
PBMCs at 24 h ^c	<0.01	ND	<0.01	63.2 ± 15.5	ND
Whole blood at 24 h	<0.01	0.85 ± 0.20	0.16	0.20 ± 0.00	0.2
Total recovery	81.10		68.96		

^a NA, not applicable; ND, not determined; <LOD, below the limit of detection (0.02 μg-eq/g).

^b Concentrations are means ± SD for groups of two dogs.

^c Calculated using typical recovery of 15 × 10⁶ cells total and mean PBMC volume of 0.2 picoliters/cell.

The detailed mechanism for the selective intracellular targeting of the amidate prodrugs is not fully understood. However, we have shown that the monoamidate prodrugs of tenofovir are more rapidly cleaved in the intracellular environment than in plasma and that the tenofovir formed within the cell is rapidly phosphorylated to give a long-lived metabolite. The selectivity exhibited between plasma and intracellular extracts is the basis for the preferential cellular loading observed in

vivo. The limited structure activity relationship presented in this study demonstrates that the intracellular degradation of the amidate prodrugs is sensitive to the stereochemistry at both the amino acid and the phosphorus, suggesting that the process is enzyme mediated. It has yet not been possible under buffer or enzymatic conditions to isolate the monophenyl monoamidate species, leading to the conclusion that this reaction is spontaneous. Work by other investigators with the monoami-

dates of nucleosides has identified a cytosolic fraction from hepatocytes thought to be responsible for cleavage of the P-N bond (27). However, in the case of GS 7160, degradation at low pH is extremely fast ($t_{1/2} < 1$ min); therefore, the spontaneous hydrolysis of the metabolite in a cellular compartment with a low pH cannot be ruled out. Investigations to characterize the specific enzymes and mechanism responsible for the conversion of the prodrug to tenofovir are in progress. The preferential distribution into PBMCs and other lymphatic tissues is likely due to the increased metabolic activity of these tissues and the long intracellular half-lives of tenofovir and its mono- and diphosphate metabolites.

In conclusion, the high concentrations of tenofovir observed in lymphatic tissues after oral administration of GS 7340 are expected to result in increased clinical potency relative to tenofovir DF and could have a profound effect on the low-level virus replication that occurs in tissues with suboptimal drug exposure during HAART. The parent drug, tenofovir, is unique in this potential, due to its long half-life as the active diphosphate in uninfected cells and the lack of significant in vivo resistance development. With GS 7340, it should be possible to reduce the total dose of tenofovir, thereby minimizing systemic exposure, while at the same time increasing antiviral activity.

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Application of the Phosphoramidate ProTide Approach to 4'-Azidouridine Confers Sub-micromolar Potency versus Hepatitis C Virus on an Inactive Nucleoside

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We report the application of our phosphoramidate ProTide technology to the ribonucleoside analogue 4'-azidouridine to generate novel antiviral agents for the inhibition of hepatitis C virus (HCV). 4'-Azidouridine did not inhibit HCV, although 4'-azidocytidine was a potent inhibitor of HCV replication under similar assay conditions. However 4'-azidouridine triphosphate was a potent inhibitor of RNA synthesis by HCV polymerase, raising the question as to whether our phosphoramidate ProTide approach could effectively deliver 4'-azidouridine monophosphate to HCV replicon cells and unleash the antiviral potential of the triphosphate. Twenty-two phosphoramidates were prepared, including variations in the aryl, ester, and amino acid regions. A number of compounds showed sub-micromolar inhibition of HCV in cell culture without detectable cytotoxicity. These results confirm that phosphoramidate ProTides can deliver monophosphates of ribonucleoside analogues and suggest a potential path to the generation of novel antiviral agents against HCV infection. The generic message is that ProTide synthesis from inactive parent nucleosides may be a warranted drug discovery strategy.

Introduction

The hepatitis C virus (HCV^a) was identified for the first time in 1989 as a single-stranded positive sense RNA virus of the Flaviviridae family.¹ According to the World Health Organization (WHO), more than 170 million people are estimated to be chronically infected by this virus, which is a major cause of severe liver disease.²

At present, treatment options comprise immunotherapy using recombinant interferon (often pegylated) in combination with ribavirin. The clinical benefit of this treatment is limited, and a vaccine has not yet been developed. The development of selective inhibitors of essential viral enzymes such as the serine protease NS3 or the RNA-dependent RNA polymerase NS5b are expected to improve the potency and tolerability of future treatment options for HCV infected patients.^{3,4}

Nucleoside analogues have already been validated as an important class of polymerase inhibitors of other viral targets, such as HCMV, HSV, HIV, and HBV.⁵ All antiviral agents acting via a nucleoside analogue mode of action need to be phosphorylated, most of them to their corresponding 5'-triphosphates, by cellular and/or viral enzymes. The nucleotide triphosphate analogues will then inhibit the requisite polymerase and/or compete with natural nucleotide triphosphates as substrates for incorporation into viral nucleic acid during viral replication.⁵

Recently, 4'-azidocytidine was discovered as a potent inhibitor of HCV replication in cell culture. The corresponding 5'-

triphosphate was described as a competitive inhibitor of cytidylate incorporation by HCV polymerase and a potent inhibitor of native, membrane-associated HCV replicase in vitro.⁶

Interestingly, the corresponding uridine analogue, 4'-azidouridine (**1**), was inactive as an inhibitor of HCV replication in the cell-based replicon system.⁷

It was hypothesized that (**1**) (**1**, Figure 1) may be a poor substrate for phosphorylation by cellular enzymes. The first phosphorylation step to produce the 5'-monophosphate has often been found to be the rate-limiting step in the pathway to intracellular nucleotide triphosphate formation, suggesting that nucleoside monophosphate analogues could be useful antiviral agents. However, as unmodified agents, nucleoside monophosphates are unstable in biological media and they also show poor membrane permeation because of the associated negative charges at physiological pH.^{8,9}

Our aryloxy phosphoramidate ProTide approach allows bypass of the initial kinase dependence by intracellular delivery of the monophosphorylated nucleoside analogue as a membrane permeable "ProTide" form.^{10,11} This technology greatly increases the lipophilicity of the nucleoside monophosphate analogue with a consequent increase of membrane permeation and intracellular availability. Previously we have demonstrated the success of our approach with the aryloxy-phosphoramidate derivatives of ddA,¹⁰ d4T,^{11,12} LCd4A,¹³ and d4A.^{10,14} These nucleotide monophosphate analogues were shown to exhibit greatly enhanced activity against HIV compared to the parent nucleoside analogues in vitro. In contrast to the parent nucleosides, full antiviral activity of the monophosphate analogues was retained in kinase-deficient cell lines, which was consistent with an efficient bypass of the first phosphorylation step in HIV infected cells. Aryloxy-phosphoramidates are considered to be efficient lipophilic prodrugs of the corresponding 5'-monophosphate species in which the two masking groups are represented by an amino acid ester and an aryl moiety. After passive diffusion

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^a Abbreviations: NS3, nonstructural protein 3; NS5B, nonstructural protein 5B; HSV, herpes simplex virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HBV, hepatitis B virus; d4A/d4T, 2',3'-dideoxy-2',3'-dideoxyadenosine/thymidine; ddA, 2',3'-dideoxyadenosine; LCd4A, (1*R*,*cis*)-4-(6-Amino-9*H*-purin-9-yl)-2-cyclopentene-1-methanol; BVdU, *E*-5-(2-bromovinyl)-2'-deoxyuridine; HCMV, human cytomegalovirus; AZU, 4'-azidouridine; NMI, *N*-methylimidazole.

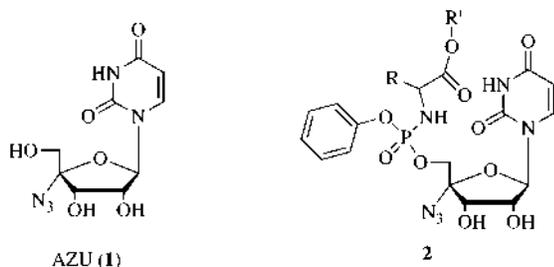


Figure 1. Structure of AZU and its corresponding phenyl-phosphoramidate ProTide.

through cell membranes, the suggested activation pathway¹⁵ involves initial enzyme-catalyzed cleavage of the carboxylic ester, followed by the internal nucleophilic attack of the acid residue on the phosphorus center, displacing the aryloxy group. The putative transient, cyclic mixed-anhydride is then rapidly hydrolyzed to the corresponding amino acid phosphomonoester. Last, a suggested phosphoramidase activity catalyzes the cleavage of the P–N bond to free the nucleoside monophosphate intracellularly. In the current study we tested the possibility to apply our ProTide approach to the inactive 4'-azidouridine (**1**) in order to achieve bypass of the first phosphorylation step and thereby generate novel antiviral agents (**2**) with potent activity against HCV.

Results and Discussion

Chemistry. The synthesis of **1** has been previously described.¹⁶ To prepare monophosphate prodrugs (**2**) of **1** we initially followed the previously described phosphorochloridate chemistry for the synthesis of ProTides developed in our laboratory, using 1-methylimidazole (NMI) as the coupling agent.^{14,17,18} Several attempts were performed using different conditions (different amino acid esters, different reaction conditions) without successful isolation of the corresponding aryloxy-phosphoramidate. These initial unsuccessful attempts might be explained considering the presence of a bulky group (azido) at the 4'-position adjacent to the coupling site at the 5'-position; in all previously published ProTide examples the 4'-position was unsubstituted.

The method of Uchiyama was investigated next.¹⁹ This approach is based on the treatment of a nucleoside with 1 equiv of a strong organometallic base, such as a solution of *tert*-butylmagnesium chloride (tBuMgCl), to form the corresponding metal alkoxide. In the case of (**1**), this reaction was observed to be very rapid and gave yields between 3% and 20% of desired products. In the first instance, we synthesized 4'-azidouridine phosphoramidates starting from an unprotected nucleoside. The apparent reactivity at the 2'- and 3'-positions was low, suggesting high regioselectivity for the reaction at the 5'-position. In this way it was also possible to synthesize compounds **13**, **21**, and **26**. In order to achieve higher solubility in the reaction solvent (tetrahydrofuran) and increase reactivity at the 5' position, the 2'- and 3'-positions of **1** were protected with a cyclopentyl group.²⁰ The final synthetic pathway (Scheme 1) involves the coupling of phenyl dichlorophosphate with different amino acid ester salts (**5**) to give the corresponding phenyloxy-phosphorochloridates (**6**), which were purified by flash chromatography and then coupled with the 2',3'-*O*,*O*-cyclopentylidene derivative **7** of (**1**) in the presence of tBuMgCl (1 M solution in THF).

The deprotection step was performed with a solution of 80% formic acid in water for 4 h at room temperature (Scheme 2). Due to the stereochemistry at the phosphorus center, the final

compounds were always isolated as mixtures of two diastereoisomers. The presence of these diastereoisomers in the final preparations was confirmed by ³¹P (two peaks), ¹H, and ¹³C NMR. A total of 22 phenyl phosphoramidates were synthesized as reported in Table 1.

We have previously reported extensive structure–activity relationship (SAR) studies of anti-HIV phosphoramidates exploring the amino acid region, including natural amino acid variation,²¹ un-natural α,α -dialkyls,²² stereochemical variation,²³ and amino acid extensions²⁴ and replacements.²⁵ In general, L-alanine and the unnatural amino acid α,α -dimethylglycine showed the best activity for the d4T parent molecule versus HIV.^{11,12}

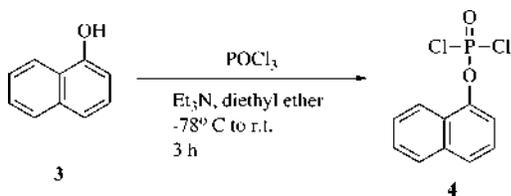
Using the previously described method (Scheme 1), we synthesized the L-alanine (**12**), α,α -dimethylglycine (**18**), cyclopentylglycine (**20**), L-phenylalanine (**22**), L-leucine (**27**), L-methionine (**29**), ethyl L-glutamate (**31**), and L-proline (**28**) phenylphosphoramidates of **1**, each bearing an ethyl ester. Further investigations on the amino acid variation were conducted on a series of benzyl esters: L-alanine (**17**), α,α -dimethylglycine (**19**), cyclopentylglycine (**21**), L-phenylalanine (**23**), L-valine (**24**), and glycine (**25**). We further compared the importance of the stereochemistry at the amino acid position by preparing a D-alanine benzyl ester phosphoramidate (**26**). On the basis of the L-alanine phenyl phosphoramidate backbone, we also explored the SAR of different esters including methyl (**11**), ethyl (**12**), butyl (**13**), 2-butyl (**14**), isopropyl (**15**), *tert*-butyl (**16**), and benzyl (**17**). In order to have an indirect proof of phenyl phosphoramidate metabolism, we synthesized the *N*-methylglycine (**30**) and β -alanine (**32**) analogues, which were considered unfavorable substrates according to the postulated mechanism of activation.¹⁵

Recently we noted an increase of *in vitro* potency of a 1-naphthyl-phosphoramidate analogue compared to the corresponding phenyl derivative while investigating the anticancer activity of BVdU phosphoramidates.²⁶ Therefore, similar phosphoramidate analogues were also generated for (**1**). The synthesis of the 1-naphthyl phosphoramidate (**33**) was performed by reacting 1-naphthol with phosphorus oxychloride in an almost quantitative reaction to give the corresponding phosphorodichloridate (Scheme 3), which was then coupled to an amino acid ester and the nucleoside analogue according to our standard procedures. In this case, the separation of the two phosphate diastereoisomers (**34** and **35**) was achieved by using a semi-preparative HPLC purification with elution conditions of 70% water/30% acetonitrile. The ³¹P NMR spectrum showed the presence of only one peak for the first of the two fractions separated, and the ¹H NMR spectrum supported the suggestion of a single diastereoisomer in this case. The second fraction contained an excess of the second diastereoisomer together with a minor proportion (estimated at 7% by ³¹P NMR integration) of the first diastereoisomer (see Supporting Information for data).

