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30th June, 2020

The Controller of Patents
The Patent Office,
Intellectual property building,
G.S.T. Road, Guindy,
Chennai – 600032

Re: Opposition u/s 25(2) of the Patent act – By Low Cost Standard Therapeutics against Indian Patent No. 319927 (Formerly Indian Patent Application No. 1328/CHENP/2013)
Patentee: GILEAD SCIENCES INC.

Respected Sir,

We submit herewith Post-Grant Opposition under Section 25(2) of the Patent Act, 2005 along with evidence and Form 7.

We crave leave of the Controller to submit additional documents or evidence, if necessary to support any averments in the representation as may be necessitated in the proceeding.

The Controller is requested to take the documents on record and proceed further in the matter and keep the Petitioner advised of each and every step taken in the matter.

Lastly, we request the Controller to grant us an opportunity of being heard before the above Opposition is finally decided.

Thanking you,

RAJESHWARI H IN/PA - 0358
AGENT FOR THE OPPONENT
FOR RAJESHWARI AND ASSOCIATES

Encl:
1. Form 7;
2. Post grant Opposition with Annexures

BEFORE THE CONTROLLER OF PATENTS, CHENNAI

IN THE MATTER OF:

The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005, and The Patents Rules, 2003, as amended by The Patents (Amendment) Rules, 2006

AND

IN THE MATTER of Post grant opposition under Section 25(2)

AND

IN THE MATTER of Indian Patent No. 319927
[Formerly Indian Patent Application No. 1328/CHENP/2013]

IN THE MATTER OF:

Low Cost Standard Therapeutics ... Opponent

Versus

Gilead Sciences Inc. ... Patentee

POST-GRANT OPPOSITION BY MSN LABORATORIES PRIVATE LIMITED

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Dated this 30th day of June, 2020

RAJESHWARI H. IN/PA – 0358
AGENT FOR THE OPPONENT
OF RAJESHWARI AND ASSOCIATES

To
The Controller of Patents
The Patent Office, Chennai

FORM 7
THE PATENTS ACT, 1970
(39 OF 1970)
AND
THE PATENTS RULES, 2003
NOTICE OF OPPOSITION
[See Section 25(2) and rule 55A]

We, Low Cost Standard Therapeutics, hereby give Notice of opposition to the grant of patent in respect of Indian Patent No. 319927 [Formerly Indian Patent Application No. 1328/CHENP/2013] made by GILEAD SCIENCES INC. on the grounds

(a) Section 25(2) (b): Lack of inventive step by prior publication

(b) Section 25(2) (c): Lack of Novelty by prior claiming

(c) Section 25(1) (e): lack of inventive step

(d) Section 25(1)(g): The complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed

(e) Section 25(1)(h): The Applicant has failed to disclose to the Controller the information required under Section 8.

(Detailed grounds are set out in the Opposition)

Our address for service in India is:

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Email: rajeshwari@ralegal.co.in; patent@ralegal.co.in

Dated this 30th day of June, 2020

RAJESHWARI H. IN/PA 0358
AGENT FOR THE OPPONENT
OF RAJESHWARI AND ASSOCIATES

To,
The Controller of Patents
The Patent Office, Chennai

BEFORE THE CONTROLLER OF PATENTS, CHENNAI

IN THE MATTER OF:

The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005, and the Patents Rules,

2003, as amended by The Patents (Amendment) Rules, 2006

AND

IN THE MATTER of Post grant opposition under Section 25(2)

AND

IN THE MATTER of Indian Patent No. 319927

[Formerly Indian Patent Application No. 1328/CHENP/2013]

IN THE MATTER OF:

Low Cost Standard Therapeutics **... OPPONENT**

VS.

GILEAD SCIENCES INC. **... PATENTEE**

STATEMENT OF OPPOSITION

We, Low Cost Standard Therapeutics (hereinafter LOCOST), are a not-for profit organisation having its office at I Floor, Premananda Sahitya Bhavan, Opposite Lakadipul, Dandia Bazar, Vadodara, 390 001, Gujarat, India, hereby submit[our representation by way of opposition of patent in respect of patent no. 319927 granted on 05/09/2019 and published on 05/09/2019 entitled "COMPOUNDS FOR TREATING PARAMYXOVIRIDAE VIRUS INFECTIONS" by GILEAD SCIENCES INC. on the following grounds.

1. Petitioner/Opponent has learnt that The Patentee was granted Indian Patent No 319227 (Application No.1328/CHENP/2013), hereinafter IN'227, on 05/09/2019. This Impugned patent is the national phase application of PCT/US2011/045102, which was filed on 22/07/2011 and claims priority from US61/366,609 dated 22/07/2010.

2. The opponent by way of this present post-grant opposition submits that the claim granted on record is not patentable under the provisions provided in the Patents Act, 1970. The granted claim, reproduced below for ready reference and annexed as **Annexure 1**, claims a compound known by the INN of Remdesivir,

We Claim:

1. A compound that is

or a pharmaceutically acceptable salt thereof.

I. LOCUS STANDI.

3. LOCOST Standard Therapeutics (hereinafter LOCOST) is a not-for profit organisation having its office at I Floor, Premananda Sahitya Bhavan, Opposite Lakadipul, Dandia Bazar, Vadodara, 390 001, Gujarat, India. LOCOST was established to make essential medicines of quality at reduced costs by eliminating the margins shared with prescribers, distributers and related market costs. LOCOST makes essential medicines for those working with urban and rural poor in India. The goal of LOCOST is to provide good quality medicines at affordable prices for those working in remote areas.

4. Registered as Low-Cost Standard Therapeutics in 1983, it is a public charitable trust that is concerned with public health aspects of medicines: their genesis, costs, clinical trials, licensing, patenting, production, distribution and consumption till it reaches the end users, namely patients. LOCOST is also concerned about the long-term impact of medicines – their use, misuse and overuse, adverse reactions and side effects - and the public regulation of all related issues.

5. LOCOST has its own well-equipped production facility in Vadodara where it produces more than 100 essential and rational medicines. The establishment makes more than 60 essential medicines in 80 formulations (liquids, capsules, tablets). LOCOST is also active in pharmaceutical policy advocacy at regional and national levels. LOCOST products are preferred by many not for profit health institutions across India The sales are directed at institutions and individuals that work on a not-for profit basis or cater to the poorest of people.

6. Manufacturing at LOCOST conforms to the highest standards of production set by the Government of India. LOCOST's production facilities have Schedule M certification of the Government of India and anytime now it is expected to have WHO-GMP certification too. LOCOST's management practises ethical business norms that further assure our long-standing clients that no corners are cut on quality standards.

7. LOCOST realizes that mere production of essential medicines is not enough. LOCOST also engages in educating prescribers as well as users. Its education cell focuses on issues related to education and training for rational use of medicines. LOCOST publishes a monthly in Gujarati, namely *Apnu Swasthya*, and other publications for the general public including but not limited to the Gujarati version of classics such as *Where there is no Doctor* and *A Lay Person's Guide to Medicine* (a guide on the use and political economy of medicines).

8. The Opponent is also engaged in the manufacture and sale of various antiviral compounds. Additionally, the Opponent has been conducting research in respect of antiviral compounds. The existence of the impugned patent is against public interest and research in respect of anti-viral compounds. Thus, the Opponent is a person interested having direct and tangible interest in filing the present opposition.

GENERAL BACKGROUND ON REMDESIVIR

9. The Indian Patent No 319927 relates to the anti-viral drug remdesivir (RDV). RDV is generally claimed to be an effective treatment against broad class of "Filoviridae virus infections", particularly for treating Ebola virus, Marbug virus and Cueva virus. The Coronavirus belongs to the same family of filoviridae virus. Originally developed for Ebola treatment, RDV is an investigational broad-spectrum anti-viral drug, which is currently (as of June 2020) undergoing clinical trials, is yet to be approved for the treatment of COVID19. However, it is being used to treat patients as part of compassionate use or clinical trial. Studies have shown that this drug works against SARS and MERS, two other coronaviruses that are more lethal but less contagious.

   RDV belongs to the nucleoside analogues class of medicines, which was developed at the University of Alabama's(UAB)Antiviral Drug Discovery and Development Centre (AD3C) with federal government funding[https://www.statnews.com/2020/03/16/remdesivir-surges-ahead-against-coronavirus/]. The AD3C received about $37.5 million "U19" grant *to study and develop a treatment for emerging infections like coronaviruses that can trigger*

*SARS, MERS, and remdesivir(GS) was developed by the researchers at the University after five years of research since 2014.[see*https://www.uab.edu/news/health/item/10307-37-5-million-grant-will-address-research-of-high-priority-infections, https://www.trialsitenews.com/university-of-alabama-multi-center-collaboration-help-develop-remdesivir-with-gilead-thanks-to-37-5m-from-nih/*]* The grant involves a public-private partnership between academic institutions and Gilead Sciences Inc. [https://www.bizjournals.com/birmingham/news/2020/03/02/drug-developed-by-uab-nih-researchers-being-used.html].Hence, the Applicant/Patentee's claim to have spent billions of dollars is misleading.

10. Despite, being an investigational drug, RDV is being used for the treatment in emergency, severe and critical COVID-19 conditions along with oxygen. RDV is being sold at an exorbitant cos [**$2340 for five day course of treatment as of June 2020**], the presence of wrongly granted patents for RDV is hindering wide-scale generic production. Hence it is highly important and extremely urgent for the Patent Office to review the grant of patents in light of the prior arts disclosed in opposition and revoke the patent.

ACCESS TO MEDICINES AND STRICT INTERPRETATION OF INDIAN PATENTABILITY STANDARDS

11. The Indian Patents (Amendment) Act, 2005 was passed to bring India into compliance with its obligations under TRIPS [full form when used for the first time?], and introduced a 20-year product patent regime. In 2001, WTO Members adopted a special Ministerial Declaration at the WTO Ministerial Conference in Doha to clarify ambiguities between the need for governments to apply the principles of public health and the terms of the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS). The Doha Declaration on the TRIPS Agreement and Public Health (the Doha Declaration) reaffirmed the right of the WTO members to make use of the flexibilities of the TRIPS Agreement in a manner that is supportive of public health objectives. In this context, patent offices play a very

critical role in making use of such flexibilities while determining the patentability of a claim on a pharmaceutical product or process..

12. The Opponent respectfully submits that the obligation to promote access to medicines for all must be upheld and that the Patents Act, 1970 must be interpreted to give effect to this aim.

The Doha Declaration should be the underlying value system that informs all patent examinations.

13. Furthermore, the Opponent submits that the Doha Declaration has been incorporated into the Patents Act by Parliament through provisions that protect public health. Patents are given to inventions in exchange for advances in science and technology. Where drug companies are granted patents for only minor improvements of existing drugs, they are at liberty to set the prices of the drugs, and often fix prices well beyond the means of an average person in the developing countries including India. Granting patents for such frivolous applications are thus injurious to both scientific advancement and to public health.

14. In 2005, while amending the Patents Act, 1970, the members of Parliament decided to deny patent protection to multiple patenting of the same substance or proliferation of patents of the same drug and rejected the practice of "evergreening". In this regard section 3(d) is perhaps the most important provision, which prohibits patents for "a new form of a known substance which does not result in the enhancement of the known efficacy of that substance" or for the mere discovery of a "new use of a known substance".

15. In this regard, the Supreme Court observed that " …………With regard to the genesis of section 3(d), and more particularly the circumstances in which section 3(d) was amended to make it even more constrictive than before, we have no doubt that the "therapeutic efficacy" of a medicine must be judged strictly and narrowly**.** Our inference that the test of enhanced efficacy in case of chemical substances, especially

medicine, should receive a narrow and strict interpretation is based not only on external factors but there is sufficient internal evidence that leads to the same view. It may be noted that the text added to section 3(d) by the 2005 amendment lays down the condition of "enhancement of the known efficacy". Further, the explanation requires the derivative to "differ significantly in properties with regard to efficacy". What is evident, therefore, is that not all advantageous or beneficial properties are relevant, but only such properties that directly relate to efficacy, which in case of medicine, as seen above, is its therapeutic efficacy." [para 180, *Novartis AG* vs *Union of India*, (2013)6SCC1]

16. Apart from section 3(d), it is also important to implement sections 2(1)(j) and (ja) more diligently and strictly to avoid frivolous and unworthy patent applications from being granted a patent. In this regard, the amended provision for inventive step sets a higher two -step standard for determining the inventive step in a patent application. The applicant has an obligation to prove that the feature of the invention has a technical advance or economic significance and the feature is non-obvious to a person skilled in the art. Thus, it is imperative for the Patent Office to seek scientific evidence for proving inventive step as well as seeking the applicant to disclose the inventive feature of the alleged invention.

17. The Opponent states that the right to health guaranteed under Article 21 of the Constitution of India is of paramount importance and that medicines required for COVID-19 treatment be made available, so that maximum number of people can benefit from the treatment and many lives can be saved. The wrongly granted patents to the Applicant would breach the right to health of a large number of COVID-19 patients languishing in the hospitals unable to access life-saving yet costly medications. It is submitted that the Hon'ble Patent Controller, may examine the Present Application with strict scrutiny, as its decision will have far reaching effect on the availability of affordable access to treatment for COVID-19 and such other infection.

18. The Opponent submits its opposition by way of representation under Section 25(2) in respect of the said Indian Patent No. **319927** on the following grounds below, which are without prejudice and in the alternative to each other.

GROUNDS OF OPPOSITION

II. Section 25(2) (b):

The claimed invention has been published before the filing date (priority date) in a specification of an application filed in India on or after January 1, 1912, or in any other documents in India or elsewhere

19. The impugned patent lacks novelty over prior art AU2009240642 (annexed as **Annexure 2**) hereinafter referred as AU'642, published on October 29, 2009.The patent document AU'642 discloses, compound of formula 1(1'-substituted carba-nucleoside analogs) for antiviral treatment;

The claimed compound of the impugned patent is covered in AU'642 in following manner:

S.No		
1.	...[II] Markush Structure	

	Page (9)	
2	In Formula II; R^1 , R^3, R^5 can be H; (Page 9)	
3	In Formula II; R^2 and R^4 represents OR^a; (Page 9) R^a represents H (Page 10) means R^2 and R^4 are OH	
4	In Formula II; R^6 represents CN (Page 10) R^8 represents $NR^{11}R^{12}$;(Page 12) R^{11} and R^{12} represents H (Page 12) means R^8 is NH_2 R^9 represents H (Page 12)	
5	In Formula II; X^1 and X^2 represents C-	

	R^{10} (Page 11) R^{10} represents H (Page 12) means X^1 and X^2 represents -CH	
6	In Formula II; R^7 represents (Page 10)	
7	In Formula II; Y represents O (Page 10)	
8	In Formula II; W^1 represents (Page 10)	

	Y^2 is O (Page 11) M2 is 0 (Page 11)	
9	In Formula II; R^x represents R^y and R^y is R and R can C_6-C_{20} aryl- that is R^x is phenyl (Page 11)	
10	In Formula II; W^2 represents (Page 10) Y^2 is NR-where R can be H, So Y^2 is NH (page 11)	
11		

	In Formula II; Rx represents Ry or formula M1a is Zero, M1c is zero, M12c is one (Page 11) Each R^y in above formula can be independent. So (from the left) R^y can be H, R^y can be R and R can be C1-C8 alkyl such as CH_3 (methyl); (Page 11) Y^1 is O (page 10) and Y^2 is O (page 11).	
12	R^y can be R and R can be substituted alkyl. (Page 11) In substituted alkyl, substituted represents R^b and Rb represents alkyl means ethyl-butyl	Remdesivir

20. Thus, the compound claimed in the impugned patent was already known through prior publication of AU'642 before the priority of the present patent. Thus, the impugned patent ought to be revoked on this ground alone.

III. Section 25(2)(c):

The invention so far as claimed in any claim of the complete specification published on or after the priority date of the claim of the patentee and filed in pursuance of an application for a patent in India, being a claim of which the priority date is earlier than that of the claim of the patentee

21. It is submitted that the subject matter of the impugned patent is prior claimed in IN275967 (annexed as **Annexure 3**), hereinafter referred to as IN'967, which has the priority of 2008 and was published in 2012.The patent document IN'967 claims compound of formula 1(1'-substituted carba-nucleoside analogs) for antiviral treatment;

Claim 1 of IN'967 claims the compound claimed in the impugned patent as shown below:

S.No		
1.	Formula I, Markush Structure	
2	In Formula I; R^1, R^3, R^5 can be H;	

3	In Formula I; R^2 and R^4 represents OR^a; R^a represents H means R^2 and R^4 are OH	
4	In Formula I; R^6 represents CN R^8 represents $NR^{11}R^{12}$; R^{11} and R^{12} represents H, means R^8 is NH_2 R^9 represents H	
5	In Formula I; X^1 and X^2 represents C-R^{10} R^{10} represents H means X^1 and X^2 represents -CH	

6	In Formula I; R^7 represents	
7	In Formula I; Y represents O	
8	In Formula I; W^1 represents Y^2 is O M2 is 0	
9	In Formula I; R^x represents R^y and R^y is R and R can C_6-C_{20} aryl- that is R^x is phenyl	

10	In Formula I; W^2 represents Y^2 is NR-where R can be H, So Y^2 is NH	
11	In Formula I; Rx represents Ry or formula M1a is Zero, M1c is zero, M12c is one Each R^y in above formula can be independent. So (from the left) R^y can be H, R^y can be R and R can be C1-C8 alkyl such as CH_3 (methyl); Y^1 is O and Y^2 is O.	

12	R^y can be R and R can be substituted alkyl. In substituted alkyl, substituted represents ethyl and alkyl means - butyl	Remdesivir

22. Thus, the compound claimed in the impugned patent was already prior claimed in IN'967. Therefore, the impugned patent ought to be revoked on this ground alone.

IV. Section 25(1) (e): lack of inventive step

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 as mentioned in clause (b) or having regard to what was used in India before the priority date of the claim.

23. The technical teaching of the impugned invention applies to limited knowledge which is well-known in art without any inventiveness. It is submitted that the claims are obvious and lack any inventive step in view of teachings, motivation and suggestion in various prior art documents listed herein below.

24. It is submitted that the impugned application is obvious in light of teachings of following:

- AU2009240642 **(annexed herewith as Annexure 2)**
- Christopher McGuigan, Dominique Cahard, Hendrika M. Sheeka, Erik De Clercq, and Jan Balzarini;" Aryl Phosphoramidate Derivatives of d4T Have Improved Anti-HIV Efficacy in Tissue Culture and May Act by the Generation of a Novel

Intracellular Metabolite"; J. *Med. Chem*. 1996, 39, 1748, published in 1996 (**annexed herewith as annexure 4**).

- Larryn W Petersen, "Prodrug approaches to improving the oral absorption of antiviral nucleotide analogues", published in 2009" **(annexed herewith as Annexure 5)**
- Drew R Bobeck et al., Advances in nucleoside monophosphate prodrugs as anti-HCV agents. Antiviral Therapy 2010 15:935-950 **(annexed herewith as Annexure 6).**

25. **AU2009240642**, hereinafter referred as AU'642, published on October 29, 2009 discloses, 1'-substituted carba-nucleoside analogs for antiviral treatment and the inventors of this document have assessed the EC50 value of certain compounds disclosed therein for viruses belonging to Flaviviridae family, also known as Flaviviruses, such as HCV and Dengue virus. For Dengue virus the best result was demonstrated by Compound 17 and for HCV the best result was demonstrated by Compound 6 closely followed by Compound 13 and 12. The said compounds are shown below:

Compound 17

17

6

Compound 13

26. Taking into consideration the common structural features of these compounds, a PSITA reaches the following structure:

27. Further, a PSITA also gets a teaching from this document that the 5'position of ribose ring may have a methyl phosphate group and that this phosphate group may be further derivatized.

28. In the **McGuigan *et al.*** publication the authors disclose that they prepared phosphoramidate derivatives of various nucleoside antivirals such as AZT and d4T.

The authors prepared various phosphoramidate derivatives and found that among these the alanine containing ester derivative named as Compound 4a was most effective. The structure of compound 4a is shown below:

(4a) R = Me (4d) R = CH_2iPr
(4b) R = H (4e) R = CH_2Ph
(4c) R = iPr (4f) R = CH_2CH_2SMe

29. This prior art teaches a person skilled in the art to apply the aryloxy phosphoramidate approach for the successful delivery of nucleoside analogue into a cell.

30. This approach of using a prodrug form known as protide, and specifically aryloxy phosphoramidates having phenyl as the aryl function, alanine as the amino acid, and methyl as the ester function emerged as the most promising combination in the said prior art.

31. Many other documents existent in the art at the time of impugned invention also teach the use of protide formation of nucleoside antivirals for their effective delivery. One among such documents is apublication by Petersen.

32. **Petersen** discloses that prodrugs ('ProTides') based on an amino acid ester promoiety, attached to the drug (as an aryl monophosphate or phosphonate) via a P-N bond are a common approach to increase the delivery of nucleotide antivirals to the cells. The author further discloses that the original approach involved in using simple alkyloxy phosphoramidates has now evolved into aryloxy phosphoramide

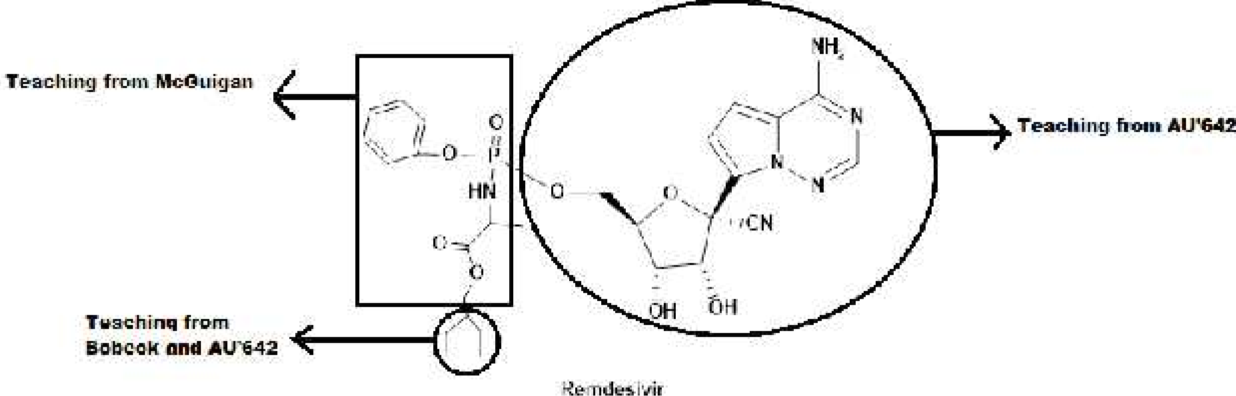
pronucleotides wherein the introduction of promoieties at the phosphorus (–[O, CH2]-P (O) (X) (Y) where X, Y = OR, OR′, NHR″) directly addresses the problem of blocking P-OH ionization *in vivo*. The attachment of a well-designed promoiety increases the delivery of the drug to its target. The author further deliberates upon the effect of changing the amino acid moiety in the prodrug and concludes that the bestantiviral activity was obtained with the L-alanine methyl ester.

33. Thus, based on the disclosures of Mcguigan and Petersen, a person skilled in the art is motivated to prepare an alanine containing phenyl protide of the basic molecule arrived at by disclosure of AU'642.

34. Meanwhile, researchers in the field such as Bobeck *et al*workedon modulating the distal alkyl group of the ester in phosphoramidate group. Upon working with various nucleosides they found that in nucleosides containing a saturated ribose ring and phenyl as aryl substituent in phosphoramidate group, Compounds 5 and 6 gave the best activity in terms of EC50 for reducing viral replication as inferred from the Table given in Fig 2 of Bobeck *et al*.

35. When read in consonance with the disclosure of AU'642, a person skilled in the art is aware that the basic structure of AU'642 gave good antiviral activity when the distal alkyl substituent on the ester of phosphoramidate is tertiary butyl as seen in Compound 6. Thus, drawing parallel between the disclosure of AU'642 and Bobeck it was an obvious conclusion for a person skilled in the art that the 2-ethylbutyl substituent disclosed in Bobeck more closely approximates the tertiary butyl substituent disclosed in AU'642 and hence, could be a viable effective alternative of the methyl group of the alanine ester.

36. Combining the teachings of the documents a person skilled in the art arrives at the claimed compound of the impugned patent in following manner:



37. Therefore, the subject matter claimed in the impugned patent is obvious in light of the prior art disclosed above. Further, the patentee has failed to disclose any data for establishing the technical advancement of the claimed invention over similar compounds which were already known in the art at the time of invention. Therefore, the subject matter claimed in the impugned patent is obvious and the impugned patent should be rejected on this basis alone.

38. Further to establish the superiority of the claimed compound, the patentee has randomly chosen certain compounds of prior art WO 2009/132135, which is the corresponding WO application of AU'642 and IN'967 and made comparisons with them without any application of reason in their response to the objections raised in FER.

39. Furthermore a bare perusal of the specification of IN'967 and IN'927 reveals that the mechanism of action described in both the documents is exactly the same. The paragraphs on pages 49 and 50 of IN'967 which describe the mechanism of cleavage of prodrug and the paragraphs on pages 44 and 45 of IN'927 which describe the mechanism of cleavage of prodrugs are verbatim copies of each other.

40. In light of these stark and obvious similarities between the prior art and IN'927, the patent ought to be revoked.

V. <u>The subject matter of claims is not patentable under Section 3(d) of the Act</u>:

Section 3(d) in of Patents Act, 1970

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.

41. There are numerous compounds disclosed in AU'642 (corresponding AU application of IN'967) which are structurally similar to the compound claimed in Claim 1.The applicant has failed to provide comparative efficacy data of the compounds disclosed in IN'967 and the compound claimed in Claim 1.

42. As per the Supreme Court's direction in *Novartis AG v Union of India*[paras 180-189 (2013)6SCC1], the applicant has the responsibility to provide comparative data on enhanced efficacy by *in vitro* and *in vivo* tests. The applicant has failed to provide any such data. Regardless of the data, which is a statutory requirement, the grant of the patent is erroneous. Hence the patent ought to be revoked.