Antiviral Activity. The phenyl phosphoramidates described above (**11–32**) were characterized *in vitro* as inhibitors of HCV replication in a HCV replicon assay as previously reported.^{6,7} Data are presented in Table 1 as EC₅₀ values (representing the concentration of compounds reducing HCV replication by 50%) and CC₅₀ values (representing the concentration of compounds reducing cell viability by 50%) as determined using the WST assay. All compounds showed CC₅₀ values greater than 100 μ M. The parent compound **1** did not inhibit HCV replication significantly in the replicon system (EC₅₀ > 100 μ M). In contrast, a number of phosphoramidate derivatives showed

Table 1. Anti HCV Activity and Cytotoxicity Data for (1) and Phenyl Phosphoramidate Nucleotide Analogues

compound	amino acid	ester	EC ₅₀ (μM)	CC ₅₀ (μM)
11	L-Ala	Me	3.1	>100
12	L-Ala	Et	1.3	>100
13	L-Ala	Bu	1.2	>100
14	L-Ala	2-Bu	0.63	>100
15	L-Ala	iPr	0.77	>100
16	L-Ala	tBu	5.1	>100
17	L-Ala	Bn	0.61	>100
18	Me ₂ Gly	Et	10.3	>100
19	Me ₂ Gly	Bn	3.4	>100
20	cPntGly	Et	>100	>100
21	cPntGly	Bn	<100	>100
22	Phe	Et	1.37	>100
23	Phe	Bn	<100	>100
24	Val	Bn	<100	>100
25	Gly	Bn	1.6	>100
26	D-Ala	Bn	1.2	>100
27	Leu	Et	2.3	>100
28	Pro	Et	6.0	>100
29	Met	Et	14	>100
30	N-MeGly	Et	>100	>100
31	EtGlu	Et	>100	>100
32	β-Ala	Et	>100	>100
4'-azidouridine (1)	—	—	>100	>100

Scheme 3. Synthetic Pathway for the Synthesis of 1-naphthylphosphorodichloridate**Table 2.** Inhibition of HCV Polymerase (NS5B) Activity in Vitro

enzyme	IC ₅₀ [μM]	
	R1479-TP (4'-azido CTP)	4'-azido UTP
NS5B570n-BK	0.29 ± 0.13	0.23 ± 0.01
NS5B570-Con1	0.32 ± 0.11	0.22 ± 0.02

inhibitors in the L-alanine series, all having μM inhibition of HCV. The antiviral activity of these three phosphoramidates was exceptional if compared to the parent compound **1** (EC₅₀ > 100 μM), providing strong support for the notion of ProTide-mediated kinase bypass.

In the benzyl ester family, L-alanine (**17**) provided the most active compound with D-alanine (**26**) and glycine (**25**) being only slightly less potent. These results were striking when compared to the 60–70 fold reduction in anti-HIV potency for d4T ProTides with an L-alanine to glycine replacement and a 20–40 fold reduction for the corresponding abacavir ProTides.^{21,27} This reinforces our earlier conclusion that a separate ProTide motif optimization process is needed for each nucleoside analogue versus a given target. It may be that cell line dependent enzyme expression may determine different phosphoramidate SARs.

The presence of a methyl (D- and L-alanine, **26** and **17**) or α,α-dimethyl (**19**) enhanced the activity if compared to larger and hydrophobic amino acid side chain residues such as L-valine (**24**), L-phenylalanine (**23**), and cyclopentylglycine (**21**), which were weakly active in the replicon assay.

An unexpected correlation was found between amino acid and ester function. While the L-phenylalanine derivative was substantially inactive as a benzyl ester (**23**), the corresponding ethyl ester (**22**) showed a significantly increased antiviral

Table 3. Anti HCV Activity and Cytotoxicity Data for (1) and 1-Naphthyl Nucleotide Analogues

compound	phosphorus configuration	amino acid	ester	EC ₅₀ (μM)	CC ₅₀ (μM)
33	S/R	L-Ala	Bn	0.22	>100
34	R	L-Ala	Bn	0.39	>100
35	S	L-Ala	Bn	0.43	>100
17 (Phenyl ProTide)	S/R	L-Ala	Bn	0.61	>100
4'-azidouridine (1)				>100	>100

activity, displaying an EC₅₀ value of 3.4 μM. Therefore, matrix-based optimization of amino acid and ester functions may be preferred over stepwise approaches.

The inactivity of the β-alanine (**32**) and of the N-methyl glycine (**30**) compounds might underline the presence of an α-amino acid and a free NH as a minimum requirement in the amino acid structure to enable the metabolic activation of aryloxy-phosphoramidates. However, the proline compound (with a blocked NH) did show modest (**28**) activity, pointing to a complex amino acid SAR.

In conclusion, ester variation was widely tolerated except for the *tert*-butyl which gave a slight reduction in potency in the L-alanine series (**16**) and the benzyl in the case of the L-phenylalanine derivative (**23**). L-Alanine remained the most effective amino acid, with glycine and D-alanine showing only slightly reduced potency. Dimethylglycine, L-leucine, and L-proline also provided compounds with antiviral potencies in a low micromolar range. It therefore appears that the amino acid core could be considerably varied to give antiviral agents with potencies within a 10-fold range in replicon cells. Importantly, potency optimization requires consideration of both amino acid and ester moieties as most clearly shown for the ethyl and benzyl esters of the L-phenylalanine analogues. Moreover, quite distinct SARs emerged from this family versus HCV as compared to our prior studies in other families.

We also explored the possibility to replace the phenyl substituent on the phosphate with a more hydrophobic moiety, 1-naphthyl. Previously, we noted an increase of *in vitro* potency of 1-naphthylphosphoramidates compared to the corresponding phenyl phosphoramidates when investigating BVdU phosphoramidates in an anticancer assay.²⁶ We synthesized **33**, the 1-naphthyl analogue of **17** (L-alanine benzyl ester). As shown in Table 3, compound **33** inhibited HCV replication with an EC₅₀ of 0.22 μM, leading to a further increase in antiviral activity (>450-fold) in comparison to 4'-azidouridine (Table 3). One of the two phosphorus diastereoisomers could be purified using a C-18 reverse phase semipreparative HPLC. One of the two main fractions obtained showed only one ³¹P NMR peak. The second fraction was less pure, although the second diastereoisomer appeared as the major component of the mixture. We have previously reported a method for the prediction of the phosphorus configuration of such diastereoisomers based on a different ¹H NMR profile of the methylene protons of the benzyl ester.²⁶ Applying this concept to compounds **34** and **35**, we noticed that in one case (more polar, **35**) a clear AB-system was observed while, for the other diastereoisomer (less polar, **34**), the two protons displayed an apparent doublet. Conformational studies were performed using the Sybyl 7.0 software package. The lowest energy conformation found for each diastereoisomer is shown in Figure 2. These differences in proton profiles can be explained by the ability of one, but not the other, diastereoisomer to form π–π interactions between the naphthyl and the phenyl group of the benzyl ester resulting in a constrained conformation. This interaction can only occur with the S phosphorus configuration (**35**) with the two methylene

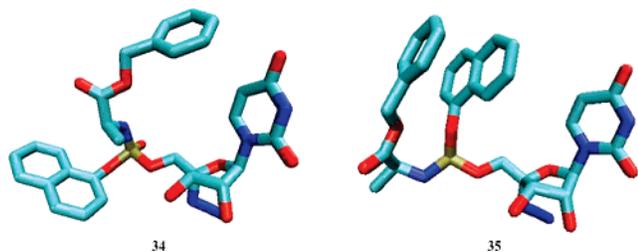


Figure 2. Lowest energy conformations of compounds **34** and **35**.

protons becoming nonmagnetically equivalent (AB system). For the diastereoisomer with *R* phosphorus configuration (**34**), this interaction does not occur and the higher degree of flexibility around the methylene renders its protons more magnetically similar (apparent doublet). The biological activities of the separated diastereoisomers (**34** and **35**) were comparable to each other and to the mixture (**33**) (Table 3).

Interestingly, application of similar ProTide methods to the active 4'-azidocytidine gave little or no boost in anti-HCV activity (data not shown), implying a rather efficient phosphorylation of this nucleoside analogue, with which ProTide methods presumably cannot compete.

Conclusion

A series of phosphoramidate ProTides of 4'-azidouridine were prepared and evaluated as inhibitors of HCV replication *in vitro*. The phosphoramidate approach provided novel compounds with highly increased potency in the replicon assay when compared to the inactive parent compound, corresponding to boosts in anti-HCV potency of >450-fold. All phosphoramidates tested were nontoxic in the replicon assay ($CC_{50} > 100 \mu\text{M}$). The most active compound prepared in the series was the 1-naphthyl *L*-alanine benzyl ester phosphoramidate with an EC_{50} of 0.22 μM in the replicon assay. The diastereoisomers of this compound were separated by HPLC and their absolute phosphorus configurations predicted by modeling and NMR. However, they did not show any differences in biological activity. This report demonstrates the ability of the ProTide approach to successfully bypass the rate limiting initial phosphorylation of a ribonucleoside analogue and thus confer significant antiviral activity on an inactive parent nucleoside.

Experimental Section

Biology. HCV replicon assay was performed in the stable replicon cell line 2209-23 derived from Huh-7 cells stably transfected with a bicistronic HCV replicon (genotype 1b) expressing the renilla luciferase reporter gene, as described.⁷ The RNA synthesis activity of recombinant HCV polymerase proteins was measured as incorporation of radiolabeled UMP into acid-insoluble RNA products using HCV genome derived cIRES RNA as a template in a primer-independent RNA synthesis assay.⁷ Recombinant proteins used were truncated at amino acid position 570 and derived from genotype 1b strain BK (NS5B570n-BK) or Con1 (NS5B570-Con1).

Chemistry. General Procedures. All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Anhydrous tetrahydrofuran and dichloromethane were purchased from Aldrich. Proton, carbon, and phosphorus Nuclear Magnetic Resonance (^1H , ^{13}C , ^{31}P NMR) spectra were recorded on a Bruker Avance spectrometer operating at 500, 125, and 202 MHz, respectively. All ^{13}C and ^{31}P spectra were recorded proton-decoupled. All NMR spectra were recorded in CD_3OD at room temperature ($20 \text{ }^\circ\text{C} \pm 3 \text{ }^\circ\text{C}$). Chemical shifts for ^1H and ^{13}C spectra are quoted in parts per million downfield from tetramethylsilane. Coupling constants are referred to as *J* values. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet

(q), broad signal (br), doublet of doublet (dd), doublet of triplet (dt), or multiplet (m). Chemical shifts for ^{31}P spectra are quoted in parts per million relative to an external phosphoric acid standard. Many proton and carbon NMR signals were split due to the presence of (phosphate) diastereoisomers in the samples. The mode of ionization for mass spectroscopy was fast atom bombardment (FAB) using MNOBA as matrix. Column chromatography refers to flash column chromatography carried out using Merck silica gel 60 (40–60 μM) as stationary phase.

For convenience, standard procedures have been given, as similar procedures were employed for reactions concerning the synthesis of precursors and derivatives of ProTides. Variations from these procedures and individual purification methods are given in the main text. Preparative and spectroscopic data on individual precursor, blocked nucleosides are given as Supporting Information only (see below), excluding only the first example.

Standard Procedure 1: Preparation of 2',3'-*O*-Cyclopentylidene-4'-azidouridine Phosphoramidates. $^i\text{BuMgCl}$ (2.0 mol equiv) and 2',3'-*O*-cyclopentylidene-4'-azidouridine (1.0 mol equiv) were dissolved in dry THF (31 mol equiv) and stirred for 15 min. Then a 1 M solution of the appropriate phosphorochloridate (2.0 mol equiv) in dry THF was added dropwise and then stirred overnight. A saturated solution of NH_4Cl was added, and the solvent was removed under reduced pressure to give a yellow solid, which was consequently purified by chromatography.

Standard Procedure 2: Deprotection of 2',3'-Protected 4'-Azidouridine Phosphoramidates. The appropriate 2',3'-*O*-cyclopentylidene-4'-azidouridine phosphoramidate was added to a solution 80% of formic acid in water. The reaction was stirred at room temperature for 4 h. The solvent was removed under reduced pressure, and the obtained oil was purified by chromatography.

Standard Procedure 3: Preparation of 4'-Azidouridine Phosphoramidates via Free Nucleoside. $^i\text{BuMgCl}$ (2.0 mol equiv) and 4'-azidouridine (1.0 mol equiv) were dissolved in dry THF (31 mol equiv) and stirred for 15 min. Then a 1 M solution of the appropriate phosphorochloridate (2.0 mol equiv) in dry THF was added dropwise and then stirred overnight. A saturated solution of NH_4Cl was added, and the solvent was removed under reduced pressure to give a yellow solid, which was purified by chromatography.

HPLC Method Used for the Separation of Compound 34 and 35. Varian ProStar instrument using a Polaris C18-A 10 μ column; elution was performed using a mobile phase consisting of water/ acetonitrile 70% H_2O /30% CH_3CN , 17 min elution time with a flow of 20 mL/min. Optimal loading on column: 8 mg of phosphoramidate per run.

Synthesis of 2',3'-*O*-Cyclopentylidene-4'-azidouridine 5'-*O*-[Phenyl(methoxy-*L*-alaninyl)] Phosphate (Methyl *N*-[1-[(3*aR*,4*R*,6*R*,6*aS*)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-*d*]^{1,3}dioxol-6-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-*L*-alaninate). Prepared according to the standard procedure 1, from 2',3'-*O*-cyclopentylidene-4'-azidouridine (150 mg, 0.427 mmol), $^i\text{BuMgCl}$ (0.85 mL, 1 M solution in THF, 0.854 mmol), and phenyl(methoxy-*L*-alaninyl) phosphorochloridate (0.85 mL of solution 1 M in THF, 0.854 mmol). The crude product was purified by column chromatography, using as eluent $\text{CHCl}_3/\text{MeOH}$ (95/5). The pure product was a white solid (156 mg, 0.263 mmol, 61%). δ_{P} ($d_4\text{-CH}_3\text{OH}$): 3.14, 3.04; δ_{H} ($d_4\text{-CH}_3\text{OH}$): 7.66 (1H, t, H6-uridine), 7.35 (2H, t, 2 CH-phenyl), 7.28–7.19 (3H, m, 3 CH-phenyl), 5.97 (1H, dd, H1'-uridine), 5.70 (1H, dd, H5-uridine), 5.12–5.04 (2H, m, H2'-uridine, H3'-uridine), 4.31–4.27 (2H, m, H5'-uridine), 4.01 (1H, m, $\text{CH}\alpha$), 3.70 (3H, d, CH_3 -methyl), 2.21–2.11 (2H, m, CH_2 -cyclopentyl), 1.79–1.73 (6H, m, 3 CH_2 -cyclopentyl), 1.37 (3H, t, CH_3 -alanine, *J* = 9.5 Hz).

Synthesis of 4'-Azidouridine 5'-*O*-[Phenyl(methoxy-*L*-alaninyl)] Phosphate (Methyl *N*-[1-(2*R*,3*S*,4*R*,5*R*)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-*L*-alaninate) (11**).** Prepared according to the standard procedure 2, from 2',3'-*O*-cyclopentylidene-4'-azidouridine 5'-*O*-[phenyl(methoxy-*L*-alaninyl)] phosphate (135 mg, 0.222 mmol), and a solution 80% of HCOOH in water (10 mL). The crude was purified by column chromatography,

using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (65 mg, 0.116 mmol, 54%). δ_P (*d*₄-CH₃OH): 3.50, 3.31; δ_H (*d*₄-CH₃OH): 7.65 (1H, dd, H6-uridine), 7.38 (2H, m, 2 CH-phenyl), 7.28–7.20 (3H, m, 3 CH-phenyl), 6.15 (1H, m, H1'-uridine), 5.72 (1H, m, H5-uridine), 4.42–4.36 (2H, m, H2'-uridine, H3'-uridine), 4.26–4.18 (2H, m, H5'-uridine), 4.00 (1H, q, CH α), 3.70 (3H, d, CH₃-methyl), 1.35 (3H, dd, CH₃-alanine). MS (E/I): 549.1124 (MNa⁺); C₁₉H₂₃N₆O₁₀NaP requires 549.1111. Anal. (C₁₉H₂₃N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(ethoxy-L-alanyl)] Phosphate (Ethyl N-[[1-[(3*R*,4*R*,6*R*,6*S*)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-*d*]^{1,3}dioxol-6-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-L-alaninate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(ethoxy-L-alanyl)] Phosphate (Ethyl N-[[1-(2*R*,3*S*,4*R*,5*R*)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-L-alaninate) (12). Prepared according to the standard procedure 2, from 2',3'-O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethoxy-L-alanyl)]-phosphate (135 mg, 0.222 mmol), and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (65 mg, 0.116 mmol, 54%). δ_P (*d*₄-CH₃OH): 3.56, 3.35; δ_H (*d*₄-CH₃OH): 7.65 (1H, m, H6-uridine), 7.37 (2H, m, 2 CH-phenyl), 7.29–7.21 (3H, m, 3 CH-phenyl), 6.17 (1H, m, H1'-uridine), 5.70 (1H, m, H5-uridine), 4.41–4.35 (2H, m, H2'-uridine, H3'-uridine), 4.26–4.15 (4H, m, H5'-uridine, CH₂-ethyl), 3.97 (1H, m, CH α), 1.35 (3H, m, CH₃-ethyl), 1.26 (3H, m, CH₃-alanine). MS (E/I): 563.1267 (MNa⁺), C₂₀H₂₅N₆O₁₀NaP requires 563.1257. Anal. (C₂₀H₂₅N₆O₁₀P) C, H, N.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(butoxy-L-alanyl)] Phosphate (Butyl N-[[1-(2*R*,3*S*,4*R*,5*R*)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-L-alaninate) (13). Prepared according to the standard procedure 3, from 4'-azidouridine (300 mg, 0.986 mmol), ⁴BuMgCl (2.46 mL 1 M solution of THF, 2.465 mmol) and phenyl(butoxy-L-alanyl) phosphorochloridate (2.46 mL of solution 1 M in THF, 2.465 mmol). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (95/5), a preparative TLC using as eluent CHCl₃/MeOH (85/15). The obtained pure product was a white solid (16.8 mg, 0.030 mmol, 3%). δ_P (*d*₄-CH₃OH): 4.91, 4.35; δ_H (*d*₄-CH₃OH): 7.65 (1H, m, H6-uridine, *J* = 8.1 Hz), 7.46–7.22 (5H, m, CH-phenyl), 6.16 (1H, m, H1'-uridine, *J* = 3.6 Hz), 5.72 (1H, m, H5-uridine, *J* = 8.1 Hz), 4.39–4.27 (2H, m, H2'-uridine, H3'-uridine), 4.24–4.09 (5H, m, CH α , H5'-uridine, CH₂-butyl), 1.67–1.59 (3H, m, CH₃-alanine), 1.48–1.30 (4H, m, 2 CH₂-butyl), 0.95 (3H, m, CH₃-butyl). δ_C *dept* (*d*₄-CH₃OH): 143.60, 142.98 (1C, C6-uridine), 131.57, 131.32 (1C, CH-phenyl), 121.72, 121.65 (2C, CH-phenyl), 104.04 (1C, C5-uridine), 92.68–92.38 (1C, C1'-uridine), 74.25, 74.08 (1C, C3'-uridine), 73.95, 73.78 (1C, C2'-uridine), 71.77 (1C, CH₂-butyl), 69.30 (1C, C5'-uridine), 68.86 (1C, CH₂-butyl), 52.12, 51.90 (1C, CH- α), 32.15 (1C, CH₂-butyl), 20.92, 20.83 (1C, CH₃-lateral chain), 20.71 (1C, CH₂-butyl), 14.41 (1C, CH₃-butyl). Anal. (C₂₂H₂₉N₆O₁₀P) C, H, N.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(2-butoxy-L-alanyl)] Phosphate (2-Butyl N-[[1-(2*R*,3*S*,4*R*,5*R*)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-L-alaninate) (14). Prepared according to the standard procedure 2, from 2',3'-O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(2-butoxy-L-alanyl)] phosphate (135 mg, 0.208 mmol), and 10 mL of a solution 80% of HCOOH in water. The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (110 mg, 0.189 mmol, 94%). δ_P (*d*₄-CH₃OH): 3.59, δ_P (*d*₄-CH₃OH): 3.60, 3.40; δ_H (*d*₄-CH₃OH): 7.65 (1H, dd, H6-uridine), 7.38 (2H, br, 2 CH-phenyl), 7.28–7.22 (3H, m, 3 CH-phenyl), 6.15 (1H, dd, H1'-uridine), 5.70 (1H, m, H5-uridine), 4.80 (1H, m, CH-2-butyl), 4.38–4.34 (2H, m, H2'-

uridine, H3'-uridine), 4.22–4.15 (2H, m, H5'-uridine), 3.97 (1H, q, CH α), 1.61–1.56 (2H, m, CH₂-2-butyl), 1.36 (3H, d, CH₃-alanine, *J* = 5.4 Hz), 1.22 (3H, t, CH₃-2-butyl, *J* = 2.1 Hz), 0.92 (3H, CH₃-2-butyl, *J* = 7.3 Hz). MS (E/I) 591.1580 (MNa⁺), C₂₂H₂₉N₆O₁₀NaP requires 591.1580. Anal. (C₂₂H₂₉N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O-Cyclopentylidene-4'-Azidouridine 5'-O-[Phenyl(isopropoxy-L-alanyl)] Phosphate (2-Propyl N-[[1-[(3*R*,4*R*,6*R*,6*S*)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-*d*]^{1,3}dioxol-6-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-L-alaninate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(isopropoxy-L-alanyl)] Phosphate (2-Propyl N-[[1-(2*R*,3*S*,4*R*,5*R*)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-L-alaninate) (15). Prepared according to the standard procedure 2, from 2',3'-O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(isopropoxy-L-alanyl)] phosphate (171 mg, 0.275 mmol), and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (144 mg, 0.260 mmol, 94%). δ_P (*d*₄-CH₃OH): 3.59, 3.38; δ_H (*d*₄-CH₃OH): 7.64 (1H, dd, H6-uridine), 7.37 (2H, d, 2 CH-phenyl), 7.28–7.22 (3H, m, 3 CH-phenyl), 6.15 (1H, dd, H1'-uridine), 5.70 (1H, dd, H5-uridine), 5.00 (1H, q, CH-isopropyl), 4.40–4.35 (2H, m, H2'-uridine, H3'-uridine), 4.24–4.18 (2H, m, H5'-uridine), 3.94 (1H, q, CH α), 1.34 (3H, dd, CH₃-alanine), 1.24 (6H, m, 2 CH₃-isopropyl). MS (E/I) 577.1427 (MNa⁺), C₂₁H₂₇N₆O₁₀NaP requires 577.1424. Anal. (C₂₁H₂₇N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(*tert*-butyloxy-L-alanyl)] Phosphate (*tert*-Butyl N-[[1-[(3*R*,4*R*,6*R*,6*S*)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-*d*]^{1,3}dioxol-6-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-L-alaninate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(*tert*-butyloxy-L-alanyl)] Phosphate (*tert*-Butyl N-[[1-(2*R*,3*S*,4*R*,5*R*)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-L-alaninate) (16). Prepared according to the standard procedure 2, from 2',3'-O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(*tert*-butyloxy-L-alanyl)] phosphate (180 mg, 0.283 mmol), and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (90 mg, 0.158 mmol, 56%). δ_P (*d*₄-CH₃OH): 3.63, 3.59; δ_H (*d*₄-CH₃OH): 7.65 (1H, m, H6-uridine), 7.37 (2H, m, 2 CH-phenyl), 7.28–7.20 (3H, m, 3 CH-phenyl), 6.14 (1H, m, H1'-uridine), 5.71 (1H, m, H5-uridine), 4.41–4.34 (2H, m, H2'-uridine, H3'-uridine), 4.24–4.19 (2H, m, H5'-uridine), 3.87–3.84 (1H, m, CH α), 1.46 (9H, s, 3 CH₃-*tert*-butyl), 1.32 (3H, d, CH₃-alanine, *J* = 7.4 Hz). MS (E/I) 591.1580 (MNa⁺), C₂₂H₂₉N₆O₁₀NaP requires 591.1586. Anal. (C₂₀H₂₅N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(benzyloxy-L-alanyl)] Phosphate (Benzyl N-[[1-[(3*R*,4*R*,6*R*,6*S*)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-*d*]^{1,3}dioxol-6-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-L-alaninate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(benzyloxy-L-alanyl)] Phosphate (Benzyl N-[[1-(2*R*,3*S*,4*R*,5*R*)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1*H*,3*H*)-dione} (Phenoxy)-phosphoryl]-L-alaninate) (17). Prepared according to the standard procedure 2, from 2',3'-O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(benzyloxy-L-alanyl)] phosphate (140 mg, 0.209 mmol), and a solution 80% of HCOOH in water (10 mL). The crude was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (70 mg, 0.116 mmol, 55%). δ_P (*d*₄-CH₃OH): 3.53, 3.28; δ_H (*d*₄-CH₃OH): 7.61 (1H, m, H6-uridine), 7.36 (6H, m, 3

CH-phenyl, 3 CH-benzyl), 7.31–7.19 (4H, m, 2 CH-phenyl, 2 CH-benzyl), 6.13 (1H, m, H1'-uridine), 5.68 (1H, m, H5-uridine), 5.15 (2H, s, CH₂-benzyl), 4.36 (2H, m, H2'-uridine, H3'-uridine), 4.21–4.14 (2H, m, H5'-uridine), 4.05 (1H, m, CH α), 1.37 (3H, m, CH₃-alanine). MS (E/I) 625.1424 (MNa⁺), C₂₅H₂₇N₆O₁₀NaP requires 625.1426. Anal. (C₂₅H₂₇N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-Azidouridine 5'-O-[Phenyl(ethoxy-dimethylglycyl)] Phosphate (Ethyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-dimethylglycinate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(ethoxy-dimethylglycyl)] Phosphate (Ethyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-dimethylglycinate) (18). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethoxy-dimethylglycyl)] phosphate (179 mg, 0.288 mmol), and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (144 mg, 0.260 mmol, 90%). δ_P (*d*₄-CH₃OH): 1.90, 1.87; δ_H (*d*₄-CH₃OH): 7.64 (1H, dd, H6-uridine), 7.39–7.35 (2H, m, 2 CH-phenyl), 7.26 (2H, d, 2 CH-phenyl), 7.20 (1H, d, CH-phenyl), 6.14 (1H, d, H1'-uridine, *J* = 2.6 Hz), 5.67 (1H, dd, H5-uridine), 4.40–4.37 (2H, m, H2'-uridine, H3'-uridine), 4.33 (2H, br, H5'-uridine), 4.23–4.15 (1H, m, CH₂-ethyl), 1.49 (6H, d, 2 CH₃-lateral chain), 1.27 (3H, t, CH₃-ethyl). MS (E/I) 577.1431 (MNa⁺), C₂₁H₂₇N₆O₁₀NaP requires 577.1424. Anal. (C₂₁H₂₇N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(benzyloxy-dimethylglycyl)] Phosphate (Benzyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-dimethylglycinate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(benzyloxy-dimethylglycyl)] Phosphate (Benzyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-dimethylglycinate) (19). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(benzyloxy-dimethylglycyl)] phosphate (148 mg, 0.217 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (110 mg, 0.250 mmol, 82%). δ_P (*d*₄-CH₃OH): 1.86, 1.83; δ_H (*d*₄-CH₃OH): 7.60 (1H, dd, H6-uridine), 7.36–7.32 (7H, m, 2 CH-phenyl, 5 CH-benzyl), 7.24–7.17 (3H, m, 3 CH-phenyl), 6.12 (1H, dd, H1'-uridine), 5.65 (1H, dd, H5-uridine), 5.15 (2H, dd, CH₂-benzyl), 4.38–4.29 (2H, m, H2'-uridine, H3'-uridine), 4.19–4.16 (2H, dd, H5'-uridine), 1.50 (6H, s, 2 CH₃-lateral chain). MS (E/I) 639.1574 (MNa⁺), C₂₆H₂₉N₆O₁₀NaP requires 639.1580. Anal. (C₂₆H₂₉N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(ethoxy-L-cyclopentylglycyl)] Phosphate (Ethyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-cyclopentylglycinate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(ethoxy-L-cyclopentylglycyl)] Phosphate (Ethyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-cyclopentylglycinate) (20). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(benzyloxy-L-cyclopentylglycyl)] phosphate (188 mg, 0.290 mmol), and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (100 mg, 0.172 mmol, 59%). δ_P (*d*₄-CH₃OH): 2.57, 2.55; δ_H (*d*₄-CH₃-