43. It is submitted that the subject matter claimed in the impugned patent is not novel and inventive. As submitted in preceding paragraphs the compound claimed in the impugned patent is already anticipated by prior publication in AU'642 as well as prior claimed in IN'967. The claimed compound also lacks inventive step in view of the prior art documents cited above. Moreover, the patentee has failed to show any enhancement in therapeutic efficacy of the claimed compound over similar compounds known in the prior art. In the absence of comparative data in the specification as filed establishing the enhanced therapeutic efficacy of the claimed

compound over the compounds already known in art at the time of the invention, the patent is non-patentable as the patentee has failed to crossover the bar set under Section 3(d) of the Act. Therefore, the subject matter claimed in the impugned patent falls squarely within the purview of Section 3(d) of the Act and the impugned patent should be revoked on this ground alone.

VI. Section 25(1)(g): The complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed

44. It is submitted that Section 10(4) of the Act mandates that a complete specification must not only fully and particularly describe an invention, and the method by which it is to be performed, it must also disclose the best method of performing the invention known to the Applicant.

45. Claim 1 of the impugned patent pertains to a compound and its pharmaceutically active salt. The complete specification discloses a number of compounds having varied structural modifications. However, no explanation is provided for the selection of claimed compound from among such laundry of compounds. Also no efficacy data is provided for the claimed compound. The description has failed to provide activity of the claimed compound with specific test under specific conditions and with specific results. Further, the specification does not provide even a single example of the salt form of the claimed compound nor does it disclose which salt forms of the compound possess the desired activity as per the invention and which don’t. Therefore, the impugned patent does not provide adequate teaching to a person skilled in the art to practice the invention.

46. In light of above, it is clear that the impugned patent does not sufficiently and clearly describe the invention. Therefore, the impugned patent should be rejected on this ground alone.

VII. Section 25(1)(h): The Applicant has failed to disclose to the Controller the information required under Section 8.

47. It is submitted that the Applicant has filed large number of corresponding patents in many countries outside India. However, the Form 3 details submitted by the applicant do not disclose the details of this entire corresponding patent at the Indian Patent Office.

48. The opponent submits that the applicant has purposefully and with malafide intention refrained from disclosing details of all the corresponding patents filed outside India and the prosecution details of the same to the Learned Controller and therefore, on this ground alone the patent patents should be rejected.

49. The opponents crave leave to file further submissions and evidence with respect to this ground.

VIII. PRAYER

In the fact and circumstances of the case, the Opponent prays as follows:

i. that the Indian Patent **319927** made by **GILEAD SCIENCES INC.,** be revoked under Section 25(2) of the Patents (Amendment) Act, 2005;

ii. the Opponent may be allowed to file further documents as evidence if necessary to support its averments;

iii. the Opponent may be allowed to make further submissions in case the applicant makes any amendments in the claims;

Dated this 30th day of June, 2020

S. Sriniwasan
Managing Trustee of Low Cost Standard Therapeutis

The Controller of Patents
The Patent Office, Chennai

Indian Patent Application Number: 1328/CHENP/2013
As Amended on 26 August 2019 – Clean Claims

We Claim:

1. A compound that is

or a pharmaceutically acceptable salt thereof.

Dated this 19 Day of February 2013

--Digitally signed--

(Shakira N)
REG. No: IN/PA-972
of De Penning & De Penning
Agent for the Applicants

ANNEXURE 2

(12) STANDARD PATENT (11) Application No. **AU 2009240642 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
1' -substituted carba-nucleoside analogs for antiviral treatment

(51) International Patent Classification(s)
C07D 487/04 (2006.01) ***A61K 31/7052*** (2006.01)
A61K 31/395 (2006.01) ***A61P 31/12*** (2006.01)
A61K 31/41 (2006.01) ***C07H 19/23*** (2006.01)
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(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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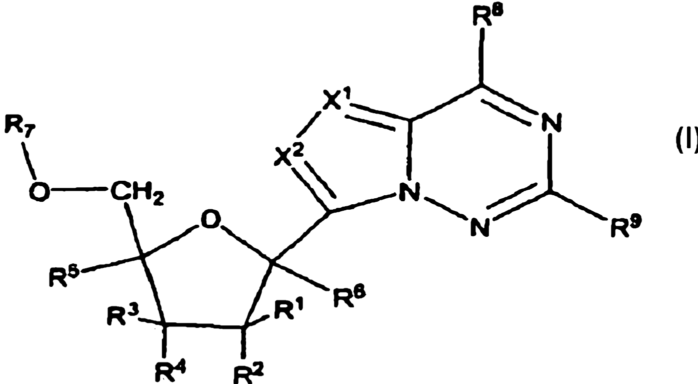
(54) Title: 1' -SUBSTITUTED CARBA-NUCLEOSIDE ANALOGS FOR ANTIVIRAL TREATMENT



(57) **Abstract**: Provided are pyrrolo[1,2-f][1,2,4]triazinyl, imidazo[1,5-f][1,2,4]triazinyl, imidazo[1,2-f][1,2,4]triazinyl, and [1,2,4]triazolo[4,3-f][1,2,4]triazinyl nucleosides, nucleoside phosphates and prodrugs thereof, wherein the I ' position of the nucleoside sugar is substituted. The compounds, compositions, and methods provided are useful for the treatment of *Flaviviridae* virus infections, particularly hepatitis C infections. Formula (I), each X^1 or X^2 is independently C-R^{10} or N.

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1'-SUBSTITUTED CARBA-NUCLEOSIDE ANALOGS FOR ANTIVIRAL TREATMENT

15

FIELD OF THE INVENTION

The invention relates generally to compounds with antiviral activity, more particularly nucleosides active against *Flaviviridae* infections and most particularly to inhibitors of hepatitis C virus RNA-dependent RNA polymerase.

BACKGROUND OF THE INVENTION

20 Viruses comprising the *Flaviviridae* family comprise at least three distinquishable genera including *pestiviruses*, *flaviviruses*, and *hepaciviruses* (Calisher, *et al.*, J. Gen. Virol., 1993, 70, 37-43). While *pestiviruses* cause many economically important animal diseases such as bovine viral diarrhea virus (BVDV), classical swine fever virus (CSFV, hog cholera) and border disease of sheep (BDV), their importance in human disease is
25 less well characterized (Moennig, V., *et al.*, Adv. Vir. Res. 1992, 48, 53-98). *Flaviviruses* are responsible for important human diseases such as dengue fever and yellow fever while *hepaciviruses* cause hepatitis C virus infections in humans. Other important viral infections caused by the *Flaviviridae* family include West Nile virus (WNV) Janpanese encephalitis virus (JEV), tick-borne encephalitis virus, Junjin virus,
30 Murray Valley encephalitis, St Louis enchaplitis, Omsk hemorrhagic fever virus and Zika virus. Combined, infections from the *Flaviviridae* virus family cause significant

5 mortality, morbidity and economic losses throughout the world. Therefore, there is a need to develop effective treatments for *Flaviviridae* virus infections.

The hepatitis C virus (HCV) is the leading cause of chronic liver disease worldwide (Boyer, N. et al. J Hepatol. 32:98-112, 2000) so a significant focus of current antiviral research is directed toward the development of improved methods of treatment
10 of chronic HCV infections in humans (Di Besceglie, A.M. and Bacon, B. R., Scientific American, Oct.: 80-85, (1999); Gordon, C. P., et al., *J. Med. Chem.* **2005**, *48*, 1-20; Maradpour, D.; et al., *Nat. Rev. Micro.* **2007**, *5*(6), 453-463). A number of HCV treatments are reviewed by Bymock et al. in Antiviral Chemistry & Chemotherapy, 11:2; 79-95 (2000).

15 RNA-dependent RNA polymerase (RdRp) is one of the best studied targets for the development of novel HCV therapeutic agents. The NS5B polymerase is a target for inhibitors in early human clinical trials (Sommadossi, J., WO 01/90121 A2, US 2004/0006002 A1). These enzymes have been extensively characterized at the biochemical and structural level, with screening assays for identifying selective
20 inhibitors (De Clercq, E. (2001) J. Pharmacol. Exp.Ther. 297:1-10; De Clercq, E. (2001) J. Clin. Virol. 22:73-89). Biochemical targets such as NS5B are important in developing HCV therapies since HCV does not replicate in the laboratory and there are difficulties in developing cell-based assays and preclinical animal systems.

Currently, there are primarily two antiviral compounds, ribavirin, a nucleoside
25 analog, and interferon-alpha (α) (IFN), which are used for the treatment of chronic HCV infections in humans. Ribavirin alone is not effective in reducing viral RNA levels, has significant toxicity, and is known to induce anemia. The combination of IFN and ribavirin has been reported to be effective in the management of chronic hepatitis C (Scott, L. J., et al. *Drugs* **2002**, *62*, 507-556) but less than half the patients given this
30 treatment show a persistent benefit. Other patent applications disclosing the use of nucleoside analogs to treat hepatitis C virus include WO 01/32153, WO 01/60315, WO 02/057425, WO 02/057287, WO 02/032920, WO 02/18404, WO 04/046331, WO2008/089105 and WO2008/141079 but additional treatments for HCV infections have not yet become available for patients. Therefore, drugs having improved antiviral

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and pharmacokinetic properties with enhanced activity against development of HCV resistance, improved oral bioavailability, greater efficacy, fewer undesirable side effects and extended effective half-life *in vivo* (De Francesco, R. *et al.* (2003) Antiviral Research 58:1-16) are urgently needed.

Certain ribosides of the nucleobases pyrrolo[1,2-f][1,2,4]triazine, imidazo[1,5-
5 f][1,2,4]triazine, imidazo[l ,2-f][1 ,2,4]triazine, and [1 ,2,4]triazolo[4,3-f][1 ,2,4]triazine have been disclosed in *Carbohydrate Research* 2001, 331 (1), 77-82; *Nucleosides & Nucleotides* (1996),15(1-3),793-807; *Tetrahedron Letters* (1994), 35(30), 5339-42; *Heterocycles* (1992), 34(3), 569-74; *J Chem. Soc. Perkin Trans. 1* 1985,3,621-30; *J Chem. Soc. Perkin Trans. 1* 1984,2,229-38; WO 2000056734; *Organic Letters (2001),* 15 3(6),839-842; *J Chem. Soc. Perkin Trans. 1* 1999,20,2929-
10 2936; and *J Med. Chem.* 1986, 29(11), 2231-5. However, these compounds have not been disclosed as useful for the treatment of HCV. Babu, Y. S., W02008/089105 and W02008/141079, discloses ribosides of pyrrolo[1,2-f][1,2,4]triazine nucleobases with antiviral, anti-HCV, and anti-RdRp activity.

Reference to any prior art in the specification is not, and should not be taken as, an
15 acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction or that this prior art could reasonably be expected to be ascertained, understood and regarded as relevant by a person skilled in the art.

As used herein, except where the context requires otherwise the term 'comprise' and variations of the term, such as 'comprising', 'comprises' and 'comprised', are not intended to
20 exclude other additives, components, integers or steps.

SUMMARY OF THE INVENTION

The instant invention provides compounds that inhibit viruses of the *Flaviviridae* family. The invention also comprises compounds that inhibit viral nucleic acid polymerases, particularly HCV RNA-dependent RNA polymerase (RdRp), rather than cellular nucleic acid polymerases.
25 Therefore, the compounds of the instant invention are useful for treating *Flaviviridae* infections in humans and other animals.

In one aspect, this invention provides a compound of Formula I:

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Formula I

or a pharmaceutically acceptable salt, thereof;

wherein:

each R^1, R^2, R^3, R^4, or R^5 is independently H, OR^a, $N(R^a)_2$, N_3, CN, NO_2,

10 $S(O)_nR^a$, halogen, (C_1-C_8)alkyl, (C_4-C_8)carbocyclylalkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl, or aryl(C_1-C_8)alkyl;

or any two R^1, R^2, R^3, R^4, or R^5 on adjacent carbon atoms when taken together are –O(CO)O- or when taken together with the ring carbon atoms to which they are attached

15 form a double bond;

R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, (C_1-C_8)alkyl, (C_4-C_8)carbocyclylalkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted

20 alkynyl, or aryl(C_1-C_8)alkyl or R^6 and either R^1 or R^2 when taken together are –O(CO)O-;

each n is independently 0, 1, or 2;

each R^a is independently H, (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, aryl(C_1-C_8)alkyl, (C_4-C_8)carbocyclylalkyl, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, -

25 $C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, or $-SO_2NR^{11}R^{12}$;

R^7 is H, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, or

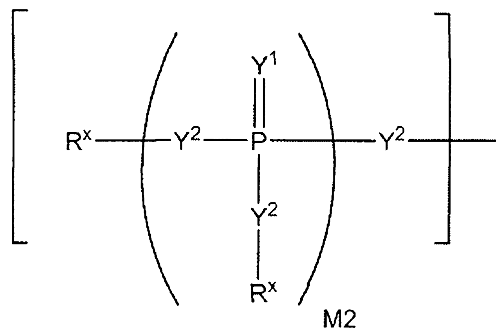
Y

W1—P—

W2 ;

each Y or Y^1 is, independently, O, S, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, or N–NR_2;

W^1 and W^2, when taken together, are $-Y^3(C(R^y)_2)_3Y^3-$; or one of W^1 or W^2 together with either R^3 or R^4 is $-Y^3-$ and the other of W^1 or W^2 is Formula Ia; or W^1 and W^2 are each, independently, a group of the Formula Ia:



Formula Ia

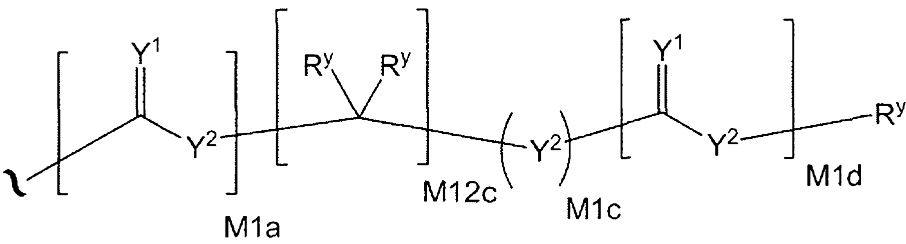
wherein:

each Y^2 is independently a bond, O, CR_2, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, N–NR_2, S, S–S, S(O), or $S(O)_2$;

each Y^3 is independently O, S, or NR;

M2 is 0, 1 or 2;

each R^x is independently R^y or the formula:



wherein:

each M1a, M1c, and M1d is independently 0 or 1;

M12c is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

5 each R^y is independently H, F, Cl, Br, I, OH, R, -C(=Y^1)R, -C(=Y^1)OR, -C(=Y^1)N(R)$_2$, -N(R)$_2$, -$^+$N(R)$_3$, -SR, -S(O)R, -S(O)$_2$R, -S(O)(OR), -S(O)$_2$(OR), -OC(=Y^1)R, -OC(=Y^1)OR, -OC(=Y^1)(N(R)$_2$), -SC(=Y^1)R, -SC(=Y^1)OR, -SC(=Y^1)(N(R)$_2$), -N(R)C(=Y^1)R, -N(R)C(=Y^1)OR, -N(R)C(=Y^1)N(R)$_2$, $-SO_2NR_2$, –CN, $-N_3$, $-NO_2$, –OR, or W^3; or when taken together, two R^y on the same carbon atom form a 10 carbocyclic ring of 3 to 7 carbon atoms;

each R is independently H, (C_1-C_8) alkyl, (C_1-C_8) substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8) substituted alkenyl, (C_2-C_8) alkynyl, (C_2-C_8) substituted alkynyl, C_6–C_{20} aryl, C_6–C_{20} substituted aryl, C_2–C_{20} heterocyclyl, C_2–C_{20} substituted heterocyclyl, arylalkyl or substituted arylalkyl;

15 W^3 is W^4 or W^5; W^4 is R, -C(Y^1)R^y, -C(Y^1)W^5, -SO_2R^y, or -SO_2W^5; and W^5 is a carbocycle or a heterocycle wherein W^5 is independently substituted with 0 to 3 R^y groups;

each X^1 or X^2 is independently C-R^{10} or N;

each R^8 is halogen, $NR^{11}R^{12}$, N(R^{11})OR^{11}, $NR^{11}NR^{11}R^{12}$, N_3, NO, NO_2, CHO, 20 CN, -CH(=NR^{11}), -CH=NNHR^{11}, -CH=N(OR^{11}), -CH(OR^{11})$_2$, -C(=O)$NR^{11}R^{12}$, -C(=S)$NR^{11}R^{12}$, -C(=O)OR^{11}, (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, (C_4–C_8)carbocyclylalkyl, optionally substituted aryl, optionally substituted heteroaryl, -C(=O)(C_1-C_8)alkyl, -S(O)$_n$(C_1-C_8)alkyl, aryl(C_1-C_8)alkyl, OR^{11} or SR^{11};

each R^9 or R^{10} is independently H, halogen, $NR^{11}R^{12}$, N(R^{11})OR^{11}, $NR^{11}NR^{11}R^{12}$, 25 N_3, NO, NO_2, CHO, CN, -CH(=NR^{11}), -CH=NHNR^{11}, -CH=N(OR^{11}), -CH(OR^{11})$_2$, -C(=O)$NR^{11}R^{12}$, -C(=S)$NR^{11}R^{12}$, -C(=O)OR^{11}, R^{11}, OR^{11} or SR^{11};

each R^{11} or R^{12} is independently H, (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, (C_4–C_8)carbocyclylalkyl, optionally substituted aryl, optionally substituted heteroaryl, -C(=O)(C_1-C_8)alkyl, -S(O)$_n$(C_1-C_8)alkyl or aryl(C_1-C_8)alkyl; or R^{11} and R^{12} taken together 30 with a nitrogen to which they are both attached form a 3 to 7 membered heterocyclic ring wherein any one carbon atom of said heterocyclic ring can optionally be replaced with -O-, -S- or –NR^a-;

wherein each (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl or aryl(C_1-C_8)alkyl of each R^1, R^2, R^3, R^4, R^5, R^6, R^{11} or R^{12} is, independently, optionally substituted with one

5 or more halo, hydroxy, CN, N_3, $N(R^a)_2$ or OR^a; and wherein one or more of the non-terminal carbon atoms of each said (C_1-C_8)alkyl may be optionally replaced with -O-, -S- or $-NR^a-$.

In another aspect, the present invention includes compounds of Formula I and pharmaceutically acceptable salts thereof and all racemates, enantiomers, diastereomers, 10 tautomers, polymorphs, pseudopolymorphs and amorphous forms thereof.

In another aspect, the present invention provides novel compounds of Formula I with activity against infectious *Flaviviridae* viruses. Without wishing to be bound by theory, the compounds of the invention may inhibit viral RNA-dependent RNA polymerase and thus inhibit the replication of the virus. They are useful for treating 15 human patients infected with a human virus such as hepatitis C.

In another aspect, the invention provides a pharmaceutical composition comprising an effective amount of a Formula I compound, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable diluent or carrier.

20 In another embodiment, the present application provides for combination pharmaceutical agent comprising:

a) a first pharmaceutical composition comprising a compound of Formula I; or a pharmaceutically acceptable salt, solvate, or ester thereof; and

b) a second pharmaceutical composition comprising at least one additional 25 therapeutic agent selected from the group consisting of interferons, ribavirin analogs, NS3 protease inhibitors, NS5a inhibitors, alpha-glucosidase 1 inhibitors, cyclophilin inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV.

In another embodiment, the present application provides for a method of 30 inhibiting HCV polymerase, comprising contacting a cell infected with HCV with an effective amount of a compound of Formula I; or a pharmaceutically acceptable salts, solvate, and/or ester thereof.

In another embodiment, the present application provides for a method of inhibiting HCV polymerase, comprising contacting a cell infected with HCV with an

5 effective amount of a compound of Formula I; or a pharmaceutically acceptable salts, solvate, and/or ester thereof; and at least one additional therapeutic agent.

In another embodiment, the present application provides for a method of treating and/or preventing a disease caused by a viral infection wherein the viral infection is caused by a virus selected from the group consisting of dengue virus, yellow fever virus, 10 West Nile virus, Japanese encephalitis virus, tick-borne encephalitis virus, Junjin virus, Murray Valley encephalitis virus, St Louis encephalitis virus, Omsk hemorrhagic fever virus, bovine viral disarrhea virus, Zika virus and Hepatitis C virus; by administering to a subject in need thereof a therapeutically effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof.

15 In another embodiment, the present application provides for a method of treating HCV in a patient, comprising administering to said patient a therapeutically effective amount of a compound of Formula I; or a pharmaceutically acceptable salt, solvate, and/or ester thereof.

In another embodiment, the present application provides for a method of treating 20 HCV in a patient, comprising administering to said patient a therapeutically effective amount of a compound of Formula I; or a pharmaceutically acceptable salt, solvate, and/or ester thereof; and at least one additional therapeutic agent.

Another aspect of the invention provides a method for the treatment or prevention of the symptoms or effects of an HCV infection in an infected animal which comprises 25 administering to, i.e. treating, said animal with a pharmaceutical combination composition or formulation comprising an effective amount of a Formula I compound, and a second compound having anti-HCV properties.

In another aspect, the invention also provides a method of inhibiting HCV, comprising administering to a mammal infected with HCV an amount of a Formula I 30 compound, effective to inhibit the replication of HCV in infected cells in said mammal.

In another aspect, the invention also provides processes and novel intermediates disclosed herein which are useful for preparing Formula I compounds of the invention.

In other aspects, novel methods for synthesis, analysis, separation, isolation, purification, characterization, and testing of the compounds of this invention are

5 provided.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

Reference will now be made in detail to certain embodiments of the invention,
10 examples of which are illustrated in the accompanying description, structures and formulas. While the invention will be described in conjunction with the enumerated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present
15 invention.

In another aspect, compounds of Formula I are represented by Formula II:

R8 X1 N R7 X2 O N O N R9 R5 R6 R3 R1 R4 R2

Formula II

or a pharmaceutically acceptable salt, thereof;

20 wherein:

each R^1, R^2, R^3, R^4, or R^5 is independently H, OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, halogen, $(C_1–C_8)$alkyl, $(C_4–C_8)$carbocyclylalkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, $(C_2–C_8)$substituted alkynyl, or aryl$(C_1$-$C_8)$alkyl;

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5 or any two R^1, R^2, R^3, R^4, or R^5 on adjacent carbon atoms when taken together are –O(CO)O- or when taken together with the ring carbon atoms to which they are attached form a double bond;

R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$,

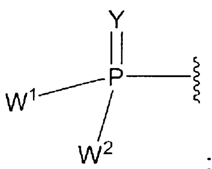
10 $-SO_2NR^{11}R^{12}$, halogen, (C_1-C_8)alkyl, (C_4-C_8)carbocyclylalkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl, or aryl(C_1-C_8)alkyl or R^6 and either R^1 or R^2 when taken together are –O(CO)O-;

each n is independently 0, 1, or 2;

each R^a is independently H, (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl,

15 aryl(C_1-C_8)alkyl, (C_4-C_8)carbocyclylalkyl, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, or $-SO_2NR^{11}R^{12}$;

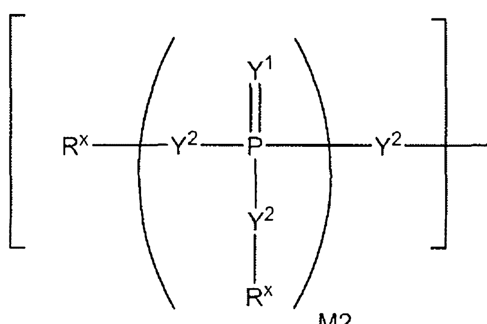
R^7 is H, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, or



;

20 each Y or Y^1 is, independently, O, S, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, or $N-NR_2$;

W^1 and W^2, when taken together, are $-Y^3(C(R^y)_2)_3Y^3-$; or one of W^1 or W^2 together with either R^3 or R^4 is $-Y^3-$ and the other of W^1 or W^2 is Formula Ia; or W^1 and W^2 are each, independently, a group of the Formula Ia:



25

Formula Ia

wherein:

each Y^2 is independently a bond, O, CR_2, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, $N{-}NR_2$, S, S–S, S(O), or $S(O)_2$;

each Y^3 is independently O, S, or NR;

M2 is 0, 1 or 2;

each R^x is independently R^y or the formula:

wherein:

each M1a, M1c, and M1d is independently 0 or 1;

M12c is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

each R^y is independently H, F, Cl, Br, I, OH, R, $-C(=Y^1)R$, $-C(=Y^1)OR$, $-C(=Y^1)N(R)_2$, $-N(R)_2$, $-^+N(R)_3$, -SR, -S(O)R, $-S(O)_2R$, -S(O)(OR), $-S(O)_2(OR)$, $-OC(=Y^1)R$, $-OC(=Y^1)OR$, $-OC(=Y^1)(N(R)_2)$, $-SC(=Y^1)R$, $-SC(=Y^1)OR$, $-SC(=Y^1)(N(R)_2)$, $-N(R)C(=Y^1)R$, $-N(R)C(=Y^1)OR$, $-N(R)C(=Y^1)N(R)_2$, $-SO_2NR_2$, –CN, $-N_3$, $-NO_2$, –OR, or W^3; or when taken together, two R^y on the same carbon atom form a carbocyclic ring of 3 to 7 carbon atoms;

each R is independently H, $(C_1\text{-}C_8)$ alkyl, $(C_1\text{-}C_8)$ substituted alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$ substituted alkenyl, $(C_2\text{-}C_8)$ alkynyl, $(C_2\text{-}C_8)$ substituted alkynyl, C_6–C_{20} aryl, C_6–C_{20} substituted aryl, C_2–C_{20} heterocyclyl, C_2–C_{20} substituted heterocyclyl, arylalkyl or substituted arylalkyl;

W^3 is W^4 or W^5; W^4 is R, $-C(Y^1)R^y$, $-C(Y^1)W^5$, $-SO_2R^y$, or $-SO_2W^5$; and W^5 is a carbocycle or a heterocycle wherein W^5 is independently substituted with 0 to 3 R^y groups;

X^2 is $C\text{-}R^{10}$ and each X^1 is independently $C\text{-}R^{10}$ or N;

5 each R^8 is independently halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, N_3, NO, NO_2, CHO, CN, $-CH(=NR^{11})$, $-CH=NHNR^{11}$, $-CH=N(OR^{11})$, $-CH(OR^{11})_2$, $-C(=O)NR^{11}R^{12}$, $-C(=S)NR^{11}R^{12}$, $-C(=O)OR^{11}$, $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl, $(C_4\text{–}C_8)$carbocyclylalkyl, optionally substituted aryl, optionally substituted heteroaryl, $-C(=O)(C_1\text{-}C_8)$alkyl, $-S(O)_n(C_1\text{-}C_8)$alkyl, aryl$(C_1\text{-}C_8)$alkyl, OR^{11} or SR^{11};

10 each R^9 or R^{10} is independently H, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, N_3, NO, NO_2, CHO, CN, $-CH(=NR^{11})$, $-CH=NHNR^{11}$, $-CH=N(OR^{11})$, $-CH(OR^{11})_2$, $-C(=O)NR^{11}R^{12}$, $-C(=S)NR^{11}R^{12}$, $-C(=O)OR^{11}$, R^{11}, OR^{11} or SR^{11};

each R^{11} or R^{12} is independently H, $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl, $(C_4\text{–}C_8)$carbocyclylalkyl, optionally substituted aryl, optionally substituted heteroaryl, -

15 $C(=O)(C_1\text{-}C_8)$alkyl, $-S(O)_n(C_1\text{-}C_8)$alkyl or aryl$(C_1\text{-}C_8)$alkyl; or R^{11} and R^{12} taken together with a nitrogen to which they are both attached form a 3 to 7 membered heterocyclic ring wherein any one carbon atom of said heterocyclic ring can optionally be replaced with -O-, -S- or $-NR^a$-;

wherein each $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl or aryl$(C_1\text{-}C_8)$alkyl of

20 each R^1, R^2, R^3, R^4, R^5, R^6, R^{11} or R^{12} is, independently, optionally substituted with one or more halo, hydroxy, CN, N_3, $N(R^a)_2$ or OR^a; and wherein one or more of the non-terminal carbon atoms of each said $(C_1\text{-}C_8)$alkyl may be optionally replaced with -O-, -S- or $-NR^a$-.