OH): 7.69 (1H, dd, H6-uridine), 7.40–7.36 (2H, m, 2 CH-phenyl), 7.27–7.20 (3H, m, 3 CH-phenyl), 6.16 (1H, d, H1'-uridine, *J* = 5.3 Hz), 5.67 (1H, m, H5-uridine), 4.40–4.33 (2H, m, H2'-uridine, H3'-uridine), 4.26–4.16 (4H, m, 2 H5'-uridine, CH₂-ethyl), 2.15–1.96 (4H, m, 2 CH₂-cyclopentyl), 1.75–1.62 (4H, m, 2 CH₂-cyclopentyl), 1.25 (3H, q, CH₃-ethyl). MS (E/I) 603.1585 (MNa⁺), C₂₃H₂₉N₆O₁₀NaP requires 603.1580. Anal. (C₂₃H₂₉N₆O₁₀P) C, H, N.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(benzyloxy-L-cyclopentylglycyl)] Phosphate (Benzyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-cyclopentylglycinate) (21). Prepared according to the standard procedure 3, from 4'-azidouridine (300 mg, 1.052 mmol), BuMgCl (2.10 mL of solution 1 M in THF, 2.10 mmol), and phenyl(benzyloxy-L-cyclopentylglycyl) phosphorochloridate (6.7) (2.10 mL of solution 1 M in THF, 2.10 mmol). The crude was purified by column chromatography, using as eluent CHCl₃/MeOH (9/1). The obtained pure product was a white solid (130 mg, 0.202 mmol, 20%). δ_P (*d*₄-CH₃OH): 3.77, 3.74; δ_H (*d*₄-CH₃OH): 7.58 (1H, m, H6-uridine, *J* = 8.13 Hz), 7.28 (7H, m, 5 CH-phenyl, 2 CH-benzyl), 7.15 (3H, m, CH-benzyl), 6.09 (1H, m, H1'-uridine), 5.55 (1H, m, H5-uridine, *J* = 8.13 Hz), 5.08 (2H, s, CH₂-phenyl), 4.29 (1H, m, H2'-uridine), 4.24 (1H, m, H3'-uridine), 4.09 (2H, m, H5'-uridine), 2.04 (2H, m, CH₂-cyclopentyl), 1.98 (2H, m, CH₂-cyclopentyl), 1.64 (2H, m, CH₂-cyclopentyl), 1.55 (2H, m, CH₂-cyclopentyl); δ_C (*d*₄-CH₃-OH): 176.83 (1C, C=O ester), 166.20 (1C, C4-uridine), 152.62, 152.48, 152.39 (1C, C2-uridine), 143.00, 142.88 (1C, C6-uridine), 137.75, 137.73 (1C, C-phenyl), 131.22 (2C, CH-phenyl), 129.98 (1C, C-benzyl), 129.73 (2C, CH-phenyl), 129.69 (1C, CH-phenyl), 126.74 (2C, CH-benzyl), 122.00 (1C, CH-benzyl), 121.98, 121.93 (2C, CH-benzyl), 103.99, 103.96 (1C, C5-uridine), 99.23, 99.20, 99.06 (1C, C4'-uridine), 92.32, 92.13 (1C, C1'-uridine), 74.86 (1C, C3'-uridine), 69.32 (1C, C2'-uridine), 69.25, 68.79, (1C, C5'-uridine), 68.75, 68.62 (2C, CH₂-benzyl), 40.35, 40.24 (1C, CH₂-cyclopentyl), 40.07, 39.55 (1C, CH₂-cyclopentyl), 25.10 (1C, CH₂-cyclopentyl), 24.99 (1C, CH₂-cyclopentyl). Anal. (C₂₈H₃₁N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(ethoxy-L-phenylalaninyl)] Phosphate (Ethyl N-[[1-[(3aR,4R,6R,6aSS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-phenylalaninate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(ethoxy-L-phenylalaninyl)] Phosphate (Ethyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-phenylalaninate) (22). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethoxy-L-phenylalaninyl)] phosphate (190 mg, 0.278 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (152 mg, 0.246 mmol, 88%). δ_P (*d*₄-CH₃OH): 3.28, 3.06; δ_H (*d*₄-CH₃OH): 7.55 (1H, dd, H6-uridine), 7.34–7.07 (10H, m, 5 CH-phenyl, 5 CH-lateral chain), 6.14 (1H, dd, H1'-uridine), 5.70 (1H, dd, H5-uridine), 4.27–4.23 (2H, m, H2'-uridine, H3'-uridine), 4.15–4.00 (3H, m, H5'-uridine, CH α), 3.81–3.78 (2H, CH₂-ethyl), 3.10 (1H, q, CH₂-lateral chain), 2.89 (1H, q, CH₂-lateral chain), 1.20 (3H, m, CH₃-ethyl). MS (E/I) 639.1594 (MNa⁺), C₂₆H₂₉N₆O₁₀NaP requires 639.1580. Anal. (C₂₆H₂₉N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(benzyloxy-L-phenylalaninyl)] Phosphate (Benzyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-phenylalaninate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(benzyloxy-L-phenylalaninyl)] Phosphate (Benzyl N-[[1-(2R,3S,4R,5R)-5-

Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl-pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-phenyl-alaninate) (23). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(benzyloxy-L-phenylalaninyl) phosphate (163 mg, 0.219 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (130 mg, 0.192 mmol, 87%). δ_P (*d*₄-CH₃OH): 3.21, 3.02; δ_H (*d*₄-CH₃OH): 7.51 (1H, dd, H6-uridine), 7.32–7.23 (6H, m, 2 CH-phenyl, 2 CH-lateral chain, 2 CH-benzyl), 7.18–7.03 (9H, m, 3 CH-phenyl, 3 CH-lateral chain, 3 CH-benzyl), 6.14 (1H, dd, H1'-uridine), 5.64 (1H, dd, H5-uridine), 5.16–5.09 (4H, m, H2'-uridine, H3'-uridine, CH₂benzyl), 4.24–4.12 (3H, m, H5'-uridine, CH α), 3.09 (1H, m, CH₂-lateral chain), 2.91–2.87 (2H, m, CH₂-lateral chain). MS (E/I) 701.1732 (MNa⁺), C₃₁H₃₁N₆O₁₀NaP requires 701.1737. Anal. (C₃₁H₃₁N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(benzyloxy-L-valinyl)] Phosphate (Benzyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-valinate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(benzyloxy-L-valinyl)] Phosphate (Benzyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-valinate) (24). Prepared according to Standard Procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(benzyloxy-L-valinyl)] phosphate (173 mg, 0.248 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (70 mg, 0.116 mmol, 55%). δ_P (*d*₄-CH₃OH): 4.45, 4.14; δ_H (*d*₄-CH₃OH): 7.62 (1H, m, H6-uridine, *J* = 6.8 Hz), 7.39–7.32 (7H, m, 4 CH-phenyl, 3 CH-benzyl), 7.24–7.18 (3H, m, CH-phenyl, 2 CH-phenyl), 6.14 (1H, m, H1'-uridine), 5.68 (1H, m, H5-uridine, *J* = 6.8 Hz), 5.19–5.10 (2H, m, CH₂-benzyl), 4.37–4.30 (2H, m, H2'-uridine, H3'-uridine), 4.22–4.14 (2H, m, H5'-uridine), 3.76 (2H, m, CH α), 2.07 (1H, m, CH-valine), 0.90 (3H, t, CH₃-valine, *J* = 8.6 Hz), 0.84 (3H, t, 3 CH₃-valine, *J* = 7.8 Hz). MS (E/I) 653.1737 (MNa⁺), C₂₇H₃₁N₆O₁₀NaP requires 653.1754. Anal. (C₂₇H₃₁N₆O₁₀NaP) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(benzyloxy-glycyl)] Phosphate (Benzyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-glycinate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(benzyloxy-glycyl)] Phosphate (Benzyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-glycinate) (25). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(benzyloxy-glycyl)] phosphate (173 mg, 0.264 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (70 mg, 0.116 mmol, 43%). δ_P (*d*₄-CH₃OH): 3.53, 3.28; δ_H (*d*₄-CH₃OH): 7.63 (1H, m, H6-uridine), 7.36 (6H, m, 3 CH-phenyl, 3 CH-benzyl), 7.34–7.19 (4H, m, 2 CH-phenyl, 2 CH-benzyl), 6.15 (1H, m, H1'-uridine), 5.69 (1H, m, H5-uridine), 5.18 (2H, s, CH₂-benzyl), 4.39–4.18 (4H, m, H2'-uridine, H3'-uridine, H5'-uridine), 3.83 (2H, d, CH₂-glycine). MS (E/I) 611.1267 (MNa⁺), C₂₄H₂₅N₆O₁₀NaP requires 611.1271. Anal. (C₂₄H₂₅N₆O₁₀P) C, H, N.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(benzyloxy-D-alanyl)] Phosphate (Benzyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-D-alaninate) (26). Prepared according to the standard procedure 3, from 4'-azidouridine (200 mg, 0.701 mmol), ¹BuMgCl (1.4 mL of solution 1 M in THF, 1.402

mmol), and phenyl(benzyloxy-D-alanyl) phosphorochloridate (2.10 mL of solution 1 M in THF, 2.10 mmol). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (9/5) and then a preparative TLC using as eluent CHCl₃/MeOH (9/1). The obtained pure product was a white solid (100 mg, 0.1723 mmol, 16%). δ_P (*d*₄-CH₃OH): 4.89, 4.29; δ_H (*d*₄-CH₃OH): 7.61 (1H, m, H6-uridine), 7.36 (7H, m, 2 CH-phenyl, 5 CH-benzyl), 7.25 (3H, m, CH-phenyl), 6.15 (1H, m, H1'-uridine), 5.68 (1H, m, H5-uridine), 5.17 (2H, s, CH₂-benzyl), 4.38 (1H, m, H3'-uridine), 4.32 (1H, m, H2'-uridine), 4.23 (2H, m, H5'-uridine), 4.05 (1H, m, CH α), 1.36 (3H, m, CH₃-alanine); δ_C (*d*₄-CH₃OH): 175.34, 175.29, 175.07, 175.01 (1C, C=O ester), 166.22 (1C, C4-uridine), 152.65, 152.56, 152.40, 152.36, 152.31, 152.27 (1C, C2-uridine), 142.94, 142.86 (1C, C6-uridine), 137.60, 137.54 (1C, C-phenyl), 131.31 (2C, CH-phenyl), 130.00 (2C, CH-phenyl), 129.79, 129.76, 129.72 (2C, CH-phenyl), 126.79 (1C, CH-phenyl), 121.83, 121.77, 121.71, 121.64 (2C, CH-phenyl), 104.03, 103.99 (1C, C5-uridine), 99.11, 98.98 (1C, C4'-uridine), 92.69, 92.43 (1C, C1'-uridine), 74.22, 74.16 (1C, C3'-uridine), 74.13, 73.93 (1C, C2'-uridine), 69.28, 69.21 (1C, CH₂-benzyl), 68.71, 68.65, 68.54, 68.48 (1C, C5'-uridine), 52.17, 51.92 (1C, CH α), 20.80, 20.70, 20.59 (1C, CH₃-lateral chain). MS (E/I) 625.1424 (MNa⁺), C₂₅H₂₇N₆O₁₀NaP requires 625.1424. Anal. (C₂₅H₂₇N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(ethyloxy-L-leucinyl)] Phosphate (Ethyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-leucinate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(ethyloxy-L-leucinyl)] Phosphate (Ethyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-leucinate) (27). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethyloxy-L-leucinyl)] phosphate (135 mg, 0.208 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (111 mg, 0.190 mmol, 91%). δ_P (*d*₄-CH₃OH): 3.83, 3.47; δ_H (*d*₄-CH₃OH): 7.64 (1H, dd, H6-uridine), 7.36 (2H, t, 2 CH-phenyl), 7.25–7.20 (3H, m, 3 CH-phenyl), 6.16 (1H, d, H1'-uridine), 5.72 (1H, dd, H5-uridine), 4.40–4.35 (2H, m, H2'-uridine, H3'-uridine), 4.22 (2H, br, H5'-uridine), 4.18–4.11 (1H, m, CH₂-ethyl), 3.90 (1H, br, CH α), 1.54 (3H, m CH-lateral chain, CH₂-lateral chain), 1.27–1.19 (3H, m, CH₃-ethyl), 0.86 (3H, t, CH₃-lateral chain), 0.80 (3H, t, CH₃-lateral chain). MS (E/I) 605.1733 (MNa⁺), C₂₃H₃₁N₆O₁₀NaP requires 605.1737. Anal. (C₂₃H₃₁N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(ethyloxy-L-prolinyl)] Phosphate (Ethyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-prolinate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(ethyloxy-L-prolinyl)] Phosphate (Ethyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-prolinate) (28). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethyloxy-L-prolinyl)] phosphate (121 mg, 0.190 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (101 mg, 0.178 mmol, 94%). δ_P (*d*₄-CH₃OH): 1.60, 1.25; δ_H (*d*₄-CH₃OH): 7.65 (1H, dd, H6-uridine), 7.39 (2H, t, 2 CH-phenyl), 7.30–7.23 (3H, m, 3 CH-phenyl), 6.12 (1H, dd, H1'-uridine), 5.71 (1H, dd, H5-uridine), 4.39–4.29 (2H, m, H2'-uridine, H3'-uridine), 4.25–4.13 (24H, m, H5'-uridine, CH₂-ethyl), 3.42 (1H, m, CH α), 2.23–2.17 (2H, m, CH₂-proline), 2.02–1.84 (4H, m 2