In one embodiment of the invention of Formula II, R^1 is $(C_1\text{–}C_8)$alkyl, $(C_2\text{–}C_8)$

25 alkenyl or $(C_2\text{–}C_8)$alkynyl. In another aspect of this embodiment, R^1 is $(C_1\text{–}C_8)$alkyl. In another aspect of this embodiment, R^1 is methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In a preferred aspect of this embodiment, R^1 is methyl. In another preferred aspect of this embodiment, R^1 is H.

In one embodiment of Formula II, R^2 is H, OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$,

30 halogen, $(C_1\text{–}C_8)$alkyl, $(C_1\text{–}C_8)$substituted alkyl, $(C_2\text{–}C_8)$alkenyl, $(C_2\text{–}C_8)$substituted alkenyl, $(C_2\text{–}C_8)$alkynyl, or $(C_2\text{–}C_8)$substituted alkynyl. In another aspect of this embodiment, R^2 is H, OR^a, $N(R^a)_2$, N_3, CN, SR^a or halogen. In another aspect of this embodiment, R^2 is H, OH, NH_2, N_3, CN, or halogen. In another aspect of this embodiment, R^2 is OR^a or halogen and R^1 is H, $(C_1\text{–}C_8)$alkyl, $(C_2\text{–}C_8)$ alkenyl or

5 (C_2-C_8)alkynyl. In another aspect of this embodiment, R^2 is OR^a or F and R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In a preferred aspect of this embodiment, R^2 is OH and R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In another preferred aspect of this embodiment, R^2 is OR^a and R^1 is H. In another preferred aspect of this embodiment, R^2 is OH and R^1 is H. In another preferred aspect of this embodiment, R^2 is
10 F and R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In another preferred aspect of this embodiment, R^2 is OR^a and R^1 is methyl. In a particularly preferred aspect of this embodiment, R^2 is OH and R^1 is methyl.

In one embodiment of Formula II, R^3 is H, OR^a, $N(R^a)_2$, N_3, CN, SR^a, halogen, (C_1-C_8)alkyl, (C_2-C_8)alkenyl or (C_2-C_8)alkynyl. In one aspect of this embodiment, R^3 is
15 H or F. In a preferred aspect of this embodiment, R^3 is H. In another preferred aspect of this embodiment, R^3 is H, R^2 is OR^a or halogen and R^1 is H, (C_1-C_8)alkyl, (C_2-C_8) alkenyl or (C_2-C_8)alkynyl. In another aspect of this embodiment, R^3 is H, R^2 is OR^a or F and R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In another aspect of this embodiment, R^3 is H, R^2 is OR^a and R^1 is methyl. In another aspect of this embodiment,
20 R^3 is H, R^2 is OH and R^1 is methyl. In another aspect of this embodiment, R^3 is H, R^2 is OR^a or F and R^1 is H. In another aspect of this embodiment, R^3 is H, R^2 is OH and R^1 is H. In another aspect of this embodiment, each R^1, R^3 and R^5 is H and R^2 is OH.

In one embodiment of Formula II, R^4 is H, OR^a, $N(R^a)_2$, N_3, CN, SR^a, halogen, (C_1-C_8)alkyl, (C_2-C_8)alkenyl or (C_2-C_8)alkynyl. In a preferred aspect of this
25 embodiment, R^4 is OR^a. In another preferred aspect of this embodiment, R^4 is OR^a, R^2 is OR^a or halogen and R^1 is H, (C_1-C_8)alkyl, (C_2-C_8) alkenyl or (C_2-C_8)alkynyl. In another preferred aspect of this embodiment, R^4 is OR^a, R^2 is OR^a or halogen and R^1 is H. In another preferred aspect of this embodiment, R^4 is OR^a, R^2 is OR^a or halogen, R^3 is H and R^1 is H, (C_1-C_8)alkyl, (C_2-C_8) alkenyl or (C_2-C_8)alkynyl. In another preferred
30 embodiment R^4 is OR^a, R^2 is OR^a or F and R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In another preferred aspect of this embodiment, R^4 is OR^a, R^2 is OR^a or F, R^3 is H and R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In another preferred aspect of this embodiment, R^4 and R^2 are, independently, OR^a and R^1 is methyl. In another preferred aspect of this embodiment, R^4 and R^2 are, independently OR^a, R^3 is H and R^1 is

5 methyl. In another preferred aspect of this embodiment, R^4 and R^2, taken together, are –O(CO)O-, R^3 is H and R^1 is methyl. In another preferred aspect of this embodiment, one of R^4 or R^2 is OR^a and the other of R^4 or R^2 is OH. . In another preferred aspect of this embodiment, one of R^4 or R^2 is OR^a wherein R^a is not H and the other of R^4 or R^2 is OH, R^3 is H, and R^1 is methyl. In another preferred aspect of this embodiment, R^4 and R^2 are 10 OH, R^3 is H, and R^1 is methyl. In another preferred aspect of this embodiment, R^4 is OR^a, R^2 is OR^a or F, and each R^1 and R^3 is H. In another preferred aspect of this embodiment, R^4 and R^2 are, independently, OR^a and R^1 is H. In another preferred aspect of this embodiment, R^4 and R^2 are, independently OR^a and each R^1 and R^3 is H. In another preferred aspect of this embodiment, R^4 and R^2, taken together, are –O(CO)O-, and each 15 R^1 and R^3 is H.

In one embodiment of Formula II, R^5 is H, OR^a, $N(R^a)_2$, N_3, CN, SR^a, halogen, $(C_1–C_8)$alkyl, $(C_2–C_8)$alkenyl or $(C_2–C_8)$alkynyl. In another aspect of this embodiment, R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $–SO_2NR^{11}R^{12}$, halogen, 20 $(C_1–C_8)$alkyl, $(C_4–C_8)$carbocyclylalkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, $(C_2–C_8)$substituted alkynyl, or aryl(C_1-C_8)alkyl. In another aspepct of this embodiment, R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $–SO_2NR^{11}R^{12}$, halogen, $(C_1–C_8)$alkyl, 25 $(C_4–C_8)$carbocyclylalkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, $(C_2–C_8)$substituted alkynyl, or aryl(C_1-C_8)alkyl and R^5 is H, N_3, CN, $(C_1–C_8)$alkyl, $(C_2–C_8)$alkenyl or $(C_2–C_8)$alkynyl. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $–SO_2NR^{11}R^{12}$, halogen, 30 $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, or $(C_2–C_8)$substituted alkynyl; R^5 is H, N_3, CN, methyl, ethenyl or ethynyl; R^4 is OR^a and R^3 is H. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, -

5 $S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^5 is H or N_3, R^4 is OR^a, R^3 is H, and R^2 is F or OR^a. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or
10 substituted ethynyl, R^5 is H or N_3, R^4 is OR^a, R^3 is H, R^2 is OR^a and R^1 is methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^3 and R^5 are H, R^2 and R^4
15 are, independently, OR^a, and R^1 is methyl. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, each R^1, R^3 and R^5 is H, and R^2 and R^4 are, independently, OR^a. In another aspect of this embodiment, R^6 is OR^a, N_3,
20 CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^3 and R^5 are H, R^2 and R^4 are OH, and R^1 is methyl. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, -
25 $S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^1, R^3 and R^5 are each H and R^2 and R^4 are OH. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl,
30 ethynyl, or substituted ethynyl, R^3 and R^5 are H, R^2 and R^4, taken together, are $-O(CO)O-$, and R^1 is methyl. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, - $S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^1, R^3 and R^5 are each H and R^2 and

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5 R^4, taken together, are –O(CO)O-. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^1 and R^3 are each H, R^2 and R^4 are independently OR^a and R^5 is N_3.

10 In one embodiment of Formula II, R^2 and R^4 are each OR^a and at least one of R^1, R^3, or R^5 is not H. In another aspect of this embodiment, R^2 and R^4 are each OR^a and R^1 is $(C_1–C_8)$alkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, $(C_2–C_8)$substituted alkynyl or aryl(C_1-C_8)alkyl. In another embodiment, R^2 and R^4 are each OR^a and R^3 is $(C_1–C_8)$alkyl, $(C_1–C_8)$substituted alkyl,

15 $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, $(C_2–C_8)$substituted alkynyl or aryl(C_1-C_8)alkyl. In another aspect of this embodiment, R^2 and R^4 are each OR^a and R^5 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, halogen, $(C_1–C_8)$alkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, $(C_2–C_8)$substituted alkynyl or aryl(C_1-C_8)alkyl. In another aspect of this embodiment, R^2 and R^4 are each

20 OR^a and R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, $(C_1–C_8)$alkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, $(C_2–C_8)$substituted alkynyl or aryl(C_1-C_8)alkyl. In another aspect of this embodiment, R^2 and R^4 are both OH and R^6 is OR^a,

25 N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, $(C_1–C_8)$alkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, or $(C_2–C_8)$substituted alkynyl.

In another embodiment of Formula II, each R^1 and R^2 is H, one of R^3 or R^4 is OR^a

30 and the other of R^3 or R^4 is $(C_1–C_8)$alkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, $(C_2–C_8)$substituted alkynyl or aryl(C_1-C_8)alkyl. In another aspect of this embodiment, each R^1 and R^2 is H, one of R^3 or R^4 is OH and the other of R^3 or R^4 is $(C_1–C_8)$alkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl,

5 (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl or aryl(C_1-C_8)alkyl.

In another embodiment of Formula II, R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, (C_1-C_8)alkyl, (C_4-C_8)carbocyclylalkyl,

10 (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl, or aryl(C_1-C_8)alkyl. In another aspect of this embodiment, R^5 is H, OR^a, $N(R^a)_2$, N_3, CN, SR^a, halogen, (C_1-C_8)alkyl, (C_2-C_8)alkenyl or (C_2-C_8)alkynyl. In another aspect of this embodiment, R^5 is H, N_3, CN, (C_1-C_8)alkyl, (C_2-C_8)alkenyl or (C_2-C_8)alkynyl. In another aspect of this embodiment, R^1 is H,

15 methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In another aspect of this embodiment, R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl and R^2 and R^4 are each OR^a. In another aspect of this embodiment, R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl; R^2 and R^4 are each OR^a; and R^3 and R^5 are each H. In another aspect of this embodiment, R^5 is H, N_3, CN, methyl, ethenyl or ethynyl; R^4 is OR^a and R^3 is H. In another aspect of this

20 embodiment, R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl; R^5 is H or N_3; R^4 is OR^a; R^3 is H; and R^2 is OR^a. In another aspect of this embodiment, R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl; R^3 and R^5 are H and R^2 and R^4 are, independently, OR^a. In another aspect of this embodiment, R^6 is OR^a, N_3,

25 halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl; R^3 and R^5 are H; and R^2 and R^4 are each OH. In another aspect of this embodiment, R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl; R^3 and R^5 are H; and R^2 and R^4, taken together, are –O(CO)O-. In another aspect of this embodiment, R^6 is OR^a, N_3, halogen,

30 CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl; R^3 is H; R^2 and R^4 are independently OR^a and R^5 is N_3. In another aspect of this of this embodiment, R^6 is N_3, halogen, CN, methyl, hydroxymethyl, ethenyl or ethynyl. In another aspect of this of this embodiment, R^6 is N_3, halogen, CN, methyl, hydroxymethyl, ethenyl or ethynyl; R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl;

and R^2 and R^4 are each OR^a. In another aspect of this of this embodiment, R^6 is N_3, halogen, CN, methyl, hydroxymethyl, ethenyl or ethynyl; R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl; R^2 and R^4 are each OR^a; and R^3 and R^5 are each H.

In one embodiment of Formula II, R^7 is H, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)SR^{11}$ or

O
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W1—P—
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W2

. In a preferred aspect of this embodiment, R^7 is H. In another preferred aspect of this embodiment, R^7 is $-C(=O)R^{11}$. In another preferred aspect of this embodiment, R^7 is $-C(=O)R^{11}$ wherein R^{11} is $(C_1\text{-}C_8)$alkyl. In another preferred aspect of this embodiment, R^7 is

O
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W2

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In one embodiment of Formula II, X^1 is N or $C\text{-}R^{10}$. In another aspect of this embodiment, X^1 is N. In another aspect of this embodiment, X^1 is $C\text{-}R^{10}$. In another aspect of this embodiment, X^2 is C-H. In another aspect of this embodiment, X^1 is N and X^2 is C-H. In another aspect of this embodiment, X^1 is $C\text{-}R^{10}$ and X^2 is CH. In another aspect of this embodiment, X^1 is C-H and X^2 is CH. In another aspect of this embodiment, X^1 is CR^{10} and R^6 is OR^a, N_3, halogen, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl. In another aspect of this embodiment, X^1 is CR^{10}; X^2 is CH; and R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl. In another aspect of this embodiment, X^1 is CR^{10}; X^2 is CH; R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl; R^3 is H; R^2 and R^4 are each OR^a; and R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl. In another aspect of this embodiment, X^1 is $C\text{-}R^{10}$; X^2 is CH; R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl; each R^3 and R^5 is H;

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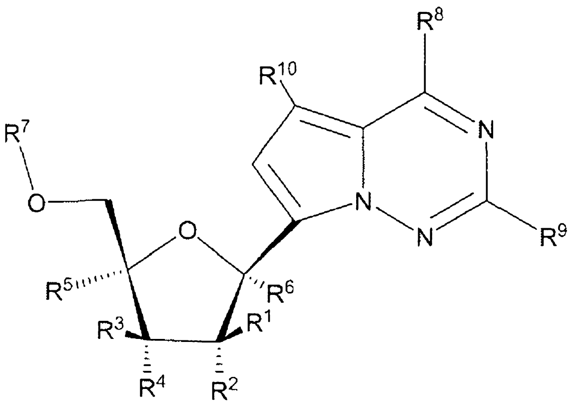
5 R^2 and R^4 are each OR^a; and R^6 is methyl, hydroxymethyl, N_3, halogen or CN. In another aspect of this embodiment, X^1 is N and R^6 is OR^a, N_3, halogen, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl. In another aspect of this embodiment, X^1 is N; X^2 is CH;
10 and R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl. In another aspect of this embodiment, X^1 is N; X^2 is CH; R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl; R^3 is H; R^2 and R^4 are each OR^a; and R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl. In another aspect of this embodiment, X^1 is N; X^2 is CH;
15 R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl; each R^3 and R^5 is H; R^2 and R^4 are each OR^a; and R^6 is methyl, hydroxymethyl, N_3, halogen or CN.

In another embodiment of Formula II, each R^8 is independently halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, N_3, NO, NO_2, CHO, CN, $-CH(=NR^{11})$, $-CH=NHNR^{11}$, $-CH=N(OR^{11})$, $-CH(OR^{11})_2$, $-C(=O)NR^{11}R^{12}$, $-C(=S)NR^{11}R^{12}$, $-C(=O)OR^{11}$, (C_1-C_8)alkyl,
20 (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, (C_4-C_8)carbocyclylalkyl, optionally substituted aryl, optionally substituted heteroaryl, $-C(=O)(C_1-C_8)$alkyl, $-S(O)_n(C_1-C_8)$alkyl, aryl(C_1-C_8)alkyl, OR^{11} or SR^{11}. In another aspect of this embodiment, each R^8 is, independently, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, OR^{11} or SR^{11}. In another aspect of this embodiment, each R^8 is, independently, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$,
25 OR^{11} or SR^{11} and R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In another aspect of this embodiment, each R^8 is, independently, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, OR^{11} or SR^{11} and R^9 is H, halogen, or $NR^{11}R^{12}$. In another aspect of this embodiment, each R^8 is, independently, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, OR^{11} or SR^{11} and R^9 is H, halogen, or $NR^{11}R^{12}$ and R^1 is H, methyl, CH_2OH, CH_2F,
30 ethenyl, or ethynyl. In another preferred aspect of this embodiment, R^8 is NH_2 and R^9 is H or halogen. In another preferred aspect of this embodiment, R^8 is NH_2 and R^9 is H or halogen and R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl. In another preferred aspect of this embodiment, R^8 and R^9 are each NH_2. In another preferred aspect of this embodiment, R^8 and R^9 are each NH_2 and R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or

5 ethynyl. In another preferred aspect of this embodiment, R^8 is OH and R^9 is NH_2. In another preferred aspect of this embodiment, R^8 is OH and R^9 is NH_2 and R^1 is H, methyl, CH_2OH, CH_2F, ethenyl, or ethynyl.

In another embodiment of Formula II, each R^{10} is, independently, H, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, N_3, NO, NO_2, CHO, CN, $-CH(=NR^{11})$,
10 $-CH=NHNR^{11}$, $-CH=N(OR^{11})$, $-CH(OR^{11})_2$, $-C(=O)NR^{11}R^{12}$, $-C(=S)NR^{11}R^{12}$, $-C(=O)OR^{11}$, R^{11}, OR^{11} or SR^{11}. In another aspect of this embodiment, each R^{10} is H, halogen, CN or optionally substituted heteroaryl.

In another aspect, compounds of Formula I are represented by Formula III:



15 Formula III

or a pharmaceutically acceptable salt, thereof;

wherein:

R^1 is H or CH_3;

each R^2, R^3, R^4, or R^5 is independently H, OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$,
20 halogen, (C_1-C_8)alkyl, (C_4-C_8)carbocyclylalkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl, or aryl(C_1-C_8)alkyl;

or any two R^2, R^3, R^4, or R^5 on adjacent carbon atoms when taken together are –O(CO)O- or when taken together with the ring carbon atoms to which they are attached
25 form a double bond;

R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, -$C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$,

5 $-SO_2NR^{11}R^{12}$, halogen, (C_1-C_8)alkyl, (C_4-C_8)carbocyclylalkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl, or aryl(C_1-C_8)alkyl or R^6 and R^2 when taken together are –O(CO)O-;

wherein each (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl or aryl(C_1-C_8)alkyl of each R^2, R^3, R^4, R^5, R^6, R^{11} or R^{12} is, independently, optionally substituted with one or 10 more halo, hydroxy, CN, N_3, $N(R^a)_2$ or OR^a; and wherein one or more of the non-terminal carbon atoms of each said (C_1-C_8)alkyl may be optionally replaced with -O-, -S- or $-NR^a$-; and

all remaining variables are defined as for Formula I.

In one embodiment of Formula III, R^1 is H.

15 In one embodiment of Formula III, R^1 is CH_3.

In one embodiment of Formula III, R^2 is H, OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, halogen, (C_1-C_8)alkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, or (C_2-C_8)substituted alkynyl. In another aspect of this embodiment, R^2 is H, OR^a, $N(R^a)_2$, N_3, CN, SR^a or halogen. In another aspect of this 20 embodiment, R^2 is H, OH, NH_2, N_3, CN, or halogen. In another aspect of this embodiment, R^2 is OR^a or halogen and R^1 is methyl. In another aspect of this embodiment, R^2 is OR^a or halogen and R^1 is H. In another aspect of this embodiment, R^2 is OR^a or F and R^1 is methyl. In another aspect of this embodiment, R^2 is OR^a or F and R^1 is H. In a preferred aspect of this embodiment, R^2 is OH and R^1 is methyl. In another 25 preferred aspect of this embodiment, R^2 is OR^a and R^1 is H. In another preferred aspect of this embodiment, R^2 is OH and R^1 is H. In another preferred aspect of this embodiment, R^2 is F. In another preferred aspect of this embodiment, R^2 is OR^a and R^1 is methyl.

In one embodiment of Formula III, R^3 is H, OR^a, $N(R^a)_2$, N_3, CN, SR^a, halogen, 30 (C_1-C_8)alkyl, (C_2-C_8)alkenyl or (C_2-C_8)alkynyl. In one aspect of this embodiment, R^3 is H or F. In a preferred aspect of this embodiment, R^3 is H. In another preferred aspect of this embodiment, R^3 is H, R^2 is OR^a or halogen and R^1 is methyl. In another preferred aspect of this embodiment, R^3 is H, R^2 is OR^a or halogen and R^1 is H. In another aspect of this embodiment, R^3 is H, R^2 is OR^a or F and R^1 is methyl. In another aspect of this

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5 embodiment, R^3 is H, R^2 is OR^a or F and R^1 is H. In another aspect of this embodiment, R^3 is H, R^2 is OR^a and R^1 is methyl. In another aspect of this embodiment, R^3 is H, R^2 is OH and R^1 is methyl. In another aspect of this embodiment, R^3 is H, R^2 is OR^a and R^1 is H. In another aspect of this embodiment, R^3 is H, R^2 is OH and R^1 is H. In another aspect of this embodiment, each R^1, R^3 and R^5 is H and R^2 is OH.

10 In one embodiment of Formula III, R^4 is H, OR^a, $N(R^a)_2$, N_3, CN, SR^a, halogen, $(C_1–C_8)$alkyl, $(C_2–C_8)$alkenyl or $(C_2–C_8)$alkynyl. In a preferred aspect of this embodiment, R^4 is OR^a. In another preferred aspect of this embodiment, R^4 is OR^a, R^2 is OR^a or halogen and R^1 is methyl. In another preferred aspect of this embodiment, R^4 is OR^a, R^2 is OR^a or halogen and R^1 is H. In another preferred aspect of this embodiment, 15 R^4 is OR^a, R^2 is OR^a or halogen, R^3 is H and R^1 is methyl. In another preferred aspect of this embodiment, R^4 is OR^a, R^2 is OR^a or halogen, R^3 is H and R^1 is H. In another preferred embodiment R^4 is OR^a, R^2 is OR^a or F and R^1 is methyl. In another preferred embodiment R^4 is OR^a, R^2 is OR^a or F and R^1 is H. In another preferred aspect of this embodiment, R^4 is OR^a, R^2 is OR^a or F, R^3 is H and R^1 is methyl. In another preferred 20 aspect of this embodiment, R^4 and R^2 are, independently, OR^a and R^1 is methyl. In another preferred aspect of this embodiment, R^4 and R^2 are, independently OR^a, R^3 is H and R^1 is methyl. In another preferred aspect of this embodiment, R^4 and R^2, taken together, are –O(CO)O-, R^3 is H and R^1 is methyl. In another preferred aspect of this embodiment, R^4 and R^2, taken together, are –O(CO)O-, R^3 is H and R^1 is H. In another 25 preferred aspect of this embodiment, one of R^4 or R^2 is OR^a and the other of R^4 or R^2 is OH. In another preferred aspect of this embodiment, one of R^4 or R^2 is OR^a wherein R^a is not H and the other of R^4 or R^2 is OH, R^3 is H, and R^1 is methyl. In another preferred aspect of this embodiment, one of R^4 or R^2 is OR^a wherein R^a is not H and the other of R^4 or R^2 is OH, R^3 is H, and R^1 is H. In another preferred aspect of this embodiment, R^4 and 30 R^2 are OH, R^3 is H, and R^1 is methyl. In another preferred aspect of this embodiment, R^4 and R^2 are OH, R^3 is H, and R^1 is H. In another preferred aspect of this embodiment, R^4 is OR^a, R^2 is OR^a or F, and each R^1 and R^3 is H. In another preferred aspect of this embodiment, R^4 and R^2 are, independently, OR^a and R^1 is H. In another preferred aspect of this embodiment, R^4 and R^2 are, independently OR^a and each R^1 and R^3 is H.