CH₂-proline), 1.28 (3H, m, CH₃-ethyl). MS (E/I) 589.1416 (MNa⁺), C₂₂H₂₇N₆O₁₀NaP requires 589.1424. Anal. (C₂₂H₂₇N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(ethoxy-L-methioninyl)] Phosphate (Ethyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-methioninate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(ethoxy-L-methioninyl)] Phosphate (Ethyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-methioninate) (29). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethoxy-L-methioninyl)] phosphate (187 mg, 0.280 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (133 mg, 0.116 mmol, 79%). δ_P (d₄-CH₃OH): 3.81, 3.48; δ_H (d₄-CH₃OH): 7.65 (1H, t, H6-uridine), 7.38 (2H, d, 2 CH-phenyl), 7.30–7.21 (3H, m, 3 CH-phenyl), 6.15 (1H, dd, H1'-uridine), 5.72 (1H, dd, H5-uridine), 4.41–4.35 (2H, m, H2'-uridine, H3'-uridine), 4.27–4.16 (4H, m, H5'-uridine, CH₂-ethyl), 4.08 (1H, t, CHα), 2.53 (1H, m, CH₂-lateral chain), 2.42 (1H, CH₂-lateral chain), 2.03 (3H, d, CH₃-lateral chain, *J* = 15.3 Hz), 1.88–1.84 (2H, m, CH₂-lateral chain), 1.31 (3H, m, CH₃-ethyl). MS (E/I) 563.1267 (MNa⁺) C₂₀H₂₅N₆O₁₀NaP requires 563.1257. Anal. (C₂₀H₂₅N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(ethoxy-L-N-methyl-glycinyl)] Phosphate (Ethyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-N-methyl-glycinate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(ethoxy-L-N-methyl-glycinyl)] Phosphate (Ethyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-N-methyl-glycinate) (30). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethoxy-L-N-methyl-glycinyl)] phosphate (172 mg, 0.284 mmol) and a solution 80% of HCOOH in water (10 mL). The crude was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (135 mg, 0.250 mmol, 88%). δ_P (d₄-CH₃OH): 5.12, 4.93; δ_H (d₄-CH₃OH): 7.66 (1H, dd, H6-uridine), 7.42–7.38 (2H, m, 2 CH-phenyl), 7.27–7.22 (3H, m, 3 CH-phenyl), 6.15 (1H, d, H1'-uridine, *J* = 2.1 Hz), 5.70 (1H, dd, H5-uridine), 4.42–4.29 (3H, m, H2'-uridine, H3'-uridine, H5'-uridine), 4.26–4.17 (3H, m, H5'-uridine, CH₂-ethyl), 4.00 (1H, m, CH₂α), 3.80 (1H, m, CH₂α), 2.83 (3H, d, CH₃-N), 1.28 (3H, t, CH₃-ethyl, *J* = 5.0 Hz). Anal. (C₂₀H₂₅N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(ethoxy-L-ethyl-glutamyl)] Phosphate (Ethyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-ethylglutamate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(ethoxy-L-ethyl-glutamyl)] Phosphate (Ethyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-ethylglutamate) (31). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethoxy-L-ethyl-glutamyl)] phosphate (197 mg, 0.284 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (168 mg, 0.268 mmol, 94%). δ_P (d₄-CH₃OH): 3.66, 3.39; δ_H (d₄-CH₃OH): 7.60 (1H, dd, H6-uridine), 7.35 (2H, m, 2 CH-phenyl), 7.26–7.19 (3H, m, 3 CH-phenyl), 6.12 (1H, d, H1'-uridine), 5.72 (1H, dd, H5-uridine), 4.40–4.32 (2H, m, H2'-uridine, H3'-uridine), 4.29–4.08 (6H, m, H5'-

uridine, CH₂-ethyl, CH₂-ethyl lateral chain), 4.01–3.94 (1H, m, CHα), 2.42–2.16 (2H, m, CH₂-lateral chain), 2.10–1.82 (2H, m, CH₂-lateral chain), 1.28–1.22 (6H, m, CH₃-ethyl lateral chain, CH₃-ethyl). Anal. (C₂₄H₃₁N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[Phenyl(ethoxy-L-β-alaninyl)] Phosphate (Ethyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-β-alaninate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[Phenyl(ethoxy-L-β-alaninyl)] Phosphate (Ethyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (Phenoxy)-phosphoryl]-L-β-alaninate) (32). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[phenyl(ethoxy-L-β-alaninyl)] phosphate (165 mg, 0.272 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (127 mg, 0.235 mmol, 86%). δ_P (d₄-CH₃OH): 3.33, 3.27; δ_H (d₄-CH₃OH): 7.62 (1H, dd, H6-uridine), 7.38 (2H, t, 2 CH-phenyl), 7.26–7.20 (3H, m, 3 CH-phenyl), 6.12 (1H, d, H1'-uridine, *J* = 3.2 Hz), 5.72 (1H, dd, H5-uridine), 4.39–4.34 (2H, m, H2'-uridine, H3'-uridine), 4.23–4.11 (4H, m, H5'-uridine, CH₂-ethyl), 3.27 (1H, m, CH₂α), 2.54 (1H, br, CH₂β), 1.25 (3H, m, CH₃-ethyl). MS (E/I) 563.1279 (MNa⁺), C₂₀H₂₅N₆O₁₀NaP requires 563.1267. Anal. (C₂₀H₂₅N₆O₁₀P) C, H, N.

Synthesis of 2',3'-O,O-Cyclopentylidene-4'-azidouridine 5'-O-[1-Naphthyl(benzyloxy-L-alaninyl)] Phosphate (Benzyl N-[[1-[(3aR,4R,6R,6aS)-4-Azido-tetrahydro-4-(hydroxymethyl)-2,2-cyclopentylfuro[3,4-d]^{1,3}dioxol-6-yl]pyrimidine-2,4(1H,3H)-dione} (1-naphthoxy)-phosphoryl]-L-alaninate). See Supporting Information for preparative and spectroscopic data.

Synthesis of 4'-Azidouridine 5'-O-[1-Naphthyl(benzyloxy-L-alaninyl)] Phosphate (Benzyl N-[[1-(2R,3S,4R,5R)-5-Azido-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl]pyrimidine-2,4(1H,3H)-dione} (1-Naphthoxy)-phosphoryl]-cyclopentylglycinate) (33). Prepared according to the standard procedure 2, from 2',3'-O,O-cyclopentylidene-4'-azidouridine 5'-O-[1-naphthyl(benzyloxy-L-alaninyl)] phosphate (212 mg, 0.324 mmol) and a solution 80% of HCOOH in water (10 mL). The crude product was purified by column chromatography, using as eluent CHCl₃/MeOH (8/2). The obtained pure product was a white solid (161 mg, 0.246 mmol, 76%). δ_P (d₄-CH₃OH): 3.94, 3.76; δ_H (d₄-CH₃OH): 8.18 (1H, m, CH-naphthyl), 7.90 (1H, m, CH-naphthyl), 7.72 (1H, m, H6-uridine), 7.57–7.30 (11H, m, 6 CH-naphthyl, 5 CH-phenyl), 6.11 (1H, m, H1'-uridine), 5.50 (1H, m, H5-uridine), 5.11 (2H, m, CH₂-benzyl), 4.37 (1H, m, H3'-uridine), 4.31–4.19 (3H, m, H2'-uridine, H5'-uridine), 4.11 (1H, m, CHα), 1.35 (3H, d, CH₃-alanine, *J* = 7.5 Hz). MS (E/I) 675.1571 (MNa⁺), C₂₉H₂₉N₆O₁₀P requires 675.1575.

The two diastereoisomers obtained were separated by using a semipreparative HPLC with elution conditions of 70% H₂O/30% CH₃CN, 17 min elution time. Optimal loading on column: 8 mg of phosphoramidate per run.

Less polar diastereoisomer (34): δ_P (d₄-CH₃OH): 3.96; δ_H (d₄-CH₃OH): 8.16 (1H, t, CH-naphthyl, *J* = 4.2 Hz), 7.91 (1H, t, CH-naphthyl, *J* = 5.0 Hz), 7.73 (1H, d, H6-uridine, *J* = 8.1 Hz), 7.58–7.42 (6H, m, 3 CH-naphthyl, 3 CH-phenyl), 7.32–7.28 (5H, 2 CH-naphthyl, 3 CH-phenyl), 6.12 (1H, d, H1'-uridine, *J* = 5.6 Hz), 5.50 (1H, d, H5-uridine, *J* = 8.0 Hz), 5.10 (2H, d, CH₂-benzyl), 4.36 (1H, d, H3'-uridine, *J* = 5.8 Hz), 4.27–4.21 (3H, m, H2'-uridine, H5'-uridine), 4.11 (1H, m, CHα), 1.35 (3H, d, CH₃-alanine, *J* = 7.1 Hz).

More polar diastereoisomer (35): δ_P (d₄-CH₃OH): 3.77 (major); δ_H (d₄-CH₃OH): 8.17–8.15 (1H, m, CH-naphthyl), 7.91–7.89 (1H, m, CH-naphthyl), 7.72 (1H, t, H6-uridine), 7.57–7.41 (6H, m, 3 CH-naphthyl, 3 CH-phenyl), 7.33–7.29 (5H, 2 CH-naphthyl, 3 CH-phenyl), 6.12 (1H, dd, H1'-uridine), 5.50 (1H, dd, H5-uridine), 5.13–5.05 (2H, AB system, CH₂-benzyl), 4.37 (1H,

dd, H3'-uridine), 4.29–4.21 (3H, m, H2'-uridine, H5'-uridine), 4.12–4.09 (1H, m, CH α), 1.35 (3H, m, CH $_3$ -alanine).

Acknowledgment. We thank Helen Murphy for secretarial assistance.

Supporting Information Available: Analytical data on target compounds, preparative and spectroscopic data on blocked nucleoside intermediates, and figures of HPLCs of separated diastereoisomers. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JM0613370

β -D-2'-Deoxy-2'-fluoro-2'-C-methyluridine Phosphoramidates: Potent and Selective Inhibitors of HCV RNA Replication

Poster #
P-259

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Introduction

Nearly 2% of the US population and an estimated 170 million people worldwide are HCV carriers. The current standard of care, a combination of pegylated interferon and ribavirin, has limited efficacy. Consequently, there exists significant need to develop novel direct acting antivirals as either alternative therapies or for use in combination with the standard of care. Pharmasset and Roche are currently developing R7123 (RO4048), a prodrug of β -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine, PSI-6130, for the treatment of chronic hepatitis C. PSI-6130 has been shown to be a potent and non-cytotoxic inhibitor of HCV in the subgenomic replicon assay (1), and it has been demonstrated that the triphosphate of PSI-6130 is a potent inhibitor of the HCV NS5B polymerase. Cell metabolism studies have shown that PSI-6130 is converted to its uridine metabolite (PSI-6206) via cytidine deaminase (2). It has also been demonstrated that PSI-6206 is not an inhibitor of HCV in the replicon assay and is not metabolized to its monophosphate derivative, however, its triphosphate is a potent inhibitor of the HCV NS5B polymerase. Further metabolism studies have shown that the monophosphate of PSI-6130 is partially metabolized to the uridine monophosphate and that this PSI-6206 monophosphate can be converted to the triphosphate derivative via YMPK and NDPK (Figure 1). To investigate the potential for utilizing PSI-6206 as an inhibitor of HCV replication required that we bypass the first phosphorylation step. This was accomplished by the preparation of phosphoramidate derivatives at the 5'-position (3). Such a strategy has produced potent and safe inhibitors of HCV replication.

Figure 1: PSI-6130 and PSI-6206

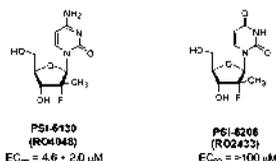
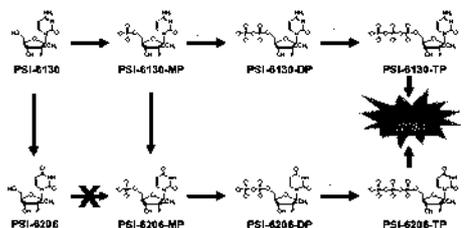
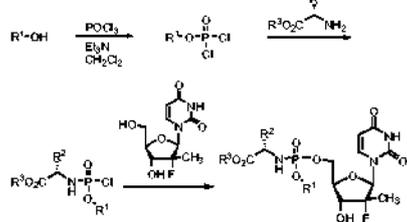


Figure 2: Proposed Intracellular Mode of Action of PSI-6130

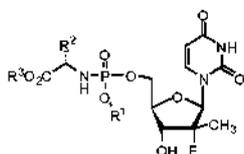


Methods & Results

Scheme 1: Preparation of Phosphoramidates



PSI-6206 Phosphoramidate



SAR Results

Table 1: Amino Acid Side Chain (R²) SAR

Cmpd No.	EC ₅₀ CloneA Cells (μM)
PSI-7672	0.90
PSI-7673	>50
PSI-7823	19.0
PSI-7834	80.1
PSI-7894	>50

Table 2: Phosphorus Ester (R¹) SAR

Cmpd No.	EC ₅₀ CloneA Cells (μM)
PSI-7672	0.90
PSI-7694	2.11
PSI-7831	0.69
PSI-7832	0.09
PSI-7840	0.69
PSI-7847	0.58
PSI-7848	0.45

Table 3: Amino Acid Ester (R³) SAR

Cmpd No.	EC ₅₀ CloneA Cells (μM)
PSI-7672	0.90
PSI-7618	0.98
PSI-7838	0.09
PSI-7839	0.13
PSI-7851	0.52
PSI-7849	0.08

Table 4: Base Modifications

Cmpd No.	Base	EC ₅₀ CloneA Cells (μM)
PSI-7672	Uracil	0.90
PSI-7693	Cytosine	14.55

HCV replicon containing cells were seeded in 96-well plates and test compounds added immediately. After incubating for 4 days, total cellular RNA was extracted and HCV replicon RNA levels were quantized by qRT-PCR.

Table 5: Cytotoxicity [CC₅₀ (μM)] Evaluated Against Several Cell Lines

Cmpd No.	EC ₅₀ CloneA Cells (μM)	Huh7	HepG2	BaPC3	GEM
PSI-6130	>100	>100	>100	>100	>100
PSI-7672	0.90	>100	>100	>100	>100
PSI-7631	0.09	50	>100	>100	>100
PSI-7635	0.09	>100	>100	>100	>100
PSI-7650	0.13	30	>100	75	50

Cytotoxicity in Huh7, HepG2, BaPC3 and GEM cells were determined after 5 days by determining the absorbance after addition of MTS dye.

Table 6: Compound Stability in Simulated Gastric Fluid (SGF), Simulated Intestinal Fluid (SIF) and Liver S9 Fraction

Cmpd No.	EC ₅₀ CloneA Cells (μM)	SGF T ₅₀ (h)	SIF T ₅₀ (h)	Liver S9 T ₅₀ (min)
PSI-7672	0.90	15.5	>20	14
PSI-7831	0.69	15.5	13	19
PSI-7838	0.09	19	>20	4.8
PSI-7839	0.13	21.75	>20	8.3

Simulated gastric fluid (pH = 1.3, without pepsin) and simulated intestinal fluid (pH = 7.5, without pancreatin) were obtained from Fisher Scientific. Compound was dissolved in acetonitrile and diluted to 50 μg/ml in 2ml of SGF or SIF and incubated at 37 °C over 24 hours. Samples were analyzed by LC/MS/MS. The % remaining is calculated based on peak area in three replicate analyses.

Conclusions

1. 5'-Phosphoramidate derivatives of PSI-6206 are potent inhibitors of HCV in the subgenomic replicon assay.
2. Selected phosphoramidates of PSI-6206 are as much as 150X more potent than the cytidine analog PSI-6130.
3. Selected PSI-6206 phosphoramidate derivatives show no cytotoxicity across several different cell lines.
4. PSI-6206 phosphoramidates show good stability under simulated gastrointestinal conditions and have the potential to be rapidly released in the target organ (the liver).
5. Good SAR was demonstrated for PSI-6206 phosphoramidates with a 100X range in potency.
6. Several PSI-6206 phosphoramidates have drug-like stability profiles that are attractive for further development.
7. 2'-Deoxy-2'-fluoro-2'-C-methyluridine phosphoramidates have emerged as promising agents for the treatment of HCV infection.
8. Conducting *in vivo* studies to select a candidate for clinical development.

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2. See Poster P-262
3. McGuffee, C., *et al*, *J. Med. Chem.*, 1995, 39, 1740-1755.

Acknowledgment

We thank Wuxi PharmaTech for assistance in preparation of the compounds in this study.

Before the Controller of Patents, New Delhi

**In the matter of pre-grant opposition under Section 25(1) of the Patents Act, 1970 and
Patents Rules, 2003**

AND

**In the matter of Patent Application No. 3658/KOLNP/2009 (“Present Application”)
filed on October 20, 2009 in the Gilead Pharmasset LLC,
titled “Nucleoside Phosphoramidate Prodrugs”**

AND

In the matter of representation

Vector BioSciences Pvt. Ltd.

...Opponent

Versus

Gilead Pharmasset LLC

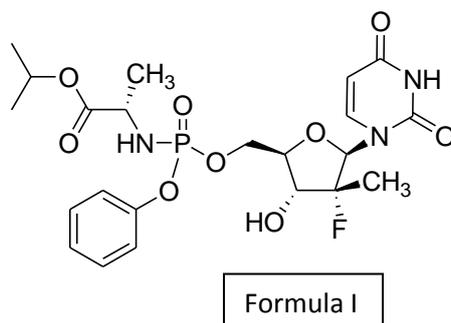
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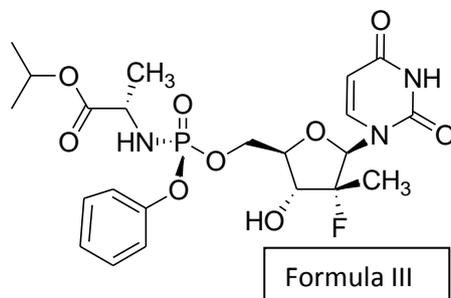
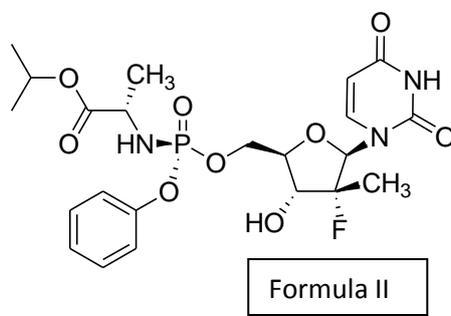
I, Sivakumar Sangarappan, aged about 42 years and residing at F-31, Brindavan Enclave, Risala Bazaar, Bolarum, Secunderabad, Telangana, India- 500 010, do hereby solemnly affirm and state as follows:

1. That, I am the Director at Vector BioSciences Pvt. Ltd. located at Hyderabad. I hold a Masters Degree and a Ph.D in Chemistry from the University of Hyderabad.
2. That, I have been working in the field of pharmaceuticals, drug discovery, particularly development of nucleoside and nucleotide analogues for the at least 10 years.
3. My curriculum vitae is annexed hereto and marked as **Annexure A**.
4. That, the Opponent is filing a pre-grant opposition against Indian Patent Application No. 3658/KOLNP/2009 (“Present Application”). The relevant documents related to opposition to the Present Application and the prior art relied upon by the Opponent in its representation has been made available to me.
5. That, I have been asked to review the complete specification of the Present Application, prior art documents on which the Opponent relies on in the opposition and the opposition made by the Opponent in the Present Application, and opine whether the claims of the Present Application are obvious to a person having ordinary skill in the art (PHOSITA) and based on the prior art documents relied upon by the Opponent whether the Present Application is liable to rejected a grant of patent.
6. That, I have been advised that the earliest priority date of March 30, 2007 from US Provisional Application No. 60/909, 315 (hereinafter referred to as “US ’315”) has been incorrectly claimed by the Applicant. Therefore, the Opponent has also relied on prior art documents dated after March 30, 2007.
7. That, the invention, as alleged in the Present Application relates to a compound of the formula (S)-2-[(2R, 3R, 4R, 5R)-5-(2, 4-Dioxo-3, 4-dihydro-2H-pyrimidin-1-yl)-4-

fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-ylmethoxy] phenoxy-phosphorylamino}-propionic acid isopropyl ester having the following structure



8. That, the Present Application also claims two other compounds that are the stereoisomers of the above disclosed compound with the chiral atom being the Phosphorus atom. The structures of these two stereoisomers are disclosed below:



9. That the Present Application also claims a method for preparing the compounds of Formula I, Formula II and Formula III.

Sufficiency of disclosure and activity of compounds

10. That on review of the complete specification of the Present Application, I am of the opinion that even though there is a general method of preparation disclosed on page 675, it is not sufficient to obtain the compounds of Formula I, Formula II and Formula III. Therefore in my opinion the PHOSITA is not given sufficient description to work the alleged invention of the Present Application.

11. That the compound of Formula I contains 6 steric centres giving the possibility of multiple stereoisomers (64 isomers) with a specific configuration. The complete specification has not given any particular process for the preparation of stereoisomers of Formula II or Formula III. Even if one is to refer to example 81 (see page 694 of the complete specification). It only identifies a slow moving isomer and a fast moving isomer and does not specify (R) and (S) nomenclature of these fast moving and slow moving isomer.
12. That the complete specification does not indicate whether the compounds of Formula II and Formula III show any activity or whether there is any advantage in producing the compounds of Formula II and Formula III at an industrial level. The complete specification does not give any reason for preference of and claiming the compounds of Formula II and Formula III. Therefore it is my opinion the complete specification doesn't make out a case for the industrial applicability of Formula II or Formula III for them to be granted a patent.

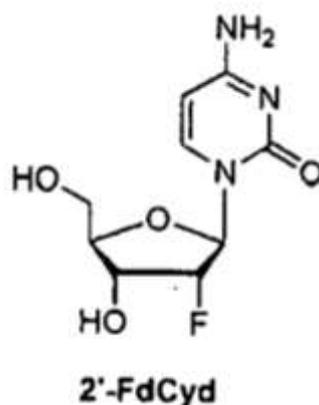
Obviousness of the claims

13. I am advised that the settled position in law that obviousness as to be determined based on the state of the art at the time of the alleged invention. The key question is whether at the time of the alleged invention, considering the state of the art and the inventive concept in the alleged invention, the PHOSITA would have considered the invention obvious.

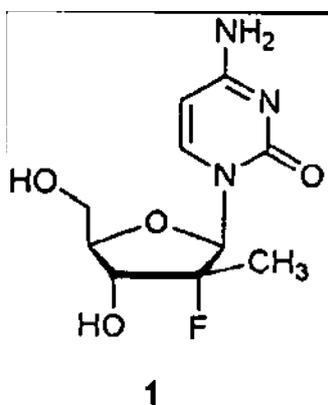
Clark *et al*

14. That the Opponent has rightly relied on a publication by **Clark *et al*** titled "*Design Synthesis, and Antiviral Activity of 2'-Deoxy-2'-fluoro-2'-C-methyleytidine, a Potent Inhibitor of Hepatitis C Virus Replication*", J. Med. Chem. 2005 48, pp. 5504-5508 (hereinafter referred as "Clark *et al*").
15. That Clark *et al* points out that despite potent HCV inhibition shown by 2'-deoxy-2'-fluorocytidine (2'-FdCyd), its therapeutic potential as an antiviral agent is diminished

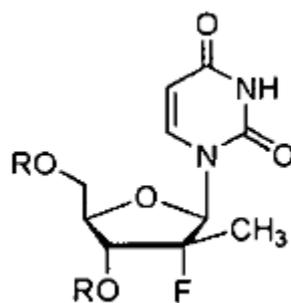
due to lack of selectivity between the cells and the target agents. The structure of 2'-FdCyd nucleoside is reproduced below for easy reference :



16. That given the lack of selectivity shown by 2'-FdCyd, Clark *et al* synthesised a novel nucleoside analogue - 2'-fluoro-2'-C-methylcytidine (**compound 1**) which demonstrated similar potency as 2'-FdCyd in the HCV replicon assay. Structure of compound is reproduced below for reference



17. That Clark *et al* teaches that “the degradation of enzymes cytidine deaminase (CDA) and deoxycytidine monophosphate deaminase (dCMP-DA) are responsible for *in vivo* metabolic conversion of cytidine or cytidine monophosphate to uridine.” (see internal page 5506, LHS column, para 2, lines 4-11). Clark *et al* tested the *in vitro* derivative of compound 1, viz. 2'-deoxy-2'-fluoro-2'-C-methyluridine (compound 9) (structure of which is reproduced below for reference)-



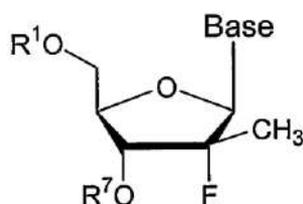
Compound 9

18. That Clark *et al* indicated that compound 9 demonstrated no activity or cytotoxicity in any assay, whereas its cytidine derivative (compound 1) showed considerable anti-HCV activity. Thereby Clark *et al* teaches that a PHOSITA considering an *in vivo* study on compound 1 would also consider its *in vivo* metabolite i.e. compound 9 for the development of an anti-HCV agent.

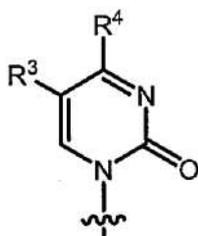
WO 2005/003147

19. That the Opponent has rightly relied on patent publication no. **WO 2005/003147** (hereinafter referred to as “WO ’147”), titled “*Modified fluorinated nucleoside analogues*” dated April 21, 2004 and bearing a priority date of May 30, 2003.