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5 In one embodiment of Formula III, R^5 is H, OR^a, $N(R^a)_2$, N_3, CN, SR^a, halogen, (C_1-C_8)alkyl, (C_2-C_8)alkenyl or (C_2-C_8)alkynyl. In another aspect of this embodiment, R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, (C_1-C_8)alkyl, (C_4-C_8)carbocyclylalkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl,
10 (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl, or aryl(C_1-C_8)alkyl and R^5 is H, OR^a, $N(R^a)_2$, N_3, CN, SR^a, halogen, (C_1-C_8)alkyl, (C_2-C_8)alkenyl or (C_2-C_8)alkynyl. In another aspect of this embodiment, R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, (C_1-C_8)alkyl,
15 (C_4-C_8)carbocyclylalkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl, or aryl(C_1-C_8)alkyl and R^5 is H, N_3, CN, (C_1-C_8)alkyl, (C_2-C_8)alkenyl or (C_2-C_8)alkynyl. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen,
20 (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, or (C_2-C_8)substituted alkynyl; R^5 is H, N_3, CN, methyl, ethenyl or ethynyl; R^4 is OR^a and R^3 is H. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl,
25 ethynyl, or substituted ethynyl, R^5 is H or N_3, R^4 is OR^a, R^3 is H, and R^2 is F or OR^a. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^5 is H or N_3, R^4 is OR^a, R^3 is H, R^2 is OR^a and R^1 is methyl. In
30 another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^3 and R^5 are H, R^2 and R^4 are, independently, OR^a, and R^1 is

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5 methyl. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^3 and R^5 are H, R^2 and R^4 are OH, and R^1 is methyl. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$,
10 $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^1, R^3 and R^5 are each H and R^2 and R^4 are OH. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen,
15 substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^3 and R^5 are H, R^2 and R^4, taken together, are –O(CO)O-, and R^1 is methyl. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^1, R^3
20 and R^5 are each H and R^2 and R^4, taken together, are –O(CO)O-. In another aspect of this embodiment, R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl, R^1 and R^3 are each H, R^2 and R^4 are independently OR^a and R^5 is N_3.

25 In one embodiment of Formula III, R^2 and R^4 are each OR^a and at least one of R^1, R^3, or R^5 is not H. In another aspect of this embodiment, R^2 and R^4 are each OR^a and R^1 methyl. In another embodiment, R^2 and R^4 are each OR^a and R^3 is $(C_1–C_8)$alkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, $(C_2–C_8)$substituted alkynyl or aryl$(C_1$-$C_8)$alkyl. In another aspect of this embodiment, R^2
30 and R^4 are each OR^a and R^5 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, halogen, $(C_1–C_8)$alkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, $(C_2–C_8)$substituted alkenyl, $(C_2–C_8)$alkynyl, $(C_2–C_8)$substituted alkynyl or aryl$(C_1$-$C_8)$alkyl. In another aspect of this embodiment, R^2 and R^4 are each OR^a and R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, -

5 $S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, (C_1-C_8)alkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl or aryl(C_1-C_8)alkyl. In another aspect of this embodiment, R^2 and R^4 are both OH and R^6 is OR^a, N_3, CN, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen,

10 (C_1-C_8)alkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, or (C_2-C_8)substituted alkynyl.

In another embodiment of Formula III, each R^1 and R^2 is H, one of R^3 or R^4 is OR^a and the other of R^3 or R^4 is (C_1-C_8)alkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl or aryl$(C_1-$

15 $C_8)$alkyl. In another aspect of this embodiment, each R^1 and R^2 is H, one of R^3 or R^4 is OR^a and the other of R^3 or R^4 is (C_1-C_8)alkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl or aryl(C_1-C_8)alkyl.

In another embodiment of Formula III, R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$,

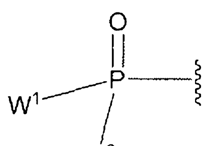
20 $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, (C_1-C_8)alkyl, (C_4-C_8)carbocyclylalkyl, (C_1-C_8)substituted alkyl, (C_2-C_8)alkenyl, (C_2-C_8)substituted alkenyl, (C_2-C_8)alkynyl, (C_2-C_8)substituted alkynyl, or aryl(C_1-C_8)alkyl. In another aspect of this embodiment, R^5 is H, N_3, CN, (C_1-C_8)alkyl, (C_2-C_8)alkenyl or

25 (C_2-C_8)alkynyl. In another aspect of this embodiment, R^1 is H. In another aspect of this embodiment, R^1 is methyl. In another aspect of this embodiment, R^1 is H and R^2 and R^4 are each OR^a. In another aspect of this embodiment, R^1 is methyl and R^2 and R^4 are each OR^a. In another aspect of this embodiment, R^1 is H; R^2 and R^4 are each OR^a; and R^3 and R^5 are each H. In another aspect of this embodiment, R^1 is methyl; R^2 and R^4 are each

30 OR^a; and R^3 and R^5 are each H. In another aspect of this embodiment, R^5 is H, N_3, CN, methyl, ethenyl or ethynyl; R^4 is OR^a and R^3 is H. In another aspect of this embodiment, R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl; R^5 is H or N_3; R^4 is OR^a; R^3 is H; and R^2 is OR^a. In

5 another aspect of this embodiment, R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl; R^3 and R^5 are H and R^2 and R^4 are, independently, OR^a. In another aspect of this embodiment, R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl; R^3 and R^5 are H; and R^2 and R^4 are each OH. In another aspect of
10 this embodiment, R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl; R^3 and R^5 are H; and R^2 and R^4, taken together, are –O(CO)O-. In another aspect of this embodiment, R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl; R^3 is H; R^2 and R^4 are independently OR^a and R^5 is N_3. In another aspect of this
15 of this embodiment, R^6 is N_3, halogen, CN, methyl, hydroxymethyl, ethenyl or ethynyl. In another aspect of this of this embodiment, R^6 is N_3, halogen, CN, methyl, hydroxymethyl, ethenyl or ethynyl; R^1 is H; and R^2 and R^4 are each OR^a. In another aspect of this of this embodiment, R^6 is N_3, halogen, CN, methyl, hydroxymethyl, ethenyl or ethynyl; R^1 is methyl; and R^2 and R^4 are each OR^a. In another aspect of this of this
20 embodiment, R^6 is N_3, halogen, CN, methyl, hydroxymethyl, ethenyl or ethynyl; R^1 is H; R^2 and R^4 are each OR^a; and R^3 and R^5 are each H. In another aspect of this of this embodiment, R^6 is N_3, halogen, CN, methyl, hydroxymethyl, ethenyl or ethynyl; R^1 is methyl; R^2 and R^4 are each OR^a; and R^3 and R^5 are each H.

In one embodiment of Formula III, R^7 is H, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)SR^{11}$
25 or



. In a preferred aspect of this embodiment, R^7 is H. In another preferred aspect of this embodiment, R^7 is H and R^1 is H. In another preferred aspect of this embodiment, R^7 is $-C(=O)R^{11}$. In another preferred aspect of this embodiment, R^7 is $-C(=O)R^{11}$ and R^1 is H. In another preferred aspect of this embodiment, R^7 is $-C(=O)R^{11}$
30 wherein R^{11} is $(C_1\text{-}C_8)$alkyl. In another preferred aspect of this embodiment, R^7 is $-C(=O)R^{11}$ wherein R^{11} is $(C_1\text{-}C_8)$alkyl and R^1 is H. In another preferred aspect of this embodiment, R^7 is

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In another preferred aspect of this embodiment, R^7 is

and R^1 is H.

In another embodiment of Formula III, each R^8 is independently halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, N_3, NO, NO_2, CHO, CN, $-CH(=NR^{11})$, $-CH=NHNR^{11}$, $-CH=N(OR^{11})$, $-CH(OR^{11})_2$, $-C(=O)NR^{11}R^{12}$, $-C(=S)NR^{11}R^{12}$, $-C(=O)OR^{11}$, (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, (C_4-C_8)carbocyclylalkyl, optionally substituted aryl, optionally substituted heteroaryl, $-C(=O)(C_1-C_8)$alkyl, -$S(O)_n(C_1-C_8)$alkyl, aryl(C_1-C_8)alkyl, OR^{11} or SR^{11}. In another aspect of this embodiment, each R^8 is, independently, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, OR^{11} or SR^{11}. In another aspect of this embodiment, each R^8 is, independently, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, OR^{11} or SR^{11} and R^1 is H. In another aspect of this embodiment, each R^8 is, independently, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, OR^{11} or SR^{11} and R^1 is methyl. In another aspect of this embodiment, each R^8 is, independently, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, OR^{11} or SR^{11} and R^9 is H, halogen, or $NR^{11}R^{12}$. In another aspect of this embodiment, each R^8 is, independently, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, OR^{11} or SR^{11} and R^9 is H, halogen, or $NR^{11}R^{12}$ and R^1 is H. In another aspect of this embodiment, each R^8 is, independently, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, OR^{11} or SR^{11} and R^9 is H, halogen, or $NR^{11}R^{12}$ and R^1 is methyl. In another preferred aspect of this embodiment, R^8 is NH_2 and R^9 is H or halogen. In another preferred aspect of this embodiment, R^8 is NH_2 and R^9 is H or halogen and R^1 is H. In another preferred aspect of this embodiment, R^8 is NH_2 and R^9 is H or halogen and R^1 is methyl. In another preferred aspect of this embodiment, R^8 and R^9 are each NH_2. In another preferred aspect of this embodiment,

5 R^8 and R^9 are each NH_2 and R^1 is H. In another preferred aspect of this embodiment, R^8 and R^9 are each NH_2 and R^1 is methyl. In another preferred aspect of this embodiment, R^8 is OH and R^9 is NH_2. In another preferred aspect of this embodiment, R^8 is OH and R^9 is NH_2 and R^1 is H. In another preferred aspect of this embodiment, R^8 is OH and R^9 is NH_2 and R^1 is methyl.

10 In another embodiment of Formula III, each R^{10} is, independently, H, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, N_3, NO, NO_2, CHO, CN, $-CH(=NR^{11})$, $-CH=NHNR^{11}$, $-CH=N(OR^{11})$, $-CH(OR^{11})_2$, $-C(=O)NR^{11}R^{12}$, $-C(=S)NR^{11}R^{12}$, $-C(=O)OR^{11}$, R^{11}, OR^{11} or SR^{11}. In another aspect of this embodiment, R^6 is OR^a, N_3, halogen, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, -
15 $S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl. In another aspect of this embodiment, each R^{10} is H, halogen, CN or optionally substituted heteroaryl and R^6 is OR^a, N_3, halogen, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, - $S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, CN, methyl, substituted methyl,
20 ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl. In another aspect of this embodiment, R^{10} is H and R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl. In another aspect of this embodiment, R^3 is H; R^2 and R^4 are each OR^a; and R^6 is OR^a, N_3, halogen, CN, methyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl. In
25 another aspect of this embodiment, each R^3 and R^5 is H; R^2 and R^4 are each OR^a; and R^6 is methyl, hydroxymethyl, N_3, halogen or CN.

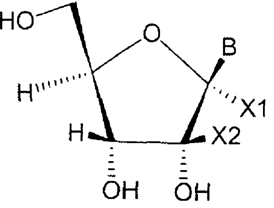
In one embodiment of Formulas I-III, R^{11} or R^{12} is independently H, $(C_1$-$C_8)$alkyl, $(C_2$-$C_8)$alkenyl, $(C_2$-$C_8)$alkynyl, $(C_4$–$C_8)$carbocyclylalkyl, optionally substituted aryl, optionally substituted heteroaryl, $-C(=O)(C_1$-$C_8)$alkyl, $-S(O)_n(C_1$-$C_8)$alkyl or
30 aryl$(C_1$-$C_8)$alkyl. In another embodiment, R^{11} and R^{12} taken together with a nitrogen to which they are both attached, form a 3 to 7 membered heterocyclic ring wherein any one carbon atom of said heterocyclic ring can optionally be replaced with -O-, -S- or $-NR^a$-. Therefore, by way of example and not limitation, the moiety $-NR^{11}R^{12}$ can be represented by the heterocycles:

and the like.

In another embodiment of Formulas I-III, R^2, R^3, R^4, R^5, R^6, R^{11} or R^{12} is, independently, $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl or aryl$(C_1\text{-}C_8)$alkyl, wherein said $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl or aryl$(C_1\text{-}C_8)$alkyl are, independently, optionally substituted with one or more halo, hydroxy, CN, N_3, $N(R^a)_2$ or OR^a. Therefore, by way of example and not limitation, R^2, R^3, R^4, R^5, R^6, R^{11} or R^{12} could represent moieties such as $-CH(NH_2)CH_3$, -CH(OH)CH2CH3, $-CH(NH_2)CH(CH_3)_2$, $-CH_2CF_3$, $-(CH_2)_2CH(N_3)CH_3$, $-(CH_2)_6NH_2$ and the like.

In another embodiment of Formula I-III, R^2, R^3, R^4, R^5, R^6, R^{11} or R^{12} is $(C_1\text{-}C_8)$alkyl wherein one or more of the non-terminal carbon atoms of each said $(C_1\text{-}C_8)$alkyl may be optionally replaced with -O-, -S- or $-NR^a$-. Therefore, by way of example and not limitation, R^2, R^3, R^4, R^5, R^6, R^{11} or R^{12} could represent moieties such as $-CH_2OCH_3$, $-CH_2OCH_2CH_3$, $-CH_2OCH(CH_3)_2$, $-CH_2SCH_3$, $-(CH_2)_6OCH_3$, $-(CH_2)_6N(CH_3)_2$ and the like.

In still another embodiment, the compounds of Formula I, Formula II, or Formula III are named below in tabular format (Table 6) as compounds of general Formula IV:



Formula IV

wherein X1 and X2, represent substituents attached to the tetrahydrofuranyl ring as defined in Tables 1-2, below; B is a purine defined in Table 4, below; and X3 represents a ring element of the purine base B as described in Table 3, below.

The point of attachment of the core structure ribose is indicated in each of the structures of X1, X2, and B. The point of attachment of the core structure purine is indicated in each of the structures X3. Each structure in Tables 1-4 is represented by an alphanumeric "code". Each structure of a compound of Formula IV can thus be

designated in tabular form by combining the "code" representing each structural moiety using the following syntax: X1.X2.X3.B. Thus, for example, X1a.X2c.X3a.B1 represents the following structure:

Table 1: X1 Structures

Code	Structure
X1a	CN
X1b	CH_3
X1c	N_3
X1d	CH_2OH

Table 2: X2 Structures

Code	Structure
X2a	H
X2b	CH_3
X2c	≡—H

Table 3: X3 Structures

Code	Structure
X3a	-N=

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Code	Structure
X3b	-CH=
X3c	-CF=

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Table 4: B Structures

Code	Structure
B1	[structure: NH_2, X3, N, N, N]
B2	[structure: OH, X3, N, N, N, NH_2]
B3	[structure: NH_2, X3, N, N, N, NH_2]
B4	[structure: $NHCH_3$, X3, N, N, N]

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5 Table 6: List of Compounds of Formula IV

X1a.X2b.X3a.B1, X1a.X2b.X3a.B2, X1a.X2b.X3a.B3, X1a.X2b.X3a.B4,
X1a.X2b.X3b.B1, X1a.X2b.X3b.B2, X1a.X2b.X3b.B3, X1a.X2b.X3b.B4,
X1a.X2b.X3c.B1, X1a.X2b.X3c.B2, X1a.X2b.X3c.B3, X1a.X2b.X3c.B4,
X1a.X2c.X3a.B1, X1a.X2c.X3a.B2, X1a.X2c.X3a.B3, X1a.X2c.X3a.B4,
10 X1a.X2c.X3b.B1, X1a.X2c.X3b.B2, X1a.X2c.X3b.B3, X1a.X2c.X3b.B4,
X1a.X2c.X3c.B1, X1a.X2c.X3c.B2, X1a.X2c.X3c.B3, X1a.X2c.X3c.B4,
X1b.X2a.X3a.B1, X1b.X2a.X3a.B2, X1b.X2a.X3a.B3, X1b.X2a.X3a.B4,
X1b.X2a.X3b.B1, X1b.X2a.X3b.B2, X1b.X2a.X3b.B3, X1b.X2a.X3b.B4,
X1b.X2a.X3c.B1, X1b.X2a.X3c.B2, X1b.X2a.X3c.B3, X1b.X2a.X3c.B4,
15 X1b.X2b.X3a.B1, X1b.X2b.X3a.B2, X1b.X2b.X3a.B3, X1b.X2b.X3a.B4,
X1b.X2b.X3b.B1, X1b.X2b.X3b.B2, X1b.X2b.X3b.B3, X1b.X2b.X3b.B4,
X1b.X2b.X3c.B1, X1b.X2b.X3c.B2, X1b.X2b.X3c.B3, X1b.X2b.X3c.B4,
X1b.X2c.X3a.B1, X1b.X2c.X3a.B2, X1b.X2c.X3a.B3, X1b.X2c.X3a.B4,
X1b.X2c.X3b.B1, X1b.X2c.X3b.B2, X1b.X2c.X3b.B3, X1b.X2c.X3b.B4,
20 X1b.X2c.X3c.B1, X1b.X2c.X3c.B2, X1b.X2c.X3c.B3, X1b.X2c.X3c.B4,
X1c.X2a.X3a.B1, X1c.X2a.X3a.B2, X1c.X2a.X3a.B3, X1c.X2a.X3a.B4,
X1c.X2a.X3b.B1, X1c.X2a.X3b.B2, X1c.X2a.X3b.B3, X1c.X2a.X3b.B4,
X1c.X2a.X3c.B1, X1c.X2a.X3c.B2, X1c.X2a.X3c.B3, X1c.X2a.X3c.B4,
X1c.X2b.X3a.B1, X1c.X2b.X3a.B2, X1c.X2b.X3a.B3, X1c.X2b.X3a.B4,
25 X1c.X2b.X3b.B1, X1c.X2b.X3b.B2, X1c.X2b.X3b.B3, X1c.X2b.X3b.B4,
X1c.X2b.X3c.B1, X1c.X2b.X3c.B2, X1c.X2b.X3c.B3, X1c.X2b.X3c.B4,
X1c.X2c.X3a.B1, X1c.X2c.X3a.B2, X1c.X2c.X3a.B3, X1c.X2c.X3a.B4,
X1c.X2c.X3b.B1, X1c.X2c.X3b.B2, X1c.X2c.X3b.B3, X1c.X2c.X3b.B4,
X1c.X2c.X3c.B1, X1c.X2c.X3c.B2, X1c.X2c.X3c.B3, X1c.X2c.X3c.B4,
30 X1d.X2a.X3a.B1, X1d.X2a.X3a.B2, X1d.X2a.X3a.B3, X1d.X2a.X3a.B4,
X1d.X2a.X3b.B1, X1d.X2a.X3b.B2, X1d.X2a.X3b.B3, X1d.X2a.X3b.B4,
X1d.X2a.X3c.B1, X1d.X2a.X3c.B2, X1d.X2a.X3c.B3, X1d.X2a.X3c.B4.

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5 In another embodiment, Formulas I-III is a compound selected from the group consisting of

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10 or ;

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5 or a pharmaceutically acceptable salt thereof.

DEFINITIONS

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

10 When trade names are used herein, applicants intend to independently include the tradename product and the active pharmaceutical ingredient(s) of the tradename product.

As used herein, "a compound of the invention" or "a compound of Formula I" means a compound of Formula I or a pharmaceutically acceptable salt, thereof. Similarly, with respect to isolatable intermediates, the phrase "a compound of Formula

15 (number)" means a compound of that formula and pharmaceutically acceptable salts, thereof.

"Alkyl" is hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. For example, an alkyl group can have 1 to 20 carbon atoms (*i.e*, C_1-C_{20} alkyl), 1 to 8 carbon atoms (*i.e.*, C_1-C_8 alkyl), or 1 to 6 carbon atoms (*i.e.*, C_1-C_6 alkyl). Examples

20 of suitable alkyl groups include, but are not limited to, methyl (Me, $-CH_3$), ethyl (Et, $-CH_2CH_3$), 1-propyl (n-Pr, n-propyl, $-CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, $-CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, $-CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, $-CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, $-CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, $-C(CH_3)_3$), 1-pentyl (n-pentyl, $-CH_2CH_2CH_2CH_2CH_3$), 2-pentyl

25 ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($-CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($-C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl ($-CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl ($-CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl ($-CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($-CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl ($-C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-

30 pentyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($-CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl ($-C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl ($-CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl ($-C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl ($-CH(CH_3)C(CH_3)_3$, and octyl ($-(CH_2)_7CH_3$).

"Alkoxy" means a group having the formula –O-alkyl, in which an alkyl group,

5 as defined above, is attached to the parent molecule via an oxygen atom. The alkyl portion of an alkoxy group can have 1 to 20 carbon atoms (*i.e.*, C_1-C_{20} alkoxy), 1 to 12 carbon atoms(*i.e.*, C_1-C_{12} alkoxy), or 1 to 6 carbon atoms(*i.e.*, C_1-C_6 alkoxy). Examples of suitable alkoxy groups include, but are not limited to, methoxy ($-O-CH_3$ or –OMe), ethoxy ($-OCH_2CH_3$ or -OEt), t-butoxy ($-O-C(CH_3)_3$ or –OtBu) and the like.

10 "Haloalkyl" is an alkyl group, as defined above, in which one or more hydrogen atoms of the alkyl group is replaced with a halogen atom. The alkyl portion of a haloalkyl group can have 1 to 20 carbon atoms (*i.e.*, C_1-C_{20} haloalkyl), 1 to 12 carbon atoms(*i.e.*, C_1-C_{12} haloalkyl), or 1 to 6 carbon atoms(*i.e.*, C_1-C_6 alkyl). Examples of suitable haloalkyl groups include, but are not limited to, $-CF_3$, $-CHF_2$, $-CFH_2$, $-CH_2CF_3$,

15 and the like.

"Alkenyl" is a hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, *i.e.* a carbon-carbon, sp^2 double bond. For example, an alkenyl group can have 2 to 20 carbon atoms (*i.e.*, C_2-C_{20} alkenyl), 2 to 8 carbon atoms (*i.e.*, C_2-C_8 alkenyl), or 2 to 6 carbon atoms (*i.e.*, C_2-C_6 alkenyl).

20 Examples of suitable alkenyl groups include, but are not limited to, ethylene or vinyl ($-CH=CH_2$), allyl ($-CH_2CH=CH_2$), cyclopentenyl ($-C_5H_7$), and 5-hexenyl ($-CH_2CH_2CH_2CH_2CH=CH_2$).

"Alkynyl" is a hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, *i.e.* a carbon-carbon, *sp* triple bond.

25 For example, an alkynyl group can have 2 to 20 carbon atoms (*i.e.*, C_2-C_{20} alkynyl), 2 to 8 carbon atoms (*i.e.*, C_2-C_8 alkyne,), or 2 to 6 carbon atoms (*i.e.*, C_2-C_6 alkynyl). Examples of suitable alkynyl groups include, but are not limited to, acetylenic ($-C\equiv CH$), propargyl ($-CH_2C\equiv CH$), and the like.

"Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon

30 radical having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. For example, an alkylene group can have 1 to 20 carbon atoms, 1 to 10 carbon atoms, or 1 to 6 carbon atoms. Typical alkylene radicals include, but are not limited to, methylene ($-CH_2-$),

5 1,1-ethyl (-CH(CH_3)-), 1,2-ethyl (-CH_2CH_2-), 1,1-propyl (-CH(CH_2CH_3)-), 1,2-propyl (-CH_2CH(CH_3)-), 1,3-propyl (-$CH_2CH_2CH_2$-), 1,4-butyl (-$CH_2CH_2CH_2CH_2$-), and the like.

"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. For 10 example, and alkenylene group can have 1 to 20 carbon atoms, 1 to 10 carbon atoms, or 1 to 6 carbon atoms. Typical alkenylene radicals include, but are not limited to, 1,2-ethylene (-CH=CH-).

"Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical having two monovalent radical centers derived by the removal of two 15 hydrogen atoms from the same or two different carbon atoms of a parent alkyne. For example, an alkynylene group can have 1 to 20 carbon atoms, 1 to 10 carbon atoms, or 1 to 6 carbon atoms. Typical alkynylene radicals include, but are not limited to, acetylene (-C≡C-), propargyl (-CH_2C≡C-), and 4-pentynyl (-$CH_2CH_2CH_2$C≡C-).

"Amino" refers generally to a nitrogen radical which can be considered a derivative 20 of ammonia, having the formula –N(X)$_2$, where each "X" is independently H, substituted or unsubstituted alkyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, etc. The hybridization of the nitrogen is approximately sp^3. Nonlimiting types of amino include –NH_2, -N(alkyl)$_2$, -NH(alkyl), -N(carbocyclyl)$_2$, -NH(carbocyclyl), -N(heterocyclyl)$_2$, -NH(heterocyclyl), -N(aryl)$_2$, -NH(aryl), -N(alkyl)(aryl), - 25 N(alkyl)(heterocyclyl), -N(carbocyclyl)(heterocyclyl), -N(aryl)(heteroaryl), -N(alkyl)(heteroaryl), etc. The term "alkylamino" refers to an amino group substituted with at least one alkyl group. Nonlimiting examples of amino groups include –NH_2, -NH(CH_3), -N(CH_3)$_2$, -NH(CH_2CH_3), - N(CH_2CH_3)$_2$, -NH(phenyl), -N(phenyl)$_2$, -NH(benzyl), -N(benzyl)$_2$, etc. Substituted alkylamino refers generally to alkylamino groups, as defined 30 above, in which at least one substituted alkyl, as defined herein, is attached to the amino nitrogen atom. Non-limiting examples of substituted alkylamino includes -NH(alkylene-C(O)-OH), -NH(alkylene-C(O)-O-alkyl), -N(alkylene-C(O)-OH)$_2$, -N(alkylene-C(O)-O-alkyl)$_2$, etc.

5 "Aryl" means an aromatic hydrocarbon radical derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. For example, an aryl group can have 6 to 20 carbon atoms, 6 to 14 carbon atoms, or 6 to 10 carbon atoms. Typical aryl groups include, but are not limited to, radicals derived from benzene (e.g., phenyl), substituted benzene, naphthalene, anthracene, biphenyl, and the like.

10 "Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group can comprise 7 to 20 carbon atoms, *e.g.*, the alkyl

15 moiety is 1 to 6 carbon atoms and the aryl moiety is 6 to 14 carbon atoms.

"Arylalkenyl" refers to an acyclic alkenyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, but also an sp^2 carbon atom, is replaced with an aryl radical. The aryl portion of the arylalkenyl can include, for example, any of the aryl groups disclosed herein, and the alkenyl portion of

20 the arylalkenyl can include, for example, any of the alkenyl groups disclosed herein. The arylalkenyl group can comprise 8 to 20 carbon atoms, *e.g.*, the alkenyl moiety is 2 to 6 carbon atoms and the aryl moiety is 6 to 14 carbon atoms.

"Arylalkynyl" refers to an acyclic alkynyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, but also an sp

25 carbon atom, is replaced with an aryl radical. The aryl portion of the arylalkynyl can include, for example, any of the aryl groups disclosed herein, and the alkynyl portion of the arylalkynyl can include, for example, any of the alkynyl groups disclosed herein. The arylalkynyl group can comprise 8 to 20 carbon atoms, *e.g.*, the alkynyl moiety is 2 to 6 carbon atoms and the aryl moiety is 6 to 14 carbon atoms.