20. That WO ’147 discloses in the twelfth embodiment a compound, including its pharmaceutically acceptable salt or its prodrug of the embodiment for treating Flaviviridae virus, in particular for HCV. The twelfth embodiment is reproduced below:



Wherein the base is a structure as reproduced below:



Where R^1 , R^3 and R^7 are H and R^4 is NH_2 or OH (see internal page 39 of WO '147)

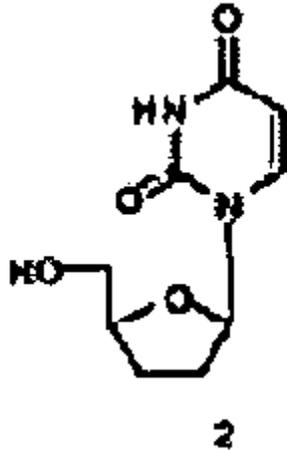
21. That WO '147 teaches a prodrug of a (2'R)-2'-deoxy-2'-fluoro-2'-C-methyl nucleoside (β -D or β -L). It also teaches that natural or synthetic D or L amino acid esters are preferred moieties at the 3' and/or the 5' positions. It particularly teaches that L-amino acid esters to be preferable at the 3' and/or 5' position.

Ma et al

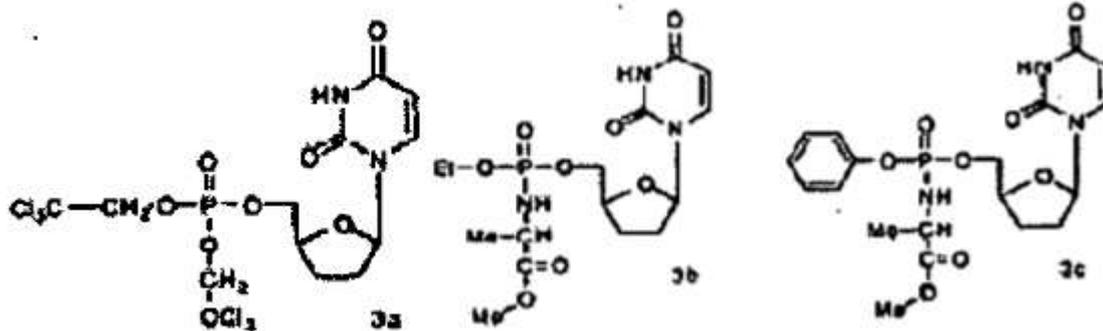
22. That the Opponent further rightly relies on a publication by *Ma et al*, titled “*Characterization of the Metabolic Activation of Hepatitis C Virus Nucleoside Inhibitor β -D-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) and Identification of a Novel Active 5'-Triphosphate Species*”, *The Journal of Biological Chemistry*, Vol. 282, No. 41, pp. 29812–29820, (hereinafter referred as “*Ma et al*”) published on October 12, 2007.
23. That *Ma et al* teaches the lack of potency of β -D-2'-deoxy-2'-fluoro-2'-C-methyluridine to be possibly related to inefficient compound phosphorylation. *Ma et al* further teaches that both β -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine-monophosphate and β -D-2'-deoxy-2'-fluoro-2'-C-methyluridine-monophosphate resulted in the complete blockage of the next nucleotide incorporation. It attributes such chain termination activity of 2-C-methyl nucleotide analogues to a steric clash of the 2-methyl group with the ribose of the next incoming nucleotide substrate.

McGuigan et al

24. That the Opponent also rightly relies on a document titled “*Certain Phosphoramidate derivatives of dideoxy uridine (ddU) are active against HIV and successfully by-pass thymidine kinase*” authored by *McGuigan et al*, *FEBS Letters* 351 (1994) 11-14 (hereinafter referred to as “*McGuigan et al*”).
25. That *McGuigan et al* tested various masked phosphates of the following compound, whose structure has been reproduced below:



26. That McGuigan *et al* teaches that of the three masked phosphates (3a, 3b and 3c reproduced below) tested for their ability to inhibit replication of HIV-1 in C8166 cells, and in the thymidine kinase deficient [JM] cells, the compound 3c showed the most activity over the parent nucleoside i.e. approximately 50-times more active than the parent nucleoside analogue.

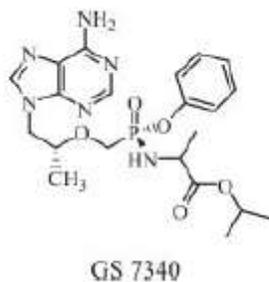


27. Therefore, McGuigan *et al* teaches that kinase by-pass by aryloxy phosphoramidates can make certain inactive nucleosides of ddU, active.

Cahard *et al*

28. That the Opponent rightly relies on a document titled “Aryloxy Phosphoramidate Triester as Pro-Tides” by Cahard *et al*, Mini-Reviews in Medicinal Chemistry, 2004, 4, pp. 371-381 (hereinafter referred as “Cahard *et al*”).

intracellular stability, and also overcame the potential rate-limiting step in the formation of nucleoside triphosphate.



34. That Lee *et al* also teaches that a non-stereospecific synthesis of GS 7340 from (R)-PMPA and (L)-isopropyl alanine ester resulting in the formation of equal amounts of two stereoisomers at phosphorus (GS 7339 and GS 7340). It further teaches that GS 7340 showed 12 fold greater activity than GS 7339 owing to the stereochemistry at phosphorus.
35. That Lee *et al* also teaches that L-alaninyl prodrug stereoisomers shows better activity as a result of greater cellular permeability, and that at least 1000 fold reduction in activity of the D-alaninyl analog relative to the L-alaninyl analog demonstrates a strong metabolic preference inside the MT-2 cells for the L-amino acid.

Perrone *et al*

36. That the Opponent rightly relies on a paper titled “*Application of the Phosphoramidate ProTide Approach to 4'-Azidouridine Confers Sub-micromolar Potency versus Hepatitis C Virus on an Inactive Nucleoside*” by Perrone *et al* (J. Med. Chem. 2007, 50, 1840-1849) (hereinafter referred as “Perrone *et al*”).
37. That Perrone *et al* teaches that isopropyl ester in phosphoramidates showed high potency and represented one of the most active phosphoramidates prepared. Further, it teaches that the 2-butyl ester, benzyl analogue and isopropyl ester phosphoramidates provided the most potent compounds of the HCV replication inhibitors in the L-alanine series when compared to the parent compound- azidouridine. This can also be seen in the table reproduced below (see internal page 1843 LHS, table 1):

Table 1. Anti HCV Activity and Cytotoxicity Data for (1) and Phenyl Phosphoramidate Nucleotide Analogues

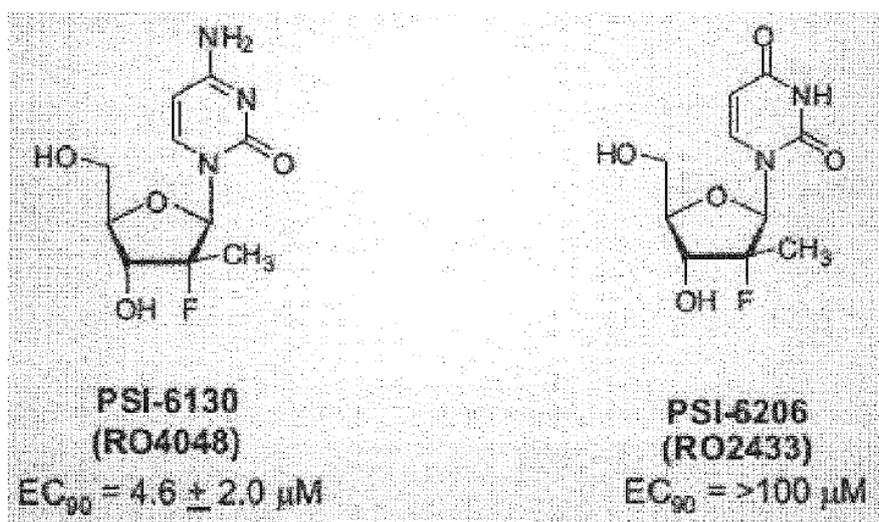
compound	amino acid	ester	EC ₅₀ (μM)	CC ₅₀ (μM)
11	L-Ala	Me	3.1	> 100
12	L-Ala	Et	1.3	> 100
13	L-Ala	Bu	1.2	> 100
14	L-Ala	2-Bu	0.63	> 100
15	L-Ala	iPr	0.77	> 100
16	L-Ala	tBu	5.1	> 100
17	L-Ala	Bn	0.61	> 100

38. That Perrone *et al* teaches that the active and non-toxic phosphoramidates indicated that ProTide approach is useful in conferring anti-viral activity on an inactive parent nucleoside.

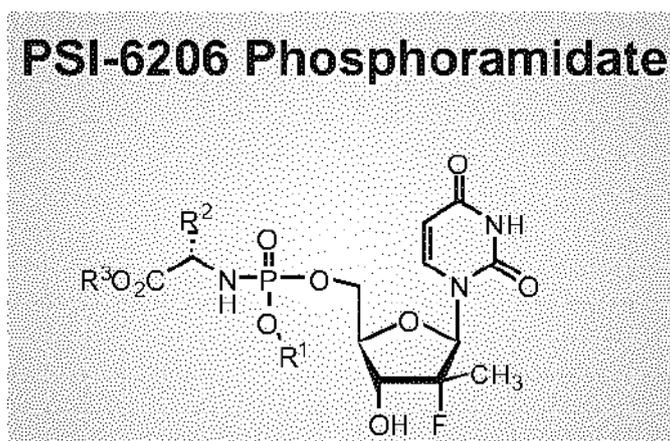
Sofia *et al*

39. That the Opponent also relies on a poster (P-259) presented at 14th International symposium on Hepatitis C virus held in Glasgow, Scotland on September 9-13, 2007 by Michael J. Sofia *et al* titled “β-D-2'-deoxy-2'-fluoro-2'-C-methyluridine Phosphoramidates: potent and Selective inhibitors of HCV RNA” (hereinafter referred as “Sofia *et al*”).

40. That Sofia *et al* discloses two compounds PSI-6206 (2'-deoxy-2'-fluoro-2'-C-methyluridine). PSI-6206 and PSI-6130 are reproduced below:



41. That Sofia *et al* further teaches that PSI-6130 is inactive *in vitro* but its triphosphate counterpart is a potent inhibitor of the HCV NS5B polymerase. It further teaches that PSI-6130 is converted into PSI-6206 *in vivo*, and that the potential of PSI-6206 as an inhibitor of the HCV replication can be investigated only by the bypass of the first phosphorylation step, using Phosphoramidate prodrugs of PSI-6206 as depicted in a markush formula below (see last box, LHS of the poster):



Where R^2 includes a variable defining the amino acid residue, R^3 includes amino acid ester radical and R^1 is the phosphate ester. Even though no particular substitutions have been identified for R^1 , R^2 , R^3 a PHOSITA could test and try rely on the prior art to find the potentially active substitutions. Sofia *et al* records EC_{90} values for the 5'-phosphoramidate derivatives of PSI-6206, which indicate that selected phosphoramidates of PSI-6206 are as much as 100x more potent than PSI-6130 and hence the phosphoramidates with amino acid esters presented attractive profiles for further development.

42. That I am advised that obviousness of an invention is determined by looking at available literature as a whole and not in isolation. Keeping the same in mind, in my opinion, a person working in the field of development of HCV medicines, i.e. a PHOSITA, would be taught by Clark *et al* and Ma *et al* that 2'-deoxy-2'-fluoro-2'-C-methyluridine has a potential in treatment of Hepatitis C Virus (HCV), and that work has to be done in the direction of overcoming the issue of lack of cell permeation of 2'-deoxy-2'-fluoro-2'-C-methyluridine. A PHOSITA would be motivated to develop a prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine for treatment of HCV, as taught in WO '147. Further, a reading of Cahard *et al* and McGuigan *et al* would teach a PHOSITA that ProTide

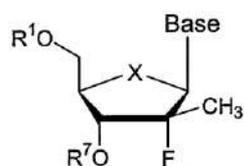
strategy can be used to overcome the problem of cell permeability. Further reading of Cahard *et al*, Lee *et al*, Perrone *et al* and Sofia *et al*, would teach that the monophosphate of the identified nucleoside does not show cell permeability and this problem may be overcome by using masked Phosphoramidate preferably containing L-alaninyl isopropyl ester. A reading of Cahard *et al* and Lee *et al* would also teach the presence of a steric centre at the phosphorus atom of the Phosphoramidate. Reading all these prior art documents together a PHOSITA would be able to arrive at a 2'-deoxy-2'-fluoro-2'-C-methyluridine prodrug which contains amino acid ester, preferably an L-alaninyl ester to overcome the issue of cell permeability of the monophosphate of the nucleoside.

43. Therefore, the claims of the Present Application would have been obvious to a PHOSITA and do not possess an inventive step.

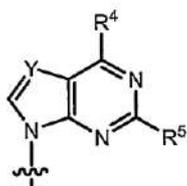
Section 3(d) and enhancement of efficacy

44. I have been advised that for the purposes of Section 3(d) of the Patents Act, salts, **esters**, ethers, polymorphs, metabolites, pure form, particle size, isomers, mixtures of isomers, complexes, combinations and other derivatives of an already known substance are considered as the same substance, unless these derivatives result in the enhancement of the known efficacy of that substance. I have also been advised that in case of pharmaceutical substances, the efficacy is to be read as therapeutic efficacy.

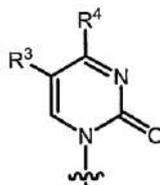
45. Accordingly, in my opinion, if one compares the claimed compounds of the Present Application with the compound disclosed in WO '147, it would be seen that the claimed compounds of the Present Application are merely an L-alanine ester derivative of the compound disclosed in the seventh embodiment of WO '147 (see internal page 36 of WO '147), reproduced below for reference:



wherein Base is selected from



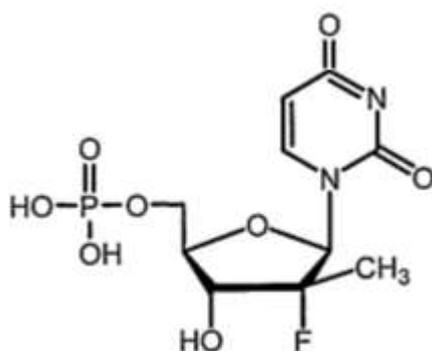
(a)



(b)

and wherein X, Y, R¹, R³, R⁴, R⁵, R⁷ and R' are as defined above

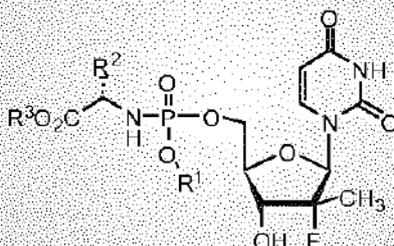
When one tries different substitutions mentioned at internal pages 31 and 34, one of the substitutions that may be considered, includes R¹ as a monophosphate, R³ and R⁷ as H and R⁴ as O. The substitution of the said elements to the general structure of the seventh embodiment results in the following compound-



46. In my opinion, the compounds of Formula I, Formula II and Formula II are mere L-alanine ester form of the seventh embodiment disclosed in the WO '147. The complete specification of the Present Application does not indicate any enhanced therapeutic efficacy of compounds of Formula I, Formula II or Formula III over the compound of seventh embodiment, and therefore are to be considered as the same substance as disclosed in WO '147.

47. Further, in my opinion, Sofia *et al* also discloses compounds which would qualify as a known compound with respect to compounds of Formula I, Formula II and Formula III of the Present Application. Attention may be drawn to the phosphoramidate nucleoside-PSI-6206 disclosed in the poster. The structure of PSI-6206 is reproduced below for easy reference-

PSI-6206 Phosphoramidate



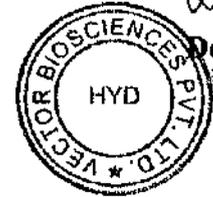
48. As Sofia *et al* clearly recorded the structure activity relationship of different amino acid ester substitutions at R³ (see Table 3 of the poster reproduced below), it is clear that efficacy of different amino acid esters of the PSI-6206 phosphoramidate was known.

Table 3: Amino Acid Ester (R³) SAR

Cmpd No.	EC ₉₀ CloneA Cells (μ M)
PSI-7672	0.90
PSI-7818	0.98
PSI-7838	0.09
PSI-7839	0.13
PSI-7851	0.52
PSI-7849	0.06

49. In my opinion, the compounds of Formula I, Formula II and Formula III are ester derivatives of PSI-6206 like those disclosed in Sofia *et al*, and the Applicant has not indicated anywhere how compounds of Formula I, Formula II or Formula III show an

enhanced therapeutic efficacy than that the known compounds disclosed in Sofia *et al.*



Deponent

VERIFICATION

I, the Deponent above named, do hereby verify that the contents of the above affidavit at paras 1- 44 are true and correct to the best of my knowledge, belief and information. No part of this Affidavit is false and nothing material has been concealed therefrom.

Verified at N.C. on this 06th day of July, 2017.

SIVAKUMAR SANGARAPPAN, Ph.D.

F-31, Brindavan Enclave,
Risala Bazaar, Bolarum
Secunderabad, Telangana
India- 500 010.
Mobile: +91-8106421818
e-mail: sangarappans@gmail.com



PERSONAL DETAILS

- Born, 22nd May 1975
- Married, 2 Children

SUMMARY OF EXPERTISE

15 years of Global Industrial, Executive, Marketing and Scientific experience in the Pharmaceutical Industry. Current interest lies in, leading a R&D team for the successful chemical development and commercialization of new and clinical API's.

Specialties:

Process R&D

Process optimisation

QBD and DOE

Technology transfer

Non infringing process

Polymorph

DMF filing

CRAMS

Business development

Analysis

Quality control

Cost control

Profile & Strengths:

- Experience in medicinal chemistry involving design and synthesis of New Chemical Entities (NCE's)
- Multi step Organic synthesis and problem solving in the area of synthesis and spectroscopy
- Proficient in Process development of active pharmaceutical ingredients for commercialization with proven expertise in enhancing the existing technology platforms (QBD and DOE) and developing new technologies and opportunities in support of the Company's business direction.
- Understanding of cGMP and ICH guideline in pharmaceutical development of manufacturing API with expertise in transfer of technology from R&D units to pilot scales and further developments to production scale.
- Coordination with cross-functional team for implementation of developed processes in commercial scale and preparation of intermediate specifications, SOPs, working standards, impurity profiling for analytical requirements & synthetic developments.
- Implementing effective cost controls on process development by fine-tuning the process and applying the recovery & reuse of solvents, capacity enhancements.
- Ability to conceptualize the processing for the product development with careful & sophisticated quality control measures in compliance to the specified standards while performing root-cause analysis to prevent any reoccurrences & defective issues.
- Excellent reporting skills, outstanding success in building and maintaining relationships with customers and colleagues. Ability to use sound decision-making skills and effectively perform in a self-directed work environment.
- Structure elucidation of compounds using spectroscopic techniques such as NMR (DEPT, NOESY & COSY), LC-MS, HRMS, GC, IR & UV.

PROFESSIONAL EXPERIENCE

Vector BioSciences Pvt. Ltd. (2007-Present)

Designation: Director

- Leading a team of scientists working on a new and clinical API development
- Coordinating with the production team for successful Tech transfer
- Closely worked with QA team for the successful audit and approval by USFDA, KFDA, PMDA and COFEPRIS
- Managing all functional teams and analytical division.
- Responsible for Business Development and also for project execution.
- Motivating the scientists and training them to handle the projects effectively.
- Planning budgets, monitoring expenses and achieving profitability.

GVK Biosciences Pvt. Ltd.

(2004-2007)

Designation: Principal Scientist

- Managed a group of 50 chemists in executing projects in lab scale synthesis and ensure accurate documentation of experiments and results.
- Worked for multinational clients for the support of their Drug Discovery Programs.
- Actively involved in successful completion of a number of medicinal chemistry related projects.
- Independently designed and synthesize novel therapeutically relevant compounds using multi-step synthesis.
- Developed novel and efficient synthetic routes for scale-up and process development.
- Managed scientific and business issues for specific clients and client needs.

Post Doctoral Experience:

Post-Doctoral Associate, Department of Chemistry, University of Kansas (2003-2004)

- Synthesis of carbohydrates and azasugar mimetics.
- Total synthesis of Taxol and Epothilone

Post-Doctoral Associate, Department of Chemistry, Penn state University (2002-2003)

- Devised an efficient solid phase synthesis of lipopeptides.
- Developed a highly fluorescence based assay for screening synthetic libraries.
- Supervised several undergraduates and graduates within the research group.

Education:

Ph. D., Chemistry, University of Hyderabad, Hyderabad, India, 2002.

Mentor: Prof. M. Periasamy

Thesis title: Synthesis, Resolution and applications of 1,2-Amino alcohols

M. Sc., Chemistry, University of Hyderabad, Hyderabad, India, 1997.

Mentor: Prof. M. Nagarajan

Project title: Application of IBX as an oxidising agent in organic synthesis

B. Sc., Gandhigram Rural University, Gandhigram, Tamilnadu, 1995.

Awards:

- Council of Scientific and Industrial Research (CSIR), New Delhi, India, **Senior Research Fellowship** (1999 - 2001).
- Council of Scientific and Industrial Research (CSIR), New Delhi, India, **Junior Research Fellowship** (1997 -1999).
- Secured 20th rank in all India Gate Exam

Selected Publications:

- A new convenient method of resolution of racemic 1,1'-bi-2-naphthol using boric acid and R- (+)- α -methylbenzylamine, M. Periasamy, L. Venkatraman, S. Sivakumar, N. S. Kumar and C. R. Ramanathan, J. Org. Chem; 1999, 64, 7643.
- New methods of resolution and purification of racemic and diastereomeric amino alcohol derivatives Using Boric Acid and Chiral 1,1'-2-bi-naphthol; M. Periasamy, N.S. Kumar, S. Sivakumar, V.D.Rao, C.R.Ramanathan and L. Venkatraman; J. Org. Chem,2001,66, 3828.
- A new convenient method of synthesis and resolution of 1,2-amino alcohols, M. Periasamy, S. Sivakumar and M. Narsireddy, Synthesis, 2003, 13, 1965.
- Toward homogeneity of chirality via selective formation of homochiral or hetero chiral aggregates, M. Periasamy, S. Sivakumar, M. Narsireddy and M. Padmaja, Org. Letters, 2004, 6, 265.
- Boraxanes: preparation and applications, M. Periasamy, S. Sivakumar, N. S. Kumar and M. Seenivasa perumal; Science of Synthesis, Houben-Weyl Methods of Molecular Transformations, Georg Thieme Verlag, Stuttgart, 2005.
- Acyloxyboranes: preparation and applications, M. Periasamy, N.S. Kumar, S. Sivakumar and M. Narsireddy; Science of Synthesis, Houben-Weyl Methods of Molecular Transformations, Georg Thieme Verlag, Stuttgart, 2005

Patents:

- Solid forms of Nucleoside phosphoramidates, WO 052995, 2016
- Nucleoside phosphoramidates piperazine cocrystal, 2016
- Improvement in the process for the synthesis of nucleoside phosphoramidates, IN201641017883
- Novel process and intermediates for the preparation of Triazole antifungal agent, 2016
- Improvement in the process for the synthesis of triazole antifungal agent, IN201641018742
- Novel process and intermediates for the synthesis of HCV inhibitor, IN201641003555

Presentations:

- Synthesis, resolution and applications of 1,2-Amino alcohols presented in OMCOS-11 international symposium held at Taipei, Taiwan, 2001.
- Solid phase synthesis of biologically active lipopeptides, American chemical society meeting held at New York, 2002.
- Invited talk given on Pharmaceutical Drug manufacturing in the Drug discovery and development workshop conducted by Hyderabad Central University, 22-28th June 2016.

Work Shops and Training Programs Attended:

- 2003: 3 day Training program on solid phase synthesis by Tom Beattie
- 2004: Project Management : One day seminar by Mr. Gopala Krishnan
- 2005: 14&15th September: Winning Edge, A Program Designed for Managers by Bala.
- 2006: 4th to 7th April: Leadership Training Program by Ahmed.
- 2007: Presentation skills by Unni.

References: Provided upon request.



తెలంగాణ తెలంగాణా TELANGANA

Sl. No. 2430 Date 9/5/17

Sold to Dr. S. Siva Kumar 20 Sangaraffan,
For Whom M.L. Vectors Biosciences Pvt Ltd, Hyd

P. Vijaya Lakshmi 120968
P. VIJAYA LAKSHMI

LICENSED STAMP VENDOR
No. 15-21-031/2011, R.No. 15-21-001/2017
Plot No. 67, APIC Colony, IDA Jeedimetla,
Medchal-Malkajgiri Dist, Cell: 9290070647

In respect of Pre-Grant opposition U/S 25(1) read with rule 55 to Indian Patent Application 3658/KOLNP/2009.

Form 26
THE PATENTS ACT, 1970
(39 of 1970)

&

The Patent Rules 2003

FORM FOR AUTHORIZATION OF A PATENT AGENT / OR ANY PERSON IN A MATTER OR PROCEEDING UNDER THE ACT
(See Sections 127 and 132 and rule 135)

We, Vector Biosciences Pvt. Ltd., having address at F-10, IDA, Gandhinagar, Hyderabad; Telangana State - 500037, India hereby authorize S. Padmaja (IN/PA 883) Indian of spiProPAT Intellectual Property Solutions, having office at 2nd Floor, Above Apollo Clinic, Suresh Square, Plot No: 1-58/91/SS, Survey No 228 & 229/1, Madinaguda, Serlingampally, Ranga Reddy District - 500 049, to act on our behalf as our

S. P.



agents for filing and representing us in respect of any opposition, whether pre-grant post grant oppositions, under the Patent Act 1970 from the Government of India in respect of Indian Patent Application No. 3658/KOLNP/2009 filed on October 20, 2009 in the name of Gilead Pharmasset LLC, titled "Nucleoside Phosphoramidate Prodrugs" and in all matters and proceedings before the Controller of Patents or the Government of India, in connection with said Opposition or Incidental thereto. Including filing of any document and payment of any fee, filing any request for amendments of any documents, filing any interlocutory petitions, filing any evidence, or any other documents in connection with said opposition, attending any discussion or interview, attending any and all official hearings in connection with said opposition(s) appointed by any authority empowered to do so and in general to do all things as may be necessary or expedient, including appointment of any substitute or substitutes. We hereby confirm and ratify any previous action of the persons authorized hereinabove in relation to this/these opposition(s), and any matters and proceedings in connection therewith and request that all notices, requisitions and communication relating thereto may be sent to such person at the address given hereunder unless otherwise specified.

spiProPAT Intellectual Property Solutions,
2nd Floor, Above Apollo Clinic,
Suresh Square, Plot No 1-58/91/SS,
Survey No 228 & 229/1, Madinaguda,
Serlingampally, Ranga Reddy District - 500 049
Email: padmaja@ipropat.com;
Tel: 040 402 401 29; 77021 21933

Date this Fifth (05th) day of July, 2017

Dr. S.

Dr. Sivakumar Sangarappan,
Director,
Vector Biosciences Pvt. Ltd.



To The Controller of Patents
The Patent Office,
Kolkata.