30 The term "substituted" in reference to alkyl, alkylene, aryl, arylalkyl, alkoxy, heterocyclyl, heteroaryl, carbocyclyl, etc. , for example, "substituted alkyl", "substituted alkylene", "substituted aryl", "substituted arylalkyl", "substituted heterocyclyl", and "substituted carbocyclyl" means alkyl, alkylene, aryl, arylalkyl, heterocyclyl, carbocyclyl respectively, in which one or more hydrogen atoms are each independently

5 replaced with a non-hydrogen substituent. Typical substituents include, but are not limited to, -X, $-R^b$, $-O^-$, =O, $-OR^b$, $-SR^b$, $-S^-$, $-NR^b_2$, $-N^+R^b_3$, $=NR^b$, $-CX_3$, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, $-NO_2$, $=N_2$, $-N_3$, $-NHC(=O)R^b$, $-OC(=O)R^b$, $-NHC(=O)NR^b_2$, $-S(=O)_2-$, $-S(=O)_2OH$, $-S(=O)_2R^b$, $-OS(=O)_2OR^b$, $-S(=O)_2NR^b_2$, $-S(=O)R^b$, $-OP(=O)(OR^b)_2$, $-P(=O)(OR^b)_2$, $-P(=O)(O^-)_2$, $-P(=O)(OH)_2$, $-P(O)(OR^b)(O^-)$,
10 $-C(=O)R^b$, -C(=O)X, $-C(S)R^b$, $-C(O)OR^b$, $-C(O)O^-$, $-C(S)OR^b$, $-C(O)SR^b$, $-C(S)SR^b$, $-C(O)NR^b_2$, $-C(S)NR^b_2$, $-C(=NR^b)NR^b_2$, where each X is independently a halogen: F, Cl, Br, or I; and each R^b is independently H, alkyl, aryl, arylalkyl, a heterocycle, or a protecting group or prodrug moiety. Alkylene, alkenylene, and alkynylene groups may also be similarly substituted. Unless otherwise indicated, when the term "substituted" is
15 used in conjunction with groups such as arylalkyl, which have two or more moieties capable of substitution, the substituents can be attached to the aryl moiety, the alkyl moiety, or both.

The term "prodrug" as used herein refers to any compound that when administered to a biological system generates the drug substance, i.e., active ingredient, as a result of
20 spontaneous chemical reaction(s), enzyme catalyzed chemical reaction(s), photolysis, and/or metabolic chemical reaction(s). A prodrug is thus a covalently modified analog or latent form of a therapeutically active compound.

One skilled in the art will recognize that substituents and other moieties of the compounds of Formula I-III should be selected in order to provide a compound which is
25 sufficiently stable to provide a pharmaceutically useful compound which can be formulated into an acceptably stable pharmaceutical composition. Compounds of Formula I-III which have such stability are contemplated as falling within the scope of the present invention.

"Heteroalkyl" refers to an alkyl group where one or more carbon atoms have been replaced with a heteroatom, such as, O, N, or S. For example, if the carbon atom of the
30 alkyl group which is attached to the parent molecule is replaced with a heteroatom (e.g., O, N, or S) the resulting heteroalkyl groups are, respectively, an alkoxy group (e.g., $-OCH_3$, etc.), an amine (e.g., $-NHCH_3$, $-N(CH_3)_2$, etc.), or a thioalkyl group (e.g., $-SCH_3$). If a non-terminal carbon atom of the alkyl group which is not attached to the parent molecule is replaced with a heteroatom (e.g., O, N, or S) the resulting heteroalkyl groups are,

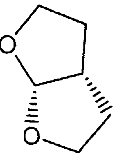
respectively, an alkyl ether (e.g., $-CH_2CH_2-O-CH_3$, etc.), an alkyl amine (e.g., $-CH_2NHCH_3$, $-CH_2N(CH_3)_2$, etc.), or a thioalkyl ether (e.g.,$-CH_2-S-CH_3$). If a terminal carbon atom of the alkyl group is replaced with a heteroatom (e.g., O, N, or S), the resulting heteroalkyl groups are, respectively, a hydroxyalkyl group (e.g., $-CH_2CH_2-OH$), an aminoalkyl group (e.g., $-CH_2NH_2$), or an alkyl thiol group (e.g., $-CH_2CH_2-SH$). A heteroalkyl group can have, for example, 1 to 20 carbon atoms, 1 to 10 carbon atoms, or 1 to 6 carbon atoms. A C_1-C_6 heteroalkyl group means a heteroalkyl group having 1 to 6 carbon atoms.

"Heterocycle" or "heterocyclyl" as used herein includes by way of example and not limitation those heterocycles described in Paquette, Leo A.; Principles of Modern Heterocyclic Chemistry (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; The Chemistry of Heterocyclic Compounds, A Series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and *J. Am. Chem. Soc.* (1960) 82:5566. In one specific embodiment of the invention "heterocycle" includes a "carbocycle" as defined herein, wherein one or more (*e.g.* 1, 2, 3, or 4) carbon atoms have been replaced with a heteroatom (*e.g.* O, N, or S). The terms "heterocycle" or "heterocyclyl" includes saturated rings, partially unsaturated rings, and aromatic rings (*i.e.*, heteroaromatic rings). Substituted heterocyclyls include, for example, heterocyclic rings substituted with any of the substituents disclosed herein including carbonyl groups. A non-limiting example of a carbonyl substituted heterocyclyl is:

N NH
O

Examples of heterocycles include by way of example and not limitation pyridyl, dihydroypyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl,

5 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazoly, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, ß-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl,
10 phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, isatinoyl, and bis-tetrahydrofuranyl:



15 By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or
20 isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-
25 pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example and not limitation, nitrogen bonded heterocycles are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2
30 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or ß-carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

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5 "Heterocyclylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heterocyclyl radical (*i.e.*, a heterocyclyl-alkylene- moiety). Typical heterocyclyl alkyl groups include, but are not limited to heterocyclyl-CH_2-, 2-(heterocyclyl)ethan-1-yl, and the like, wherein the "heterocyclyl" portion includes any of
10 the heterocyclyl groups described above, including those described in Principles of Modern Heterocyclic Chemistry. One skilled in the art will also understand that the heterocyclyl group can be attached to the alkyl portion of the heterocyclyl alkyl by means of a carbon-carbon bond or a carbon-heteroatom bond, with the proviso that the resulting group is chemically stable. The heterocyclyl alkyl group comprises 3 to 20
15 carbon atoms, *e.g.*, the alkyl portion of the arylalkyl group is 1 to 6 carbon atoms and the heterocyclyl moiety is 2 to 14 carbon atoms. Examples of heterocyclylalkyls include by way of example and not limitation 5-membered sulfur, oxygen, and/or nitrogen containing heterocycles such as thiazolylmethyl, 2-thiazolylethan-1-yl, imidazolylmethyl, oxazolylmethyl, thiadiazolylmethyl, etc., 6-membered sulfur, oxygen,
20 and/or nitrogen containing heterocycles such as piperidinylmethyl, piperazinylmethyl, morpholinylmethyl, pyridinylmethyl, pyridizylmethyl, pyrimidylmethyl, pyrazinylmethyl, etc.

"Heterocyclylalkenyl" refers to an acyclic alkenyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, but
25 also a sp^2 carbon atom, is replaced with a heterocyclyl radical (*i.e.*, a heterocyclyl-alkenylene- moiety). The heterocyclyl portion of the heterocyclyl alkenyl group includes any of the heterocyclyl groups described herein, including those described in Principles of Modern Heterocyclic Chemistry, and the alkenyl portion of the heterocyclyl alkenyl group includes any of the alkenyl groups disclosed herein. One skilled in the art will
30 also understand that the heterocyclyl group can be attached to the alkenyl portion of the heterocyclyl alkenyl by means of a carbon-carbon bond or a carbon-heteroatom bond, with the proviso that the resulting group is chemically stable. The heterocyclyl alkenyl group comprises 4 to 20 carbon atoms, *e.g.*, the alkenyl portion of the heterocyclyl alkenyl group is 2 to 6 carbon atoms and the heterocyclyl moiety is 2 to 14 carbon atoms.

5 "Heterocyclylalkynyl" refers to an acyclic alkynyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, but also an sp carbon atom, is replaced with a heterocyclyl radical (*i.e.*, a heterocyclyl-alkynylene- moiety). The heterocyclyl portion of the heterocyclyl alkynyl group includes any of the heterocyclyl groups described herein, including those described in
10 Principles of Modern Heterocyclic Chemistry, and the alkynyl portion of the heterocyclyl alkynyl group includes any of the alkynyl groups disclosed herein. One skilled in the art will also understand that the heterocyclyl group can be attached to the alkynyl portion of the heterocyclyl alkynyl by means of a carbon-carbon bond or a carbon-heteroatom bond, with the proviso that the resulting group is chemically stable.
15 The heterocyclyl alkynyl group comprises 4 to 20 carbon atoms, *e.g.*, the alkynyl portion of the heterocyclyl alkynyl group is 2 to 6 carbon atoms and the heterocyclyl moiety is 2 to 14 carbon atoms.

"Heteroaryl" refers to an aromatic heterocyclyl having at least one heteroatom in the ring. Non-limiting examples of suitable heteroatoms which can be included in the
20 aromatic ring include oxygen, sulfur, and nitrogen. Non-limiting examples of heteroaryl rings include all of those aromatic rings listed in the definition of "heterocyclyl", including pyridinyl, pyrrolyl, oxazolyl, indolyl, isoindolyl, purinyl, furanyl, thienyl, benzofuranyl, benzothiophenyl, carbazolyl, imidazolyl, thiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, quinolyl, isoquinolyl, pyridazyl, pyrimidyl, pyrazyl, etc.
25 "Carbocycle" or "carbocyclyl" refers to a saturated (i.e., cycloalkyl), partially unsaturated (e.g., cycloakenyl, cycloalkadienyl, etc.) or aromatic ring having 3 to 7 carbon atoms as a monocycle, 7 to 12 carbon atoms as a bicycle, and up to about 20 carbon atoms as a polycycle. Monocyclic carbocycles have 3 to 7 ring atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, *e.g.*, arranged
30 as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system, or spiro-fused rings. Non-limiting examples of monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, and phenyl. Non-limiting examples of bicyclo carbocycles includes

5 naphthyl, tetrahydronapthalene, and decaline.

"Carbocyclylalkyl" refers to to an acyclic akyl radical in which one of the hydrogen atoms bonded to a carbon atom is replaced with a carbocyclyl radical as described herein. Typical, but non-limiting, examples of carbocyclylalkyl groups include cyclopropylmethyl, cyclopropylethyl, cyclobutylmethyl, cyclopentylmethyl and 10 cyclohexylmethyl.

"Arylheteroalkyl" refers to a heteroalkyl as defined herein, in which a hydrogen atom (which may be attached either to a carbon atom or a heteroatom) has been replaced with an aryl group as defined herein. The aryl groups may be bonded to a carbon atom of the heteroalkyl group, or to a heteroatom of the heteroalkyl group, provided that the 15 resulting arylheteroalkyl group provides a chemically stable moiety. For example, an arylheteroalkyl group can have the general formulae -alkylene-O-aryl, -alkylene-O-alkylene-aryl, -alkylene-NH-aryl, -alkylene-NH-alkylene-aryl, -alkylene-S-aryl, -alkylene-S-alkylene-aryl, etc. In addition, any of the alkylene moieties in the general formulae above can be further substituted with any of the substituents defined or 20 exemplified herein.

"Heteroarylalkyl" refers to an alkyl group, as defined herein, in which a hydrogen atom has been replaced with a heteroaryl group as defined herein. Non-limiting examples of heteroaryl alkyl include -CH_2-pyridinyl, -CH_2-pyrrolyl, -CH_2-oxazolyl, -CH_2-indolyl, -CH_2-isoindolyl, -CH_2-purinyl, -CH_2-furanyl, -CH_2-thienyl, 25 -CH_2-benzofuranyl, -CH_2-benzothiophenyl, -CH_2-carbazolyl, -CH_2-imidazolyl, -CH_2-thiazolyl, -CH_2-isoxazolyl, -CH_2-pyrazolyl, -CH_2-isothiazolyl, -CH_2-quinolyl, -CH_2-isoquinolyl, -CH_2-pyridazyl, -CH_2-pyrimidyl, -CH_2-pyrazyl, -$CH(CH_3)$-pyridinyl, -$CH(CH_3)$-pyrrolyl, -$CH(CH_3)$-oxazolyl, -$CH(CH_3)$-indolyl, -$CH(CH_3)$-isoindolyl, -$CH(CH_3)$-purinyl, -$CH(CH_3)$-furanyl, -$CH(CH_3)$-thienyl, -$CH(CH_3)$-benzofuranyl, 30 -$CH(CH_3)$-benzothiophenyl, -$CH(CH_3)$-carbazolyl, -$CH(CH_3)$-imidazolyl, -$CH(CH_3)$-thiazolyl, -$CH(CH_3)$-isoxazolyl, -$CH(CH_3)$-pyrazolyl, -$CH(CH_3)$-isothiazolyl, -$CH(CH_3)$-quinolyl, -$CH(CH_3)$-isoquinolyl, -$CH(CH_3)$-pyridazyl, -$CH(CH_3)$-pyrimidyl, -$CH(CH_3)$-pyrazyl, etc.

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The term “optionally substituted” in reference to a particular moiety of the compound of Formula I-III (e.g., an optionally substituted aryl group) refers to a moiety wherein all substiutents are hydrogen or wherein one or more of the hydrogens of the moiety may be replaced by substituents such as those listed under the definition of “substituted”.

The term “optionally replaced” in reference to a particular moiety of the compound of Formula I-III (e.g., the carbon atoms of said (C_1-C_8)alkyl may be optionally replaced by –O-, -S-, or –NR^a-) means that one or more of the methylene groups of the (C_1-C_8)alkyl may be replaced by 0, 1, 2, or more of the groups specified (e.g., –O-, -S-, or –NR^a-).

The term “non-terminal carbon atom(s)” in reference to an alkyl, alkenyl, alkynyl, alkylene, alkenylene, or alkynylene moiety refers to the carbon atoms in the moiety that intervene between the first carbon atom of the moiety and the last carbon atom in the moiety. Therefore, by way of example and not limitation, in the alkyl moiety $-CH_2(C^*)H_2(C^*)H_2CH_3$ or alkylene moiety $-CH_2(C^*)H_2(C^*)H_2CH_2-$ the C^* atoms would be considered to be the non-terminal carbon atoms.

Certain Y and Y^1 alternatives are nitrogen oxides such as $^+N(O)(R)$ or $^+N(O)(OR)$. These nitrogen oxides, as shown here attached to a carbon atom, can also be represented by charge separated groups such as $=N^+(O^-)-R$ or $=N^+(O^-)-OR$, respectively, and are intended to be equivalent to the aforementioned representations for the purposes of describing this invention.

"Linker" or “link” means a chemical moiety comprising a covalent bond or a chain of atoms. Linkers include repeating units of alkyloxy (e.g. polyethyleneoxy, PEG, polymethyleneoxy) and alkylamino (e.g. polyethyleneamino, Jeffamine™); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide.

The terms such as “oxygen-linked”, “nitrogen-linked”, “carbon-linked”, “sulfur-linked”, or “phosphorous-linked” mean that if a bond between two moieties can be

formed by using more than one type of atom in a moiety, then the bond formed between the moieties is through the atom specified. For example, a nitrogen-linked amino acid would be bonded through a nitrogen atom of the amino acid rather than through an oxygen or carbon atom of the amino acid.

Unless otherwise specified, the carbon atoms of the compounds of Formula I-III are intended to have a valence of four. In some chemical structure representations where carbon atoms do not have a sufficient number of variables attached to produce a valence of four, the remaining carbon substitutents needed to provide a valence of four should be assumed to be hydrogen. For example,

has the same meaning as

.

"Protecting group" refers to a moiety of a compound that masks or alters the properties of a functional group or the properties of the compound as a whole. The chemical substructure of a protecting group varies widely. One function of a protecting group is to serve as an intermediate in the synthesis of the parental drug substance. Chemical protecting groups and strategies for protection/deprotection are well known in

5 the art. See: "Protective Groups in Organic Chemistry", Theodora W. Greene (John Wiley & Sons, Inc., New York, 1991. Protecting groups are often utilized to mask the reactivity of certain functional groups, to assist in the efficiency of desired chemical reactions, e.g. making and breaking chemical bonds in an ordered and planned fashion. Protection of functional groups of a compound alters other physical properties besides 10 the reactivity of the protected functional group, such as the polarity, lipophilicity (hydrophobicity), and other properties which can be measured by common analytical tools. Chemically protected intermediates may themselves be biologically active or inactive.

Protected compounds may also exhibit altered, and in some cases, optimized 15 properties *in vitro* and *in vivo*, such as passage through cellular membranes and resistance to enzymatic degradation or sequestration. In this role, protected compounds with intended therapeutic effects may be referred to as prodrugs. Another function of a protecting group is to convert the parental drug into a prodrug, whereby the parental drug is released upon conversion of the prodrug *in vivo*. Because active prodrugs may be 20 absorbed more effectively than the parental drug, prodrugs may possess greater potency *in vivo* than the parental drug. Protecting groups are removed either *in vitro,* in the instance of chemical intermediates, or *in vivo*, in the case of prodrugs. With chemical intermediates, it is not particularly important that the resulting products after deprotection, e.g. alcohols, be physiologically acceptable, although in general it is more 25 desirable if the products are pharmacologically innocuous.

"Prodrug moiety" means a labile functional group which separates from the active inhibitory compound during metabolism, systemically, inside a cell, by hydrolysis, enzymatic cleavage, or by some other process (Bundgaard, Hans, "Design and Application of Prodrugs" in Textbook of Drug Design and Development (1991), P. Krogsgaard-30 Larsen and H. Bundgaard, Eds. Harwood Academic Publishers, pp. 113-191). Enzymes which are capable of an enzymatic activation mechanism with the phosphonate prodrug compounds of the invention include, but are not limited to, amidases, esterases, microbial enzymes, phospholipases, cholinesterases, and phosphases. Prodrug moieties can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery,

5 bioavailability and efficacy.

A prodrug moiety may include an active metabolite or drug itself.

Exemplary prodrug moieties include the hydrolytically sensitive or labile acyloxymethyl esters $-CH_2OC(=O)R^{30}$ and acyloxymethyl carbonates $-CH_2OC(=O)OR^{30}$ where R^{30} is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl or
10 C_6-C_{20} substituted aryl. The acyloxyalkyl ester was used as a prodrug strategy for carboxylic acids and then applied to phosphates and phosphonates by Farquhar et al (1983) *J. Pharm. Sci.* 72: 324; also US Patent Nos. 4816570, 4968788, 5663159 and 5792756. In certain compounds of the invention, a prodrug moiety is part of a phosphate group. The acyloxyalkyl ester may be used to deliver phosphoric acids across cell
15 membranes and to enhance oral bioavailability. A close variant of the acyloxyalkyl ester, the alkoxycarbonyloxyalkyl ester (carbonate), may also enhance oral bioavailability as a prodrug moiety in the compounds of the combinations of the invention. An exemplary acyloxymethyl ester is pivaloyloxymethoxy, (POM) $-CH_2OC(=O)C(CH_3)_3$. An exemplary acyloxymethyl carbonate prodrug moiety is
20 pivaloyloxymethylcarbonate (POC) $-CH_2OC(=O)OC(CH_3)_3$.

The phosphate group may be a phosphate prodrug moiety. The prodrug moiety may be sensitive to hydrolysis, such as, but not limited to those comprising a pivaloyloxymethyl carbonate (POC) or POM group. Alternatively, the prodrug moiety may be sensitive to enzymatic potentiated cleavage, such as a lactate ester or a
25 phosphonamidate-ester group.

Aryl esters of phosphorus groups, especially phenyl esters, are reported to enhance oral bioavailability (DeLambert et al (1994) *J. Med. Chem.* 37: 498). Phenyl esters containing a carboxylic ester ortho to the phosphate have also been described (Khamnei and Torrence, (1996) *J. Med. Chem.* 39:4109-4115). Benzyl esters are
30 reported to generate the parent phosphonic acid. In some cases, substituents at the *ortho-* or *para*-position may accelerate the hydrolysis. Benzyl analogs with an acylated phenol or an alkylated phenol may generate the phenolic compound through the action of enzymes, e.g. esterases, oxidases, etc., which in turn undergoes cleavage at the benzylic C–O bond to generate the phosphoric acid and the quinone methide intermediate.

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5 Examples of this class of prodrugs are described by Mitchell et al (1992) *J. Chem. Soc. Perkin Trans. I* 2345; Brook et al WO 91/19721. Still other benzylic prodrugs have been described containing a carboxylic ester-containing group attached to the benzylic methylene (Glazier et al WO 91/19721). Thio-containing prodrugs are reported to be useful for the intracellular delivery of phosphonate drugs. These proesters contain an
10 ethylthio group in which the thiol group is either esterified with an acyl group or combined with another thiol group to form a disulfide. Deesterification or reduction of the disulfide generates the free thio intermediate which subsequently breaks down to the phosphoric acid and episulfide (Puech et al (1993) *Antiviral Res.*, 22: 155-174; Benzaria et al (1996) *J. Med. Chem.* 39: 4958). Cyclic phosphonate esters have also been
15 described as prodrugs of phosphorus-containing compounds (Erion et al, US Patent No. 6312662).

It is to be noted that all enantiomers, diastereomers, and racemic mixtures, tautomers, polymorphs, pseudopolymorphs of compounds within the scope of Formula I, Formula II, or Formula III and pharmaceutically acceptable salts thereof are embraced by
20 the present invention. All mixtures of such enantiomers and diastereomers are within the scope of the present invention.

A compound of Formula I-III and its pharmaceutically acceptable salts may exist as different polymorphs or pseudopolymorphs. As used herein, crystalline polymorphism means the ability of a crystalline compound to exist in different crystal
25 structures. The crystalline polymorphism may result from differences in crystal packing (packing polymorphism) or differences in packing between different conformers of the same molecule (conformational polymorphism). As used herein, crystalline pseudopolymorphism means the ability of a hydrate or solvate of a compound to exist in different crystal structures. The pseudopolymorphs of the instant invention may exist
30 due to differences in crystal packing (packing pseudopolymorphism) or due to differences in packing between different conformers of the same molecule (conformational pseudopolymorphism). The instant invention comprises all polymorphs and pseudopolymorphs of the compounds of Formula I-III and their pharmaceutically acceptable salts.

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5 A compound of Formula I-III and its pharmaceutically acceptable salts may also exist as an amorphous solid. As used herein, an amorphous solid is a solid in which there is no long-range order of the positions of the atoms in the solid. This definition applies as well when the crystal size is two nanometers or less. Additives, including solvents, may be used to create the amorphous forms of the instant invention. The 10 instant invention comprises all amorphous forms of the compounds of Formula I-III and their pharmaceutically acceptable salts.

Selected substituents comprising the compounds of Formula I-III are present to a recursive degree. In this context, "recursive substituent" means that a substituent may recite another instance of itself. Because of the recursive nature of such substituents, 15 theoretically, a large number of compounds may be present in any given embodiment. For example, R^x comprises a R^y substituent. R^y can be R. R can be W^3. W^3 can be W^4 and W^4 can be R or comprise substituents comprising R^y. One of ordinary skill in the art of medicinal chemistry understands that the total number of such substituents is reasonably limited by the desired properties of the compound intended. Such properties 20 include, by way of example and not limitation, physical properties such as molecular weight, solubility or log P, application properties such as activity against the intended target, and practical properties such as ease of synthesis.

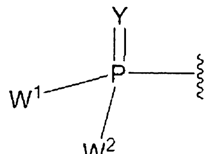
By way of example and not limitation, W^3 and R^y are recursive substituents in certain embodiments. Typically, each recursive substituent can independently occur 20, 25 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0, times in a given embodiment. More typically, each recursive substituent can independently occur 12 or fewer times in a given embodiment. Even more typically, each recursive substituent can independently occur 3 or fewer times in a given embodiment. For example, W^3 will occur 0 to 8 times, R^y will occur 0 to 6 times in a given embodiment. Even more 30 typically, W^3 will occur 0 to 6 times and R^y will occur 0 to 4 times in a given embodiment.

Recursive substituents are an intended aspect of the invention. One of ordinary skill in the art of medicinal chemistry understands the versatility of such substituents. To

5 the degree that recursive substituents are present in an embodiment of the invention, the total number will be determined as set forth above.

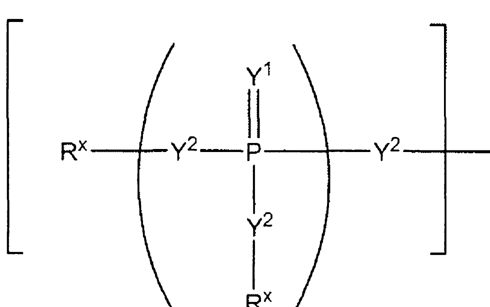
The modifier "about" used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (e.g., includes the degree of error associated with measurement of the particular quantity).

10 The compounds of the Formula I-III may comprise a phosphate group as R^7,



which may be a prodrug moiety wherein each Y or Y^1 is, independently, O, S, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, or $N{-}NR_2$; W^1 and W^2, when taken together, are $-Y^3(C(R^y)_2)_3Y^3-$; or one of W^1 or W^2 together with either R^3or R^4 is $-Y^3-$ and the other of W^1 or W^2 is Formula Ia; or W^1 and W^2 are each, independently, a

15 group of Formula Ia:



wherein:

each Y^2 is independently a bond, O, CR_2, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, $N{-}NR_2$, S, S–S, S(O), or $S(O)_2$;

20 each Y^3 is independently O, S, or NR;

M2 is 0, 1 or 2;

each R^y is independently H, F, Cl, Br, I, OH, R, $-C(=Y^1)R$, $-C(=Y^1)OR$, $-C(=Y^1)N(R)_2$, $-N(R)_2$, $-^+N(R)_3$, -SR, -S(O)R, $-S(O)_2R$, -S(O)(OR), $-S(O)_2(OR)$, $-OC(=Y^1)R$, $-OC(=Y^1)OR$, $-OC(=Y^1)(N(R)_2)$, $-SC(=Y^1)R$, $-SC(=Y^1)OR$, -

25 $SC(=Y^1)(N(R)_2)$, $-N(R)C(=Y^1)R$, $-N(R)C(=Y^1)OR$, or $-N(R)C(=Y^1)N(R)_2$, $-SO_2NR_2$,

$-CN$, $-N_3$, $-NO_2$, $-OR$, a protecting group or W^3; or when taken together, two R^y on the same carbon atom form a carbocyclic ring of 3 to 7 carbon atoms;

each R^x is independently R^y, a protecting group, or the formula:

M1a M12c M1c M1d ;

wherein:

M1a, M1c, and M1d are independently 0 or 1;

M12c is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

each R is H, halogen, $(C_1\text{-}C_8)$ alkyl, $(C_1\text{-}C_8)$ substituted alkyl, $(C_2\text{-}C_8)$ alkenyl, $(C_2\text{-}C_8)$ substituted alkenyl, $(C_2\text{-}C_8)$ alkynyl, $(C_2\text{-}C_8)$ substituted alkynyl, $C_6\text{–}C_{20}$ aryl, $C_6\text{–}C_{20}$ substituted aryl, $C_2\text{–}C_{20}$ heterocycle, $C_2\text{–}C_{20}$ substituted heterocyclyl, arylalkyl, substituted arylalkyl or a protecting group;

W^3 is W^4 or W^5; W^4 is R, $-C(Y^1)R^y$, $-C(Y^1)W^5$, $-SO_2R^y$, or $-SO_2W^5$; and W^5 is a carbocycle or a heterocycle wherein W^5 is independently substituted with 0 to 3 R^y groups.

W^5 carbocycles and W^5 heterocycles may be independently substituted with 0 to 3 R^y groups. W^5 may be a saturated, unsaturated or aromatic ring comprising a mono- or bicyclic carbocycle or heterocycle. W^5 may have 3 to 10 ring atoms, e.g., 3 to 7 ring atoms. The W^5 rings are saturated when containing 3 ring atoms, saturated or mono-unsaturated when containing 4 ring atoms, saturated, or mono- or di-unsaturated when containing 5 ring atoms, and saturated, mono- or di-unsaturated, or aromatic when containing 6 ring atoms.

A W^5 heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S). W^5 heterocyclic monocycles may have 3 to 6 ring atoms (2 to 5 carbon atoms and 1 to 2 heteroatoms selected from N, O, and S); or 5 or 6 ring atoms (3 to 5 carbon atoms and 1

5 to 2 heteroatoms selected from N and S). W^5 heterocyclic bicycles have 7 to 10 ring atoms (6 to 9 carbon atoms and 1 to 2 heteroatoms selected from N, O, and S) arranged as a bicyclo [4,5], [5,5], [5,6], or [6,6] system; or 9 to 10 ring atoms (8 to 9 carbon atoms and 1 to 2 hetero atoms selected from N and S) arranged as a bicyclo [5,6] or [6,6] system. The W^5 heterocycle may be bonded to Y^2 through a carbon, nitrogen, sulfur or
10 other atom by a stable covalent bond.

W^5 heterocycles include for example, pyridyl, dihydropyridyl isomers, piperidine, pyridazinyl, pyrimidinyl, pyrazinyl, s-triazinyl, oxazolyl, imidazolyl, thiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, furanyl, thiofuranyl, thienyl, and pyrrolyl. W^5 also includes, but is not limited to, examples such as:

15

, , ,

N , H N N , N N H ,

S N , N S , and N S .

W^5 carbocycles and heterocycles may be independently substituted with 0 to 3 R groups, as defined above. For example, substituted W^5 carbocycles include:

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Examples of substituted phenyl carbocycles include:

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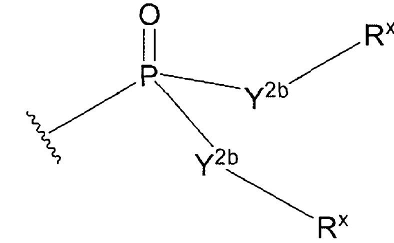
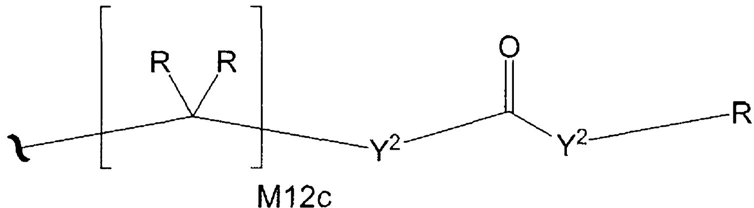
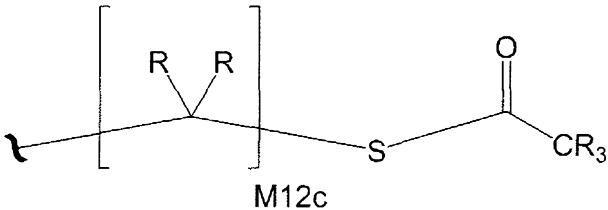
Embodiments of of Formula I-III compounds include substructures such as:



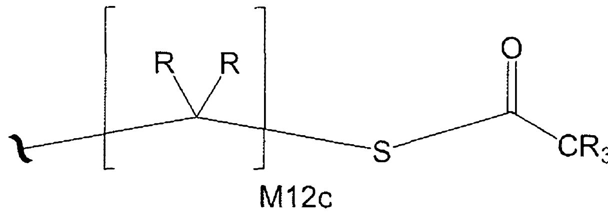
wherein each Y^{2b} is, independently, O or N(R). In a preferred aspect of this embodiment, each Y^{2b} is O and each R^x is independently:



wherein M12c is 1, 2 or 3 and each Y^2 is independently a bond, O, CR_2, or S. In another preferred aspect of this embodiment, one Y^{2b}-R^x is NH(R) and the other Y^{2b}-R^x is O-R^x wherein R^x is:



wherein M12c is 2. In another preferred aspect of this embodiment, each Y^{2b} is O and each R^x is independently:



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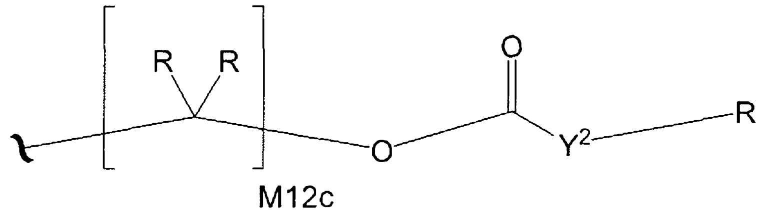
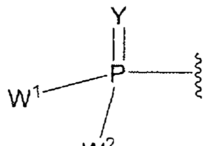
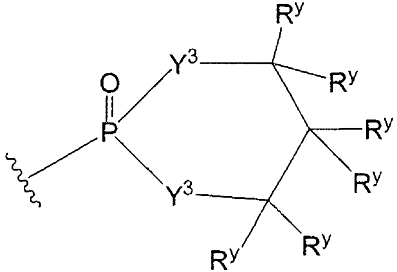
5 wherein M12c is 2. In another preferred aspect of this embodiment, each Y^{2b} is O and each R^x is independently:



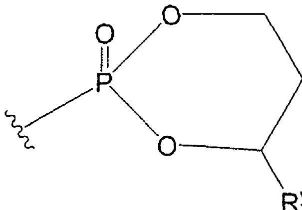
wherein M12c is 1 and Y^2 is a bond, O, or CR_2.



10 Other embodiments of [structure above] of Formulas I-III compounds include substructures such as:



wherein each Y^3 is, independently, O or N(R). In a preferred aspect of this embodiment, each Y^3 is O. In another preferred aspect of this embodiment, the substructure is:



15

wherein R^y is W^5 as defined herein.

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5 Another embodiment of [structure: W^1–P(=Y)(–W^2)–~] of Formula I-III includes the substructures:

[structure]

;

wherein each Y^{2c} is, independently, O, $N(R^y)$ or S.

Another embodiment of [structure: W^1–P(=Y)(–W^2)–~] of Formula I-III compounds includes
10 the substructures wherein one of W^1 or W^2 together with either R^3 or R^4 is $-Y^3-$ and the other of W^1 or W^2 is Formula Ia. Such an embodiment is represented by a compound of Formula Ib selected from:

[structure]

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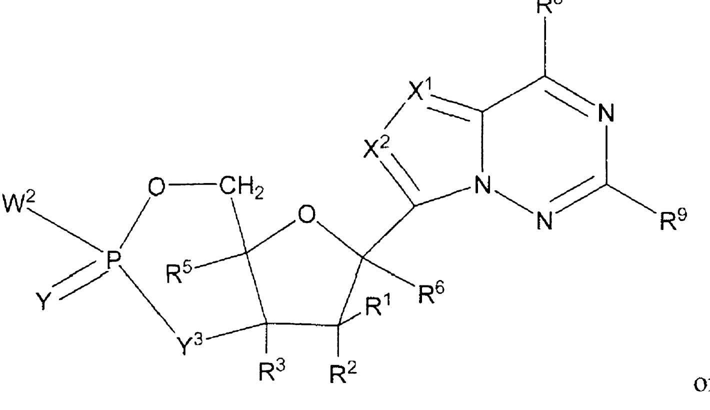


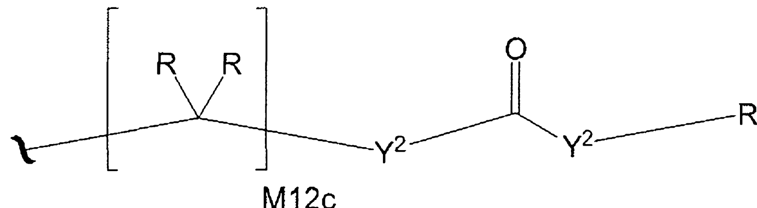
or

.

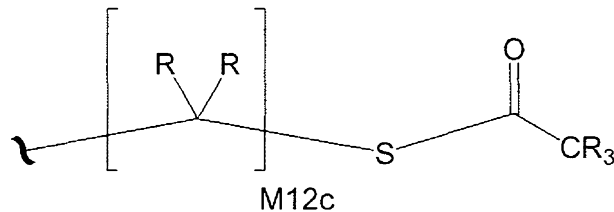
Formula Ib

In a preferred aspect of the embodiment of Formula Ib, each Y and Y^3 is O. In another

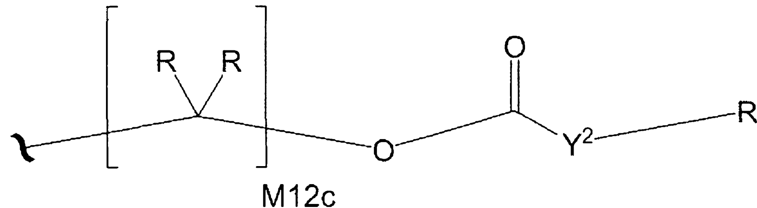
10 preferred aspect of the embodiment of Formula Ib, W^1 or W^2 is Y^{2b}-R^x; each Y, Y^3 and Y^{2b} is O and R^x is:



wherein M12c is 1, 2 or 3 and each Y^2 is independently a bond, O, CR_2, or S. In another preferred aspect of the embodiment of Formula Ib, W^1 or W^2 is Y^{2b}-R^x; each Y, Y^3 and Y^{2b} is O and R^x is:



wherein M12c is 2. In another preferred aspect of the embodiment of Formula Ib, W^1 or W^2 is Y^{2b}-R^x; each Y, Y^3 and Y^{2b} is O and R^x is:



wherein M12c is 1 and Y^2 is a bond, O, or CR_2.

Another embodiment of of Formula I-III compounds includes a substructure:

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wherein W^5 is a carbocycle such as phenyl or substituted phenyl. In another aspect of this embodiment, the substructure is:

wherein Y^{2b} is O or N(R) and the phenyl carbocycle is substituted with 0 to 3 R groups.

10 In another aspect of this embodiment of the substructure, R^x is:

wherein M12c is 1, 2 or 3 and each Y^2 is independently a bond, O, CR_2, or S.

Another embodiment of [structure] of Formula I-III includes substructures:

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5 and .

The chiral carbon of the amino acid and lactate moieties may be either the *R* or *S* configuration or the racemic mixture.

Another embodiment of of Formula I-III is substructure

10 wherein each Y^2 is, independently, –O- or -NH-. In another preferred aspect of this embodiment, R^y is (C_1-C_8) alkyl, (C_1-C_8) substituted alkyl, (C_2-C_8) alkenyl, (C_2-C_8) substituted alkenyl, (C_2-C_8) alkynyl or (C_2-C_8) substituted alkynyl. In another preferred aspect of this embodiment, R^y is (C_1-C_8) alkyl, (C_1-C_8) substituted alkyl, (C_2-C_8) alkenyl, (C_2-C_8) substituted alkenyl, (C_2-C_8) alkynyl or (C_2-C_8) substituted alkynyl; and R is CH_3.
15 In another preferred aspect of this embodiment, R^y is (C_1-C_8) alkyl, (C_1-C_8) substituted alkyl, (C_2-C_8) alkenyl, (C_2-C_8) substituted alkenyl, (C_2-C_8) alkynyl or (C_2-C_8) substituted alkynyl; R is CH_3; and each Y^2 is –NH-. In a preferred aspect of this embodiment, W^1 and W^2 are, independently, nitrogen-linked, naturally occurring amino acids or naturally occurring amino acid esters. In another preferred aspect of this embodiment, W^1 and W^2
20 are, independently, naturally-occurring 2-hydroxy carboxylic acids or naturally-

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5 occurring 2-hydroxy carboxylic acid esters wherein the acid or ester is linked to P through the 2-hydroxy group.

Another embodiment of [structure: Y=P(W1)(W2)–] of Formula I, Formula II, or Formula III is substructure:

[structure: –P(=O)(OR^x)(OR^x)]

10 In one preferred aspect of this embodiment, each R^x is, independently, $(C_1\text{-}C_8)$ alkyl. In another preferred aspect of this embodiment, each R^x is, independently, $C_6\text{-}C_{20}$ aryl or C-$_6$-C_{20} substituted aryl.

Another embodiment of [structure: Y=P(W1)(W2)–] of Formulas I-III is substructure

[structure: O=P(W1)(W2)–]

15

wherein W^1 and W^2 are independently selected from one of the formulas in Tables 20.1-20.37 and Table 30.1 below. The variables used in Tables 20.1-20.37 (e.g., W^{23}, R^{21}, etc.) pertain only to Tables 20.1-20.37, unless otherwise indicated.

The variables used in Tables 20.1 to 20.37 have the following definitions:

20 each R^{21} is independently H or $(C_1\text{-}C_8)$alkyl;

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5 each R^{22} is independently H, R^{21}, R^{23} or R^{24} wherein each R^{24} is independently substituted with 0 to 3 R^{23};

each R^{23} is independently R^{23a}, R^{23b}, R^{23c} or R^{23d}, provided that when R^{23} is bound to a heteroatom, then R^{23} is R^{23c} or R^{23d};

each R^{23a} is independently F, Cl, Br, I, -CN, N_3 or $-NO_2$;

10 each R^{23b} is independently Y^{21};

each R^{23c} is independently $-R^{2x}$, $-N(R^{2x})(R^{2x})$, $-SR^{2x}$, $-S(O)R^{2x}$, $-S(O)_2R^{2x}$, $-S(O)(OR^{2x})$, $-S(O)_2(OR^{2x})$, $-OC(=Y^{21})R^{2x}$, $-OC(=Y^{21})OR^{2x}$, $-OC(=Y^{21})(N(R^{2x})(R^{2x}))$, $-SC(=Y^{21})R^{2x}$, $-SC(=Y^{21})OR^{2x}$, $-SC(=Y^{21})(N(R^{2x})(R^{2x}))$, $-N(R^{2x})C(=Y^{21})R^{2x}$, $-N(R^{2x})C(=Y^{21})OR^{2x}$, or $-N(R^{2x})C(=Y^{21})(N(R^{2x})(R^{2x}))$;

15 each R^{23d} is independently $-C(=Y^{21})R^{2x}$, $-C(=Y^{21})OR^{2x}$ or $-C(=Y^{21})(N(R^{2x})(R^{2x}))$;

each R^{2x} is independently H, $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl, aryl, heteroaryl; or two R^{2x} taken together with a nitrogen to which they are both attached form a 3 to 7 membered heterocyclic ring wherein any one carbon atom of said heterocyclic ring can optionally be replaced with -O-, -S- or $-NR^{21}$-; and wherein one or

20 more of the non-terminal carbon atoms of each said $(C_1\text{-}C_8)$alkyl may be optionally replaced with -O-, -S- or $-NR^{21}$-;

each R^{24} is independently $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, or $(C_2\text{-}C_8)$alkynyl;

each R^{25} is independently R^{24} wherein each R^{24} is substituted with 0 to 3 R^{23} groups;

25 each R^{25a} is independently $(C_1\text{-}C_8)$alkylene, $(C_2\text{-}C_8)$alkenylene, or $(C_2\text{-}C_8)$alkynylene any one of which said $(C_1\text{-}C_8)$alkylene, $(C_2\text{-}C_8)$alkenylene, or $(C_2\text{-}C_8)$alkynylene is substituted with 0-3 R^{23} groups;

each W^{23} is independently W^{24} or W^{25};

each W^{24} is independently R^{25}, $-C(=Y^{21})R^{25}$, $-C(=Y^{21})W^{25}$, $-SO_2R^{25}$, or $-SO_2W^{25}$;

30 each W^{25} is independently carbocycle or heterocycle wherein W^{25} is independently substituted with 0 to 3 R^{22} groups; and

each Y^{21} is independently O or S.

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5 Table 20.1

1 2 3

4 5 6

7 8

Table 20.2

9 10

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5 Table 20.3

12 13 14

15 16 17

18 19

Table 20.4

20 21

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5 Table 20.5

23 24 25

26 27 28

29 30

Table 20.6

31 32

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5 Table 20.7

34 35 36

37 38 39

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Table 20.8

42 43 44

45 46 47

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Table 20.9

50 51 52

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Table 20.10

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5 Table 20.11

61 62 63

64 65 66

67 68

Table 20.12

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5 Table 20.13

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Table 20.14

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5 Table 20.15

83 84 85

86 87 88

89 90

Table 20.16

91 92 93

94 95 96

97 98

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5 Table 20.17

99 100 101

102 103 104

105 106

Table 20.18

107 108

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5 Table 20.19

110 111 112 113

114 115 116 117

Table 20.20

118 119

120

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Table 20.21

121 122 123 124

125 126 127 128

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Table 20.22

129 130 131

Table 20.23

132 133 134 135

136 137 138 139

10

Table 20.24

140 141 142 143

144 145 146 147

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5 Table 20.25

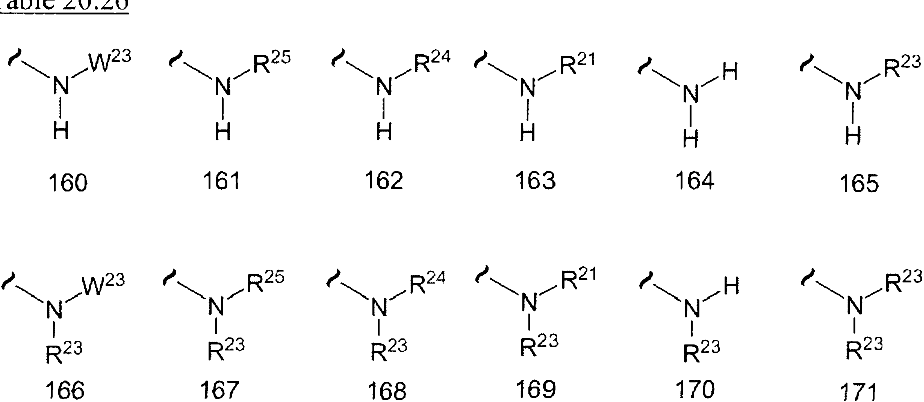
W^{23} R^{25} R^{24} R^{21} H R^{23}

148 149 150 151 152 153

O–W^{23} O–R^{25} O–R^{24} O–R^{21} O–H O–R^{23}

154 155 156 157 158 159

Table 20.26



10

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5 Table 20.27

172 173

174 175

176 177

178 179

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Table 20.28

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180 181

182 183

5 184 185

Table 20.29

186 187

188 189

190 191

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Table 20.30

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Table 20.31

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Table 20.32

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5 Table 20.33

214

215

216

217

218

219

H_3C

221

CH_3

220

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5

Table 20.34

222

223

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225

226

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Table 20.35

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229

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10 235

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5 Table 20.36

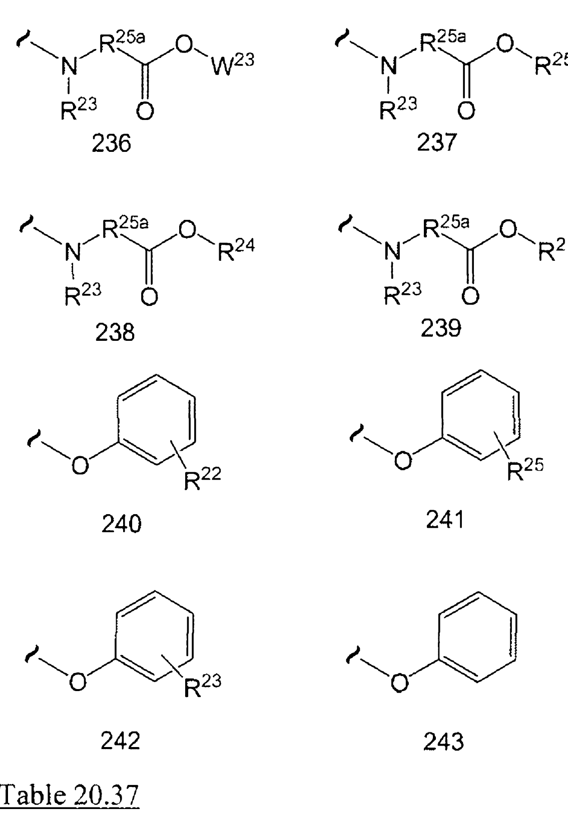
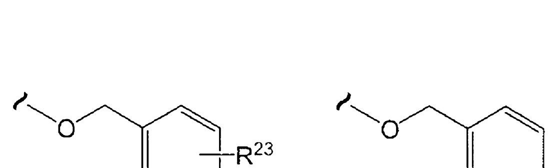


Table 20.37



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5 Table 30.1

67 68 69

70 71 258

248 249

250 251

10 252 253 254

255 256 257

Phosphate Embodiments of Compounds of Formula I-IV

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5 By way of example and not limitation, the phosphate embodiments of Formula I-IV may be represented by the general formula "MBF":

MBF

Each embodiment of MBF is depicted as a substituted nucleus (Sc). Sc is described in
10 formulae **A-G** of Table 1.1 below, wherein Sc is a generic formula for a compound of Formula I, Formula II, or Formula III and the point of attachment to $-P(O)Pd^1Pd^2$ is indicated with a wavy line.

Table 1.1

15 A , B ,

C , D ,

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NH_2 N N N N O O CN OH OH

OH N N N N NH_2 O O CN OH OH

5 E , F ,

NH_2 N N N N NH_2 O O CN OH OH

G .

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5 Combinations of "Sc" and Pd^1 and Pd^2, independently selected from Table 30.1, can be expressed in the form of $Sc.Pd^1.Pd^2$, where Sc is represented by the respective letter A-G from Table 1.1 and Pd^1 and Pd^2 are represented by the respective number from Table 30.1. Thus, A.256.256 represents the following compound:

10

Thereby, Table 7 lists many specific examples of phosphate prodrugs of Formula I-IV.

Table 7: List of Compounds of MBF

A.254.67, A.254.68, A.254.69, A.254.70, A.254.71, A.254.258, A.254.248, A.254.249,
15 A.254.250, A.254.251, A.254.252, A.254.253, B.254.67, B.254.68, B.254.69, B.254.70, B.254.71, B.254.258, B.254.248, B.254.249, B.254.250, B.254.251, B.254.252, B.254.253, C.254.67, C.254.68, C.254.69, C.254.70, C.254.71, C.254.258, C.254.248, C.254.249, C.254.250, C.254.251, C.254.252, C.254.253, D.254.67, D.254.68, D.254.69, D.254.70, D.254.71, D.254.258, D.254.248, D.254.249, D.254.250, D.254.251,
20 D.254.252, D.254.253, E.254.67, E.254.68, E.254.69, E.254.70, E.254.71, E.254.258, E.254.248, E.254.249, E.254.250, E.254.251, E.254.252, E.254.253, F.254.67, F.254.68, F.254.69, F.254.70, F.254.71, F.254.258, F.254.248, F.254.249, F.254.250, F.254.251, F.254.252, F.254.253, G.254.67, G.254.68, G.254.69, G.254.70, G.254.71, G.254.258, G.254.248, G.254.249, G.254.250, G.254.251, G.254.252, G.254.253, A.255.67,
25 A.255.68, A.255.69, A.255.70, A.255.71, A.255.258, A.255.248, A.255.249, A.255.250, A.255.251, A.255.252, A.255.253, B.255.67, B.255.68, B.255.69, B.255.70, B.255.71, B.255.258, B.255.248, B.255.249, B.255.250, B.255.251, B.255.252, B.255.253, C.255.67, C.255.68, C.255.69, C.255.70, C.255.71, C.255.258, C.255.248, C.255.249,

C.255.250, C.255.251, C.255.252, C.255.253, D.255.67, D.255.68, D.255.69, D.255.70, D.255.71, D.255.258, D.255.248, D.255.249, D.255.250, D.255.251, D.255.252, D.255.253, E.255.67, E.255.68, E.255.69, E.255.70, E.255.71, E.255.258, E.255.248, E.255.249, E.255.250, E.255.251, E.255.252, E.255.253, F.255.67, F.255.68, F.255.69, F.255.70, F.255.71, F.255.258, F.255.248, F.255.249, F.255.250, F.255.251, F.255.252, F.255.253, G.255.67, G.255.68, G.255.69, G.255.70, G.255.71, G.255.258, G.255.248, G.255.249, G.255.250, G.255.251, G.255.252, G.255.253, A.67.67, A.68.68, A.69.69, A.70.70, A.71.71, A.258.258, A.248.248, A.249.249, A.250.250, A.251.251, A252.252, A.253.253, B.67.67, B.68.68, B.69.69, B.70.70, B.71.71, B.258.258, B.248.248, B.249.249, B.250.250, B.251.251, B252.252, B.253.253, C.67.67, C.68.68, C.69.69, C.70.70, C.71.71, C.258.258, C.248.248, C.249.249, C.250.250, C.251.251, C252.252, C.253.253, D.67.67, D.68.68, D.69.69, D.70.70, D.71.71, D.258.258, D.248.248, D.249.249, D.250.250, D.251.251, D252.252, D.253.253, E.67.67, E.68.68, E.69.69, E.70.70, E.71.71, E.258.258, E.248.248, E.249.249, E.250.250, E.251.251, E252.252, E.253.253, F.67.67, F.68.68, F.69.69, F.70.70, F.71.71, F.258.258, F.248.248, F.249.249, F.250.250, F.251.251, F252.252, F.253.253, G.67.67, G.68.68, G.69.69, G.70.70, G.71.71, G.258.258, G.248.248, G.249.249, G.250.250, G.251.251, G252.252, G.253.253, A.256.257, B.256.257, C.256.257, D.256.257, E.256.257, F.256.257, G.256.257, A.256.254, B.256.254, C.256.254, D.256.254, E.256.254, F.256.254, G.256.254, A.256.250, B.256.250, C.256.250, D.256.250, E.256.250, F.256.250, G.256.250, A.256.69, B.256.69, C.256.69, D.256.69, E.256.69, F.256.69, G.256.69, A.256.71, B.256.71, C.256.71, D.256.71, E.256.71, F.256.71, G.256.71, A.256.255, B.256.255, C.256.255, D.256.255, E.256.255, F.256.255, G.256.255.

Embodiments of R^x include esters, carbamates, carbonates, thioesters, amides, thioamides, and urea groups:

R R Y^2 R^y M12a Y^1 and R R Y^1 Y^2 Y^2 R^y M12a .

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5

Any reference to the compounds of the invention described heerein also includes a reference to a physiologically acceptable salt thereof. Examples of physiologically acceptable salts of the compounds of the invention include salts derived from an appropriate base, such as an alkali metal or an alkaline earth (for example, Na^+, Li^+, K^+,
10 Ca^{+2} and Mg^{+2}), ammonium and NR_4^+ (wherein R is defined herein). Physiologically acceptable salts of a nitrogen atom or an amino group include (a) acid addition salts formed with inorganic acids, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, sulfamic acids, phosphoric acid, nitric acid and the like; (b) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid,
15 maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, isethionic acid, lactobionic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, benzenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, malonic acid, sulfosalicylic acid, glycolic acid, 2-hydroxy-3-naphthoate, pamoate, salicylic acid,
20 stearic acid, phthalic acid, mandelic acid, lactic acid, ethanesulfonic acid, lysine, arginine, glutamic acid, glycine, serine, threonine, alanine, isoleucine, leucine and the like; and (c) salts formed from elemental anions for example, chlorine, bromine, and iodine. Physiologically acceptable salts of a compound of a hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+ and NR_4^+.
25 For therapeutic use, salts of active ingredients of the compounds of the invention will be physiologically acceptable, i.e. they will be salts derived from a physiologically acceptable acid or base. However, salts of acids or bases which are not physiologically acceptable may also find use, for example, in the preparation or purification of a physiologically acceptable compound. All salts, whether or not derived form a
30 physiologically acceptable acid or base, are within the scope of the present invention.

Finally, it is to be understood that the compositions herein comprise compounds of the invention in their un-ionized, as well as zwitterionic form, and combinations with stoichiometric amounts of water as in hydrates.

The compounds of the invention, exemplified by Formula I-III may have chiral

5 centers, e.g. chiral carbon or phosphorus atoms. The compounds of the invention thus include racemic mixtures of all stereoisomers, including enantiomers, diastereomers, and atropisomers. In addition, the compounds of the invention include enriched or resolved optical isomers at any or all asymmetric, chiral atoms. In other words, the chiral centers apparent from the depictions are provided as the chiral isomers or racemic mixtures.
10 Both racemic and diastereomeric mixtures, as well as the individual optical isomers isolated or synthesized, substantially free of their enantiomeric or diastereomeric partners, are all within the scope of the invention. The racemic mixtures are separated into their individual, substantially optically pure isomers through well-known techniques such as, for example, the separation of diastereomeric salts formed with optically active
15 adjuncts, e.g., acids or bases followed by conversion back to the optically active substances. In most instances, the desired optical isomer is synthesized by means of stereospecific reactions, beginning with the appropriate stereoisomer of the desired starting material.

The term "chiral" refers to molecules which have the property of non-
20 superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

"Diastereomer" refers to a stereoisomer with two or more centers of chirality and
25 whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-
30 superimposable mirror images of one another.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., Stereochemistry of Organic Compounds (1994) John Wiley & Sons, Inc., New York. Many organic compounds

5 exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l, D and L, or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with S, (-), or l meaning that
10 the compound is levorotatory while a compound prefixed with R, (+), or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a
15 racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

Whenever a compound described herein is substituted with more than one of the same designated group, e.g., "R" or "R^1", then it will be understood that the groups may
20 be the same or different, i.e., each group is independently selected. Wavy lines, ~~~~~, indicate the site of covalent bond attachments to the adjoining substructures, groups, moieties, or atoms.

The compounds of the invention can also exist as tautomeric isomers in certain cases. Although only one delocalized resonance structure may be depicted, all such
25 forms are contemplated within the scope of the invention. For example, ene-amine tautomers can exist for purine, pyrimidine, imidazole, guanidine, amidine, and tetrazole systems and all their possible tautomeric forms are within the scope of the invention.

One skilled in the art will recognize that the pyrrolo[1,2-f][1,2,4]triazine, imidazo[1,5-f][1,2,4]triazine, imidazo[1,2-f][1,2,4]triazine, and [1,2,4]triazolo[4,3-
30 f][1,2,4]triazine nucleosides can exist in tautomeric forms. For example, but not by way of limitation, structures (a) and (b) can have equivalent tautomeric forms as shown below:

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a b.

All possible tautomeric forms of the heterocycles in all of the embodiments disclosed herein are within the scope of the invention.

Methods of Inhibition of HCV polymerase

Another aspect of the invention relates to methods of inhibiting the activity of HCV polymerase comprising the step of treating a sample suspected of containing HCV with a composition of the invention.

Compositions of the invention may act as inhibitors of HCV polymerase , as intermediates for such inhibitors or have other utilities as described below. The inhibitors will bind to locations on the surface or in a cavity of HCV polymerase having a geometry unique to HCV polymerase . Compositions binding HCV polymerase may bind with varying degrees of reversibility. Those compounds binding substantially irreversibly are ideal candidates for use in this method of the invention. Once labeled, the substantially irreversibly binding compositions are useful as probes for the detection of HCV polymerase . Accordingly, the invention relates to methods of detecting HCV

5 polymerase in a sample suspected of containing HCV polymerase comprising the steps of: treating a sample suspected of containing HCV polymerase with a composition comprising a compound of the invention bound to a label; and observing the effect of the sample on the activity of the label. Suitable labels are well known in the diagnostics field and include stable free radicals, fluorophores, radioisotopes, enzymes, 10 chemiluminescent groups and chromogens. The compounds herein are labeled in conventional fashion using functional groups such as hydroxyl, carboxyl, sulfhydryl or amino.

Within the context of the invention, samples suspected of containing HCV polymerase include natural or man-made materials such as living organisms; tissue or 15 cell cultures; biological samples such as biological material samples (blood, serum, urine, cerebrospinal fluid, tears, sputum, saliva, tissue samples, and the like); laboratory samples; food, water, or air samples; bioproduct samples such as extracts of cells, particularly recombinant cells synthesizing a desired glycoprotein; and the like. Typically the sample will be suspected of containing an organism which produces HCV 20 polymerase , frequently a pathogenic organism such as HCV. Samples can be contained in any medium including water and organic solvent\water mixtures. Samples include living organisms such as humans, and man made materials such as cell cultures.

The treating step of the invention comprises adding the composition of the invention to the sample or it comprises adding a precursor of the composition to the 25 sample. The addition step comprises any method of administration as described above.

If desired, the activity of HCV polymerase after application of the composition can be observed by any method including direct and indirect methods of detecting HCV polymerase activity. Quantitative, qualitative, and semiquantitative methods of determining HCV polymerase activity are all contemplated. Typically one of the 30 screening methods described above are applied, however, any other method such as observation of the physiological properties of a living organism are also applicable.

Organisms that contain HCV polymerase include the HCV virus. The compounds of this invention are useful in the treatment or prophylaxis of HCV infections in animals or in man.

5 However, in screening compounds capable of inhibiting human immunodeficiency viruses, it should be kept in mind that the results of enzyme assays may not correlate with cell culture assays. Thus, a cell based assay should be the primary screening tool.

Screens for HCV polymerase Inhibitors.

10 Compositions of the invention are screened for inhibitory activity against HCV polymerase by any of the conventional techniques for evaluating enzyme activity. Within the context of the invention, typically compositions are first screened for inhibition of HCV polymerase *in vitro* and compositions showing inhibitory activity are then screened for activity *in vivo*. Compositions having *in vitro* Ki (inhibitory constants)

15 of less then about 5 X 10^{-6} M, typically less than about 1 X 10^{-7} M and preferably less than about 5 X 10^{-8} M are preferred for *in vivo* use.

Useful *in vitro* screens have been described in detail and will not be elaborated here. However, the examples describe suitable *in vitro* assays.

Pharmaceutical Formulations

20 The compounds of this invention are formulated with conventional carriers and excipients, which will be selected in accord with ordinary practice. Tablets will contain excipients, glidants, fillers, binders and the like. Aqueous formulations are prepared in sterile form, and when intended for delivery by other than oral administration generally will be isotonic. All formulations will optionally contain excipients such as those set

25 forth in the "Handbook of Pharmaceutical Excipients" (1986). Excipients include ascorbic acid and other antioxidants, chelating agents such as EDTA, carbohydrates such as dextran, hydroxyalkylcellulose, hydroxyalkylmethylcellulose, stearic acid and the like. The pH of the formulations ranges from about 3 to about 11, but is ordinarily about 7 to 10.

30 While it is possible for the active ingredients to be administered alone it may be preferable to present them as pharmaceutical formulations. The formulations, both for veterinary and for human use, of the invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers therefor and optionally

5 other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and physiologically innocuous to the recipient thereof.

The formulations include those suitable for the foregoing administration routes. The formulations may conveniently be presented in unit dosage form and may be 10 prepared by any of the methods well known in the art of pharmacy. Techniques and formulations generally are found in Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, PA). Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately 15 bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or 20 a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be administered as a bolus, electuary or paste.

A tablet is made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable 25 machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient moistened with an inert liquid diluent. The tablets may optionally be coated or scored and optionally are formulated so as to provide 30 slow or controlled release of the active ingredient therefrom.

For infections of the eye or other external tissues e.g. mouth and skin, the formulations are preferably applied as a topical ointment or cream containing the active ingredient(s) in an amount of, for example, 0.075 to 20% w/w (including active ingredient(s) in a range between 0.1% and 20% in increments of 0.1% w/w such as 0.6%

5 w/w, 0.7% w/w, etc.), preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base.

If desired, the aqueous phase of the cream base may include, for example, at least 10 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG 400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration 15 enhancers include dimethyl sulphoxide and related analogs.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic 20 emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

25 Emulgents and emulsion stabilizers suitable for use in the formulation of the invention include Tween® 60, Span® 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties. The cream should preferably be a non-greasy, non-staining 30 and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three

5 being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils are used.

Pharmaceutical formulations according to the present invention comprise a combination according to the invention together with one or more pharmaceutically 10 acceptable carriers or excipients and optionally other therapeutic agents. Pharmaceutical formulations containing the active ingredient may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared. Compositions intended for oral use may 15 be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for 20 manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known 25 techniques including microencapsulation to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use may be also presented as hard gelatin capsules where 30 the active ingredient is mixed with an inert solid diluent, for example calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients

5 include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcelluose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally-occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long
10 chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxy-benzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose
15 or saccharin.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth
20 above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives.
25 Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis
30 oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally-occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as

5 polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a sterile 10 injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-15 diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in 20 the preparation of injectables.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active 25 material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight:weight). The pharmaceutical composition can be prepared to provide easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 μg of the active ingredient per milliliter of solution in 30 order that infusion of a suitable volume at a rate of about 30 mL/hr can occur.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10%, and

5 particularly about 1.5% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a
10 suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for intrapulmonary or nasal administration have a particle size for example in the range of 0.1 to 500 microns, such as 0.5, 1, 30, 35 etc., which is
15 administered by rapid inhalation through the nasal passage or by inhalation through the mouth so as to reach the alveolar sacs. Suitable formulations include aqueous or oily solutions of the active ingredient. Formulations suitable for aerosol or dry powder administration may be prepared according to conventional methods and may be delivered with other therapeutic agents such as compounds heretofore used in the treatment or
20 prophylaxis of HCV infections as described below.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-
25 aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

The formulations are presented in unit-dose or multi-dose containers, for example
30 sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions are prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit daily sub-

5 dose, as herein above recited, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

10 The invention further provides veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier therefor.

Veterinary carriers are materials useful for the purpose of administering the composition and may be solid, liquid or gaseous materials which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These 15 veterinary compositions may be administered orally, parenterally or by any other desired route.

Compounds of the invention are used to provide controlled release pharmaceutical formulations containing as active ingredient one or more compounds of the invention ("controlled release formulations") in which the release of the active 20 ingredient are controlled and regulated to allow less frequency dosing or to improve the pharmacokinetic or toxicity profile of a given active ingredient.

Effective dose of active ingredient depends at least on the nature of the condition being treated, toxicity, whether the compound is being used prophylactically (lower doses) or against an active viral infection, the method of delivery, and the pharmaceutical 25 formulation, and will be determined by the clinician using conventional dose escalation studies. It can be expected to be from about 0.0001 to about 100 mg/kg body weight per day; typically, from about 0.01 to about 10 mg/kg body weight per day; more typically, from about .01 to about 5 mg/kg body weight per day; most typically, from about .05 to about 0.5 mg/kg body weight per day. For example, the daily candidate dose for an adult 30 human of approximately 70 kg body weight will range from 1 mg to 1000 mg, preferably between 5 mg and 500 mg, and may take the form of single or multiple doses.

Routes of Administration

One or more compounds of the invention (herein referred to as the active ingredients) are administered by any route appropriate to the condition to be treated.

5 Suitable routes include oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural), and the like. It will be appreciated that the preferred route may vary with for example the condition of the recipient. An advantage of the compounds of this invention is that they are orally bioavailable and can be dosed orally.

10

Combination Therapy

Compositions of the invention are also used in combination with other active ingredients. Preferably, the other active therapeutic ingredients or agents are interferons, ribavirin analogs, NS3 protease inhibitors, NS5a inhibitors, alpha-glucosidase 1
15 inhibitors, cyclophilin inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV.

Combinations of the compounds of Formula I-III are typically selected based on the condition to be treated, cross-reactivities of ingredients and pharmaco-properties of the combination. For example, when treating an infection (e.g., HCV), the compositions
20 of the invention are combined with other active therapeutic agents (such as those described herein).

Suitable active therapeutic agents or ingredients which can be combined with the compounds of Formula I-III can include interferons, e.g., pegylated rIFN-alpha 2b, pegylated rIFN-alpha 2a, rIFN-alpha 2b, IFN alpha-2b XL, rIFN-alpha 2a, consensus
25 IFN alpha, infergen, rebif, locteron, AVI-005, PEG-infergen, pegylated IFN-beta, oral interferon alpha, feron, reaferon, intermax alpha, r-IFN-beta, infergen + actimmune, IFN-omega with DUROS, and albuferon; ribavirin analogs, e.g., rebetol, copegus, VX-497, and viramidine (taribavirin); NS5a inhibitors, e.g., A-831, A-689 and BMS-790052; NS5b polymerase inhibitors, e.g., NM-283, valopicitabine, R1626, PSI-6130 (R1656),
30 HCV-796, BILB 1941, MK-0608, NM-107, R7128, VCH-759, PF-868554, GSK625433, and XTL-2125; NS3 protease inhibitors, e.g., SCH-503034 (SCH-7), VX-950 (Telaprevir), ITMN-191, and BILN-2065; alpha-glucosidase 1 inhibitors, e.g., MX-3253 (celgosivir) and UT-231B; hepatoprotectants, e.g., IDN-6556, ME 3738, MitoQ, and LB-84451; non-nucleoside inhibitors of HCV, e.g., benzimidazole derivatives, benzo-1,2,4-

5 thiadiazine derivatives, and phenylalanine derivatives; and other drugs for treating HCV, *e.g.*, zadaxin, nitazoxanide (alinea), BIVN-401 (virostat), DEBIO-025, VGX-410C, EMZ-702, AVI 4065, bavituximab, oglufanide, PYN-17, KPE02003002, actilon (CPG-10101), KRN-7000, civacir, GI-5005, ANA-975, XTL-6865, ANA 971, NOV-205, tarvacin, EHC-18, and NIM811.

10 In yet another embodiment, the present application discloses pharmaceutical compositions comprising a compound of the present invention, or a pharmaceutically acceptable salt, solvate, and/or ester thereof, in combination with at least one additional therapeutic agent, and a pharmaceutically acceptable carrier or exipient.

According to the present invention, the therapeutic agent used in combination 15 with the compound of the present invention can be any agent having a therapeutic effect when used in combination with the compound of the present invention. For example, the therapeutic agent used in combination with the compound of the present invention can be interferons, ribavirin analogs, NS3 protease inhibitors, NS5a inhibitors, alpha-glucosidase 1 inhibitors, cyclophilin inhibitors, hepatoprotectants, non-nucleoside 20 inhibitors of HCV, and other drugs for treating HCV.

In another embodiment, the present application provides pharmaceutical compositions comprising a compound of the present invention, or a pharmaceutically acceptable salt, solvate, and/or ester thereof, in combination with at least one additional therapeutic agent selected from the group consisting of pegylated rIFN-alpha 2b, 25 pegylated rIFN-alpha 2a, rIFN-alpha 2b, IFN alpha-2b XL, rIFN-alpha 2a, consensus IFN alpha, infergen, rebif, locteron, AVI-005, PEG-infergen, pegylated IFN-beta, oral interferon alpha, feron, reaferon, intermax alpha, r-IFN-beta, infergen + actimmune, IFN-omega with DUROS, albuferon, rebetol, copegus, VX-497, viramidine (taribavirin), A-831, A-689, NM-283, valopicitabine, R1626, PSI-6130 (R1656), HCV-796, BILB 30 1941, MK-0608, NM-107, R7128, VCH-759, PF-868554, GSK625433, XTL-2125, SCH-503034 (SCH-7), VX-950 (Telaprevir), ITMN-191, and BILN-2065, MX-3253 (celgosivir), UT-231B, IDN-6556, ME 3738, MitoQ, and LB-84451, benzimidazole derivatives, benzo-1,2,4-thiadiazine derivatives, and phenylalanine derivatives, zadaxin, nitazoxanide (alinea), BIVN-401 (virostat), DEBIO-025, VGX-410C, EMZ-702, AVI

5 4065, bavituximab, oglufanide, PYN-17, KPE02003002, actilon (CPG-10101), KRN-7000, civacir, GI-5005, ANA-975, XTL-6865, ANA 971, NOV-205, tarvacin, EHC-18, and NIM811 and a pharmaceutically acceptable carrier or exipient.

In yet another embodiment, the present application provides a combination pharmaceutical agent comprising:

10 a) a first pharmaceutical composition comprising a compound of the present invention, or a pharmaceutically acceptable salt, solvate, or ester thereof; and

b) a second pharmaceutical composition comprising at least one additional therapeutic agent selected from the group consisting of HIV protease inhibiting compounds, HIV non-nucleoside inhibitors of reverse transcriptase, HIV nucleoside

15 inhibitors of reverse transcriptase, HIV nucleotide inhibitors of reverse transcriptase, HIV integrase inhibitors, gp41 inhibitors, CXCR4 inhibitors, gp120 inhibitors, CCR5 inhibitors, interferons, ribavirin analogs, NS3 protease inhibitors, NS5a inhibitors, alpha-glucosidase 1 inhibitors, cyclophilin inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV, and combinations thereof.

20 Combinations of the compounds of Formula I-III and additional active therapeutic agents may be selected to treat patients infected with HCV and other conditions such as HIV infections. Accordingly, the compounds of Formula I-III may be combined with one or more compounds useful in treating HIV, for example HIV protease inhibiting compounds, HIV non-nucleoside inhibitors of reverse transcriptase,

25 HIV nucleoside inhibitors of reverse transcriptase, HIV nucleotide inhibitors of reverse transcriptase, HIV integrase inhibitors, gp41 inhibitors, CXCR4 inhibitors, gp120 inhibitors, CCR5 inhibitors, interferons, ribavirin analogs, NS3 protease inhibitors, NS5a inhibitors, alpha-glucosidase 1 inhibitors, cyclophilin inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV.

30 More specifically, one or more compounds of the present invention may be combined with one or more compounds selected from the group consisting of 1) HIV protease inhibitors, *e.g.*, amprenavir, atazanavir, fosamprenavir, indinavir, lopinavir, ritonavir, lopinavir + ritonavir, nelfinavir, saquinavir, tipranavir, brecanavir, darunavir, TMC-126, TMC-114, mozenavir (DMP-450), JE-2147 (AG1776), AG1859, DG35, L-

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5 756423, RO0334649, KNI-272, DPC-681, DPC-684, and GW640385X, DG17, PPL-100, 2) a HIV non-nucleoside inhibitor of reverse transcriptase, *e.g.*, capravirine, emivirine, delaviridine, efavirenz, nevirapine, (+) calanolide A, etravirine, GW5634, DPC-083, DPC-961, DPC-963, MIV-150, and TMC-120, TMC-278 (rilpivirine), efavirenz, BILR 355 BS, VRX 840773, UK-453,061, RDEA806, 3) a HIV nucleoside
10 inhibitor of reverse transcriptase, *e.g.*, zidovudine, emtricitabine, didanosine, stavudine, zalcitabine, lamivudine, abacavir, amdoxovir, elvucitabine, alovudine, MIV-210, racivir (±-FTC), D-d4FC, emtricitabine, phosphazide, fozivudine tidoxil, fosalvudine tidoxil, apricitibine (AVX754), amdoxovir, KP-1461, abacavir + lamivudine, abacavir + lamivudine + zidovudine, zidovudine + lamivudine, 4) a HIV nucleotide inhibitor of
15 reverse transcriptase, *e.g.*, tenofovir, tenofovir disoproxil fumarate + emtricitabine, tenofovir disoproxil fumarate + emtricitabine + efavirenz, and adefovir, 5) a HIV integrase inhibitor, *e.g.*, curcumin, derivatives of curcumin, chicoric acid, derivatives of chicoric acid, 3,5-dicaffeoylquinic acid, derivatives of 3,5-dicaffeoylquinic acid, aurintricarboxylic acid, derivatives of aurintricarboxylic acid, caffeic acid phenethyl
20 ester, derivatives of caffeic acid phenethyl ester, tyrphostin, derivatives of tyrphostin, quercetin, derivatives of quercetin, S-1360, zintevir (AR-177), L-870812, and L-870810, MK-0518 (raltegravir), BMS-707035, MK-2048, BA-011, BMS-538158, GSK364735C, 6) a gp41 inhibitor, *e.g.*, enfuvirtide, sifuvirtide, FB006M, TRI-1144, SPC3, DES6, Locus gp41, CovX, and REP 9, 7) a CXCR4 inhibitor, *e.g.*, AMD-070, 8) an entry
25 inhibitor, *e.g.*, SP01A, TNX-355, 9) a gp120 inhibitor, *e.g.*, BMS-488043 and BlockAide/CR, 10) a G6PD and NADH-oxidase inhibitor, *e.g.*, immunitin, 10) a CCR5 inhibitor, *e.g.*, aplaviroc, vicriviroc, INCB9471, PRO-140, INCB15050, PF-232798, CCR5mAb004, and maraviroc, 11) an interferon, *e.g.*, pegylated rIFN-alpha 2b, pegylated rIFN-alpha 2a, rIFN-alpha 2b, IFN alpha-2b XL, rIFN-alpha 2a, consensus
30 IFN alpha, infergen, rebif, locteron, AVI-005, PEG-infergen, pegylated IFN-beta, oral interferon alpha, feron, reaferon, intermax alpha, r-IFN-beta, infergen + actimmune, IFN-omega with DUROS, and albuferon, 12) ribavirin analogs, e.g., rebetol, copegus, VX-497, and viramidine (taribavirin) 13) NS5a inhibitors, e.g., A-831, A-689 and BMS-790052, 14) NS5b polymerase inhibitors, e.g., NM-283, valopicitabine, R1626, PSI-6130

5 (R1656), HCV-796, BILB 1941, MK-0608, NM-107, R7128, VCH-759, PF-868554, GSK625433, and XTL-2125, 15) NS3 protease inhibitors, e.g., SCH-503034 (SCH-7), VX-950 (Telaprevir), ITMN-191, and BILN-2065, 16) alpha-glucosidase 1 inhibitors, e.g., MX-3253 (celgosivir) and UT-231B, 17) hepatoprotectants, e.g., IDN-6556, ME 3738, MitoQ, and LB-84451, 18) non-nucleoside inhibitors of HCV, e.g., benzimidazole

10 derivatives, benzo-1,2,4-thiadiazine derivatives, and phenylalanine derivatives, 19) other drugs for treating HCV, *e.g.*, zadaxin, nitazoxanide (alinea), BIVN-401 (virostat), DEBIO-025, VGX-410C, EMZ-702, AVI 4065, bavituximab, oglufanide, PYN-17, KPE02003002, actilon (CPG-10101), KRN-7000, civacir, GI-5005, ANA-975, XTL-6865, ANA 971, NOV-205, tarvacin, EHC-18, and NIM811, 19) pharmacokinetic

15 enhancers, e.g., BAS-100 and SPI452, 20)RNAse H inhibitors, e.g., ODN-93 and ODN-112, 21) other anti-HIV agents, e.g., VGV-1, PA-457 (bevirimat), ampligen, HRG214, cytolin, polymun, VGX-410, KD247, AMZ 0026, CYT 99007, A-221 HIV, BAY 50-4798, MDX010 (iplimumab), PBS119, ALG889, and PA-1050040.

It is also possible to combine any compound of the invention with one or more

20 other active therapeutic agents in a unitary dosage form for simultaneous or sequential administration to a patient. The combination therapy may be administered as a simultaneous or sequential regimen. When administered sequentially, the combination may be administered in two or more administrations.

Co-administration of a compound of the invention with one or more other active

25 therapeutic agents generally refers to simultaneous or sequential administration of a compound of the invention and one or more other active therapeutic agents, such that therapeutically effective amounts of the compound of the invention and one or more other active therapeutic agents are both present in the body of the patient.

Co-administration includes administration of unit dosages of the compounds of

30 the invention before or after administration of unit dosages of one or more other active therapeutic agents, for example, administration of the compounds of the invention within seconds, minutes, or hours of the administration of one or more other active therapeutic agents. For example, a unit dose of a compound of the invention can be administered first, followed within seconds or minutes by administration of a unit dose of one or more

5 other active therapeutic agents. Alternatively, a unit dose of one or more other therapeutic agents can be administered first, followed by administration of a unit dose of a compound of the invention within seconds or minutes. In some cases, it may be desirable to administer a unit dose of a compound of the invention first, followed, after a period of hours (e.g., 1-12 hours), by administration of a unit dose of one or more other
10 active therapeutic agents. In other cases, it may be desirable to administer a unit dose of one or more other active therapeutic agents first, followed, after a period of hours (e.g., 1-12 hours), by administration of a unit dose of a compound of the invention.

The combination therapy may provide "synergy" and "synergistic", i.e. the effect achieved when the active ingredients used together is greater than the sum of the effects
15 that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or
20 delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together. A synergistic anti-viral effect denotes an antiviral effect which is greater than the
25 predicted purely additive effects of the individual compounds of the combination.

In still yet another embodiment, the present application provides for methods of inhibiting HCV polymerase in a cell, comprising: contacting a cell infected with HCV with an effective amount of a compound of Formula I-III, or a pharmaceutically
30 acceptable salt, solvate, and/or ester thereof, whereby HCV polymerase is inhibited.

In still yet another embodiment, the present application provides for methods of inhibiting HCV polymerase in a cell, comprising: contacting a cell infected with HCV with an effective amount of a compound of Formula I-III, or a pharmaceutically

5 acceptable salt, solvate, and/or ester thereof, and at least one additional active therapeutic agent, whereby HCV polymerase is inhibited.

In still yet another embodiment, the present application provides for methods of inhibiting HCV polymerase in a cell, comprising: contacting a cell infected with HCV with an effective amount of a compound of Formula I-III, or a pharmaceutically
10 acceptable salt, solvate, and/or ester thereof, and at least one additional active therapeutic agent selected from the group consisting of interferons, ribavirin analogs, NS3 protease inhibitors, NS5a inhibitors, alpha-glucosidase 1 inhibitors, cyclophilin inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV.

In still yet another embodiment, the present application provides for methods of
15 treating HCV in a patient, comprising: administering to the patient a therapeutically effective amount of a compound of Formula I-III, or a pharmaceutically acceptable salt, solvate, and/or ester thereof.

In still yet another embodiment, the present application provides for methods of treating HCV in a patient, comprising: administering to the patient a therapeutically
20 effective amount of a compound of Formula I-III, or a pharmaceutically acceptable salt, solvate, and/or ester thereof, and at least one additional active therapeutic agent, whereby HCV polymerase is inhibited.

In still yet another embodiment, the present application provides for methods of treating HCV in a patient, comprising: administering to the patient a therapeutically
25 effective amount of a compound of Formula I-III, or a pharmaceutically acceptable salt, solvate, and/or ester thereof, and at least one additional active therapeutic agent selected from the group consisting of interferons, ribavirin analogs, NS3 protease inhibitors, NS5a inhibitors, alpha-glucosidase 1 inhibitors, cyclophilin inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV.

30 In still yet another embodiment, the present application provides for the use of a compound of the present invention, or a pharmaceutically acceptable salt, solvate, and/or ester thereof, for the preparation of a medicament for treating an HCV infection in a patient.

Metabolites of the Compounds of the Invention

5 Also falling within the scope of this invention are the *in vivo* metabolic products of the compounds described herein, to the extent such products are novel and unobvious over the prior art. Such products may result for example from the oxidation, reduction, hydrolysis, amidation, esterification and the like of the administered compound, primarily due to enzymatic processes. Accordingly, the invention includes novel and
10 unobvious compounds produced by a process comprising contacting a compound of this invention with a mammal for a period of time sufficient to yield a metabolic product thereof. Such products typically are identified by preparing a radiolabelled (e.g. ^{14}C or ^{3}H) compound of the invention, administering it parenterally in a detectable dose (e.g. greater than about 0.5 mg/kg) to an animal such as rat, mouse, guinea pig, monkey, or to
15 man, allowing sufficient time for metabolism to occur (typically about 30 seconds to 30 hours) and isolating its conversion products from the urine, blood or other biological samples. These products are easily isolated since they are labeled (others are isolated by the use of antibodies capable of binding epitopes surviving in the metabolite). The metabolite structures are determined in conventional fashion, e.g. by MS or NMR
20 analysis. In general, analysis of metabolites is done in the same way as conventional drug metabolism studies well-known to those skilled in the art. The conversion products, so long as they are not otherwise found *in vivo*, are useful in diagnostic assays for therapeutic dosing of the compounds of the invention even if they possess no HCV polymerase inhibitory activity of their own.
25 Recipes and methods for determining stability of compounds in surrogate gastrointestinal secretions are known. Compounds are defined herein as stable in the gastrointestinal tract where less than about 50 mole percent of the protected groups are deprotected in surrogate intestinal or gastric juice upon incubation for 1 hour at 37°C. Simply because the compounds are stable to the gastrointestinal tract does not mean that
30 they cannot be hydrolyzed *in vivo*. The prodrugs of the invention typically will be stable in the digestive system but may be substantially hydrolyzed to the parental drug in the digestive lumen, liver or other metabolic organ, or within cells in general.

Examples

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5 Certain abbreviations and acronyms are used in describing the experimental details. Although most of these would be understood by one skilled in the art, Table 1 contains a list of many of these abbreviations and acronyms.

Table 1. List of abbreviations and acronyms.

Abbreviation	Meaning
Ac_2O	acetic anhydride
AIBN	2,2'-azobis(2-methylpropionitrile)
Bn	benzyl
BnBr	benzylbromide
BSA	bis(trimethylsilyl)acetamide
BzCl	benzoyl chloride
CDI	carbonyl diimidazole
DABCO	1,4-diazabicyclo[2.2.2]octane
DBN	1,5-diazabicyclo[4.3.0]nonene-5
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DBU	1,5-diazabicyclo[5.4.0]undecene-5
DCA	dichloroacetamide
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMTCl	dimethoxytrityl chloride
DMSO	dimethylsulfoxide
DMTr	4, 4'-dimethoxytrityl
DMF	dimethylformamide
EtOAc	ethyl acetate
ESI	electrospray ionization
HMDS	hexamethyldisilazane

HPLC	High pressure liquid chromatography
LDA	lithium diisopropylamide
LRMS	low resolution mass spectrum
MCPBA	meta-chloroperbenzoic acid
MeCN	acetonitrile
MeOH	methanol
MMTC	mono methoxytrityl chloride
m/z or m/e	mass to charge ratio
MH^+	mass plus 1
MH^-	mass minus 1
MsOH	methanesulfonic acid
MS or ms	mass spectrum
NBS	N-bromosuccinimide
rt or r.t.	room temperature
TBAF	tetrabutylammonium fluoride
TMSCl	chlorotrimethylsilane
TMSBr	bromotrimethylsilane
TMSI	iodotrimethylsilane
TEA	triethylamine
TBA	tributylamine
TBAP	tributylammonium pyrophosphate
TBSCl	t-butyldimethylsilyl chloride
TEAB	triethylammonium bicarbonate
TFA	trifluoroacetic acid
TLC or tlc	thin layer chromatography
Tr	triphenylmethyl
Tol	4-methylbenzoyl
δ	parts per million down field from tetramethylsilane

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756.PF

5

Preparation of Compounds

Compound 1a-1f

10

AcCl

CH_3OH

1a **1b**

To a solution of **1a** (22.0 g, 54.9 mmol, prepared according to the procedures described in *J.O.C.*, **2004**, 6257) in methanol (300 mL) was dropwise added acetyl chloride (22 mL) at 0 °C using a dropping funnel over a period of 30 min. and then stirred at room temperature for 16 h. The mixture was concentrated, re-dissolved in ethyl acetate (400 mL), washed with ice-cold 2 N NaOH, and concentrated to dryness, affording the crude methyl ether **1b** as an oil. MS = 437.2 (M + Na^+).

20

$NaOCH_3$

CH_3OH

1b **1c**

To a solution of **1b** (obtained from the previous step) in methanol (300 mL) was added 0.5 M sodium methoxide solution in methanol (20 mL, 10 mmol), and stirred for 16 h at room temperature. The reaction was quenched with 4.0 N HCl solution in dioxane (2.5 mL, 10 mmol). The mixture was then concentrated, affording the crude **1c**. MS = 201.0 (M + Na^+).

756.PF

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HO, HO, OH — BnCl, KOH / Tritron X-405 / toluene →

1c 1d

A mixture of **1c** (obtained from the previous step), Tritron X-405 (70% in water, 6.0 g), 50% KOH (in water, 85 g) in toluene (500 mL) was heated to reflux with a Dean-Stark trap attached. After 1h collecting ~25 mL of water, benzyl chloride (33 g, 260
10 mmol) was added and continued to reflux with stirring for 16 h. The mixture was then cooled and partitioned between ethyl acetate (400 mL) and water (300mL). The organic layer was washed with water (300 mL), and concentrated. The residue was purified by silica gel column chromatography (~20% EtOAc / hexanes), affording the methyl ether **1d** as an oil (22.0 g, 89% in three steps). ^1H NMR (300 MHz, $CDCl_3$): δ 7.3 (m, 15H),
15 4.5 - 4.9 (m, 7H), 4.37 (m, 1H), 3.87 (d, 1H), 3.56 (m, 2H), 3.52 (s, 3H), 1.40 (s, 3H).

3M H_2SO_4 / acetic acid →

1d 1e

20 To a solution of **1d** (22.0 g, 49.0 mmol) in acetic acid (110 mL) was added ~ 3 M sulfuric acid (prepared by mixing 4.8 g of concentrated sulfuric acid with 24 mL of water) and stirred at 70 °C for 8 h. The mixture was concentrated to a volume of ~20 mL, and partitioned between ethyl acetate and ice-cold 2N NaOH. The ethyl acetate layer was concentrated, and purified by silica gel column chromatography (~35% EtOAc
25 / hexanes), affording **1e** as an oil (17.0 g, 80%). MS = 457.2 (M + Na^+).

756.PF

DMSO, Ac_2O

1e 1f

To a solution of **1e** (45 g, 104 mmol) in DMSO (135 mL) was dropwise added acetic anhydride (90 mL, 815 mmol) at room temperature under argon. The mixture was stirred for 16 h at room temperature, and then poured into ice-water (1 L) while stirring. After ice was completely melted (~30 min), ethyl acetate (~500 mL) was added. The organic layer was separated. This extraction process was repeated three times (3x500 mL). The organic extracts were combined and concentrated. The residue was purified by silica gel column chromatography (~20% EtOAc / hexanes), affording **1f** as an oil (39 g, 88%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.3 (m, 15H), 4.4 - 4.8 (m, 7H), 4.08 (d, J = 7.5 Hz, 1H), 3.75 (dd, J = 2,4, 11.4 Hz, 1H), 3.64 (dd, J = 5.4, 11.4 Hz, 1H), 1.51 (s, 3H).

Compound 2

BuLi, TMSCl

THF

1f 2a

To a dry, argon purged round bottom flask (100 mL) were added 7-bromo-pyrrolo[2,1-f][1,2,4]triazin-4-ylamine (234 mg, 1.10 mmol) (prepared according to

5 WO2007056170) and anhydrous THF (1.5 mL). TMSCl (276 μL, 2.2 mmol) was then added and the reaction mixture stirred for 2 h. The flask was placed into a dry ice/acetone bath (~ -78 °C) and BuLi (2.5 mL, 4.0 mmol, 1.6M in hexanes) was added dropwise. After 1h, a solution of **1f** (432.5 mg, 1.0 mmol) in THF was cooled to 0 °C and then added to the reaction flask dropwise. After 1 h of stirring at -78 °C, the flask
10 was warmed to 0 °C and sat. NH_4Cl (5 mL) was added to quench the reaction. The organics were extracted using EtOAc (3 x 10 mL) and the combined organic layers were dried using $MgSO_4$. The solvent was removed under reduced pressure and the crude material was purified using flash chromatography (hexanes / EtOAc). 560 mg (99 %) of **2a** was isolated as a mixture of two anomers. LC/MS = 567.2 ($M + H^+$). ^1H NMR (300
15 MHz, $CDCl_3$): δ 7.85 (m, 1H), 7.27 (m, 15H), 7.01 (m, 1H), 6.51 (m, 1H), 4.66 (m, 8H), 4.40 (m, 2H), 3.79 (m, 3H), 1.62 (s, 2'-CH_3 from the one anomer), 1.18 (s, 2'-CH_3 from the other anomer).

NH2 Bn O O OH N N N O O Bn Bn — BBr_3 / DCM → NH2 HO O OH N N N HO OH

2a **2**

20

To a dry, argon purged round bottom flask (50 mL) were added compound **2a** (185 mg, 0.33 mmol) and anhydrous dichloromethane (10 mL). The flask was placed into a dry ice/acetone bath (~ -78 °C) and the solution stirred for 10 min. BBr_3 (0.25 mL, 0.25 mmol, 1.0 M in DCM) was then added and the reaction continued to stir at -78 °C
25 until complete disappearance of the starting material. After 1 h, a solution of pyridine (2 mL) in MeOH (10 mL) was added and the flask was warmed to room temperature. The solvent was removed under reduced pressure and the crude material was re-dissolved in MeOH. After this process was repeated two more times, the crude material was then dissolved in water and purified using a Gilson Preparatory HPLC system (acetonitrile /

5 H_2O). 49 mg (50%) of **Compound 2** was isolated as an isomeric mixture. LC/MS = 297.1 (M + H^+). ^1H NMR (300 MHz, D_2O): δ 7.68 (m, 1H), 6.80 (m, 2H), 4.04 (m, 2H), 3.78 (m, 2H), 3.65 (m, 1H), 1.30 (s, 2'-CH_3), 0.80 (s, 2'-CH_3).

Compound 3

10

CH₃OH

AcOH

2 3

To a dry, argon purged round bottom flask (100 mL) were added **Compound 2** (12 mg, 0.04 mmol) (**2**) and anhydrous MeOH (5 mL). Acetic acid (5 mL) was then

15 added and the reaction stirred overnight at room temperature. Saturated $NaHCO_3$ was added to neutralize the reaction mixture and the crude material was purified using a Gilson Preparatory HPLC system (acetonitrile-H_2O). 2 mg (16%) of the desired material **Compound 3** was isolated. LC/MS = 311.2 (M +H^+). ^1H NMR (300 MHz, D_2O): δ 7.71 (s, 1H), 6.78 (s, 2H), 3.98 (m, 1H), 3.83 (dd, 1H), 3.74 (dd, 1H), 3.62 (d, 1H), 2.94 (s,

20 3H), 0.76 (s, 3H). The other alpha-isomer was also isolated; ^1H NMR (300 MHz, D_2O): δ 7.65 (s, 1H), 6.78 (d, 1H), 6.75 (d, 1H), 4.03 (m, 2H), 3.77 (dd, 1H), 3.59(d, 1H), 2.95 (s, 3H), 1.31 (s, 3H).

Compound 4

25

756.PF

2a → 4a ($AlMe_3$, BF_3-Et_2O, DCM)

5

To a dry, argon purged round bottom flask (50 mL) were added compound **2a** (220 mg, 0.39 mmol) and anhydrous dichloromethane (10 mL). The flask was placed into a dry ice/acetone bath (~ -78 °C) and the solution stirred for 10 min. BF_3-Et_2O (0.10 mL) was added dropwise and the reaction stirred for 10 min. $AlMe_3$ (0.58 mL, 1.16 mmol, 2.0 M in toluene) was then added. After a few minutes, the dry ice/acetone bath was removed and the reaction mixture warmed to room temperature over 4 h. A solution of pyridine (2mL) in MeOH (10 mL) was added and the solvent was removed under reduced pressure. The crude material was purified using flash chromatography (hexanes / EtOAc). 164 mg (74 %) of the desired material **4a** was isolated. LC/MS = 565.2 (M + H^+). ^{1}H NMR (300 MHz, CD_3OD): δ 7.71 (s, 1H), 7.32 (m, 15H), 7.02 (m, 1H), 6.78 (m, 1H), 4.62 (m, 8H), 4.21 (m, 1H), 4.04 (m, 1H), 3.84 (m, 1H), 1.95 (s, 3H), 1.10 (s, 3H).

20

4a → 4 (H_2, 10% Pd/C, AcOH)

To a dry, argon purged round bottom flask (50 mL) were added compound **4a** (164 mg, 0.29 mmol) and glacial acetic acid (10 mL). Pd/C (100 mg, 10 % by wt.) was

756.PF

5 then added and the flask was equipped with a balloon containing hydrogen gas. The flask was purged two times to ensure all of the argon had been replaced with hydrogen. The reaction was allowed to stir at room temperature overnight. The reaction mixture was then neutralized using $NaHCO_3$ and filtered to remove the catalyst. The crude material was purified using a Gilson Preparatory HPLC system (acetonitrile / H_2O). 6
10 mg (7 %) of the desired material **Compound 4** was isolated. LC/MS = 295.1 ($M + H^+$). ^1H NMR (300 MHz, D_2O): δ 7.66 (s, 1H), 6.72 (m, 1H), 6.64 (m, 1H), 3.93 (m, 1H), 3.76 (m, 3H), 1.63 (s, 3H), 0.76 (s, 3H).

Compound 5

15

TMSCN, BF_3-Et_2O / DCM

2a → 5a

To a solution of compound **2a** (1 g, 1.77 mmol) in CH_2Cl_2 (20 mL) at 0 °C was
20 added TMSCN (1.4 mL, 10.5 mmol) and BF_3-Et_2O (1 mL, 8.1 mmol). The reaction mixture was stirred at 0 °C for 0.5 h, then at room temperature for additional 0.5 h. The reaction was quenched with $NaHCO_3$ at 0 °C, and diluted with CH_3CO_2Et. The organic phase was separated, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by chromatography on silica gel, eluted with CH_3CO_2Et-
25 hexanes (1:1 to 2:1), to give the desired compound **5a** (620 mg, 61%) as an isomeric mixture. MS = 576.1 ($M + H^+$).

756.PF

5 5a $\xrightarrow[\text{DCM}]{BCl_3}$ 5

To a solution of compound **5a** (150 mg, 0.26 mmol) in CH_2Cl_2 (4 mL) at -78 °C was added BCl_3 (2 mL, 1M in CH_2Cl_2). The reaction mixture was stirred at -78 °C for 1 h. The reaction was quenched at -78 °C by dropwise addition of TEA (2 mL) and MeOH
10 (5 mL). The mixture was allowed to warm up to room temperature, evaporated, and co-evaporated with MeOH several times. The residue was treated with $NaHCO_3$ (1 g in 10 mL H_2O), concentrated and purified by HPLC to give the desired product **Compound 5** (48 mg, 60%). ^1H NMR (300 MHz, D_2O): δ 7.74 (s 1H), 6.76 (d, J = 5 Hz, 1H), 6.73 (d, J = 5 Hz, 1H), 4.1 (m, 1H), 3.9 (m, 1H), 3.8 (m, 2H), 0.84 (s, 3H). MS = 305.9 (M +
15 H^+). The other alpha-anomer was also obtained (9 mg, 11%): ^1H NMR (300 MHz, D_2O): δ 7.70 (s 1H), 6.8 (d, J = 5 Hz, 1H), 6.7 (d, J = 5 Hz, 1H), 4.25 (d, J = 9 Hz, 1H), 4.07 (m, 1H), 3.85 (m, 1H), 3.7 (m, 1H), 1.6 (s, 3H). MS = 306.1 (M + H^+).

756.PF

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Compound 6

10

To a solution of compound **5** (30 mg, 0.098 mmol) and 1H-tetrazole (30 mg, 0.43 mmol) in anhydrous CH_3CN (1 mL) at 0 °C was added 2,2-dimethyl-thiopropionic acid S-(2-{diisopropylamino-[2-(2,2-dimethyl-propionylsulfanyl)-ethoxy]-phosphanyloxy}-ethyl) ester (90 mg, 0.2 mmol) (described in *J. Med. Chem.*, **1995**, 3941). The reaction
15 mixture was stirred at 0 °C for 1 h, then H_2O_2 (30%, 80 uL) was added and stirred for 0.5 h at 0 °C. The reaction was quenched with sodium thiosulfate (1 M, 1 mL) and $NaHCO_3$, diluted with CH_3CO_2Et. The organic phase was separated, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by HPLC to give the desired **Compound 6** (28 mg, 42%). ^1H NMR (300 MHz, $CDCl_3$): δ 8.04 (s, 1H),
20 6.85 (d, J = 4.5 Hz, 1H), 6.73 (d, J = 4.5 Hz, 1H), 6.0 (brs, 2H), 4.6 (m, 1H), 4.4 (m, 2H), 4.1 (m, 4H), 4.0 (d, J = 4 Hz, 1H), 3.15 (m, 4H), 1.24 (s, 18H), 0.99 (s, 3H). ^{31}P NMR (300 MHz, $CDCl_3$): δ -1.825. MS = 673.9 ($M + H^+$), 672.1 ($M - H^-$).

756.PF

General procedure for preparation of a nucleoside triphosphate:

To a pear-shaped flask (5-15 mL) is charged with a nucleoside (~20 mg). Trimethyl phosphate (0.5-1.0 mL) is added. The solution is cooled with ice-water bath. $POCl_3$ (40-45 mg) is added and stirred at 0 °C until the reaction is complete (1 to 4 h; the reaction progress is monitored by ion-exchange HPLC; analytical samples are prepared by taking ~3 uL of the reaction mixture and diluting it with 1.0 M $Et_3NH_2CO_3$ (30-50 uL)). A solution of pyrophosphate-Bu_3N (250 mg) and Bu_3N (90-105 mg) in acetonitrile or DMF (1-1.5 mL) is then added. The mixture is stirred at 0 °C for 0.3 to 2.5 h, and then the reaction is quenched with 1.0 M $Et_3NH_2CO_3$ (~5 mL). The resulting mixture is stirred for additional 0.5-1 h while warming up to room temperature. The mixture is concentrated to dryness, re-dissolved in water (4 mL), and purified by ion exchange HPLC. The fractions containing the desired product is concentrated to dryness, dissolved in water (~5 mL), concentrated to dryness, and again dissolved in water (~5 mL). $NaHCO_3$ (30-50 mg) is added and concentrated to dryness. The residue is dissolved in water and concentrated to dryness again. This process is repeated 2-5 times. The residue is then subjected to C-18 HPLC purification, affording the desired product as a sodium salt.

Compound 7

Compound 7 was prepared by the general method using **Compound 5** as starting material. ^1H NMR (300 MHz, D_2O): δ 7.76 (s, 1H), 6.95 (d, J = 4.5 Hz, 1H), 6.8 (d, J = 4.5 Hz, 1H), 4.25 (m, 3H), 4.0 (d, J = 6 Hz, 1H), 0.92 (s, 3H). ^{31}P NMR (300 MHz, D_2O): δ -5.6, -10.7, -21.4. MS = 545.8 (M + H^+), 544.0 (M – H^-).

756.PF

5

Compound 8

2a → (TMSN$_3$, BF$_3$-Et$_2$O, DCM) → 8a (6) → (BCl$_3$, DCM) → 8

10 **Compound 8** may be obtained from **2a** in a manner similar to that described in preparation of **Compound 5** except using TMSN$_3$ instead of TMSCN.

Compound 9

756.PF

5

Compound 9 may be obtained from **2a** in a manner similar to that described in preparation of **Compound 5** except using TMS-acetylene instead of TMSCN.

10

Compound 10

15

To a suspension of 7-bromo-2,4-bis-methylsulfanyl-imidazo[2,1-f][1,2,4]triazine (prepared according to WO2008116064, 600 mg, 2.06 mmol) in anhydrous THF (6 mL) was dropwise added BuLi (1.6 M in hexanes, 1.75 mL, 2.81 mmol) at -78 °C. The suspension became red brown solution after 5 min, and then **1f** in THF (0.6 mL) was

5 added dropwise to the mixture. The mixture was then allowed to warm up to room temperature. After 30 min, saturated NH_4Cl was added to quench the reaction. The mixture was diluted with ethyl acetate; the organic layer was washed with brine and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (~40% EtOAc / hexanes), affording **10a** as an isomeric mixture (0.77 g, 64%). MS =
10 645.2 ($M + H^+$).

NH_3

10a **10b**

Compound **10a** (2.0 g, 3.10 mmol) was transferred to a steel bomb reactor, and
15 cooled at -78 °C. Liquid ammonia (~20 mL) was collected at -78 °C and added to the bomb reactor. The bomb reactor was tightly sealed and warmed up to room temperature. The mixture was then heated at 50 °C for 20 h. Complete conversion occurred. After the gas was vented, the residue was purified by silica gel column chromatography (EtOAc / hexanes), affording the product **10b** as a pale yellow solid (1.78 g, 94%). MS = 614.3
20 ($M + H^+$).

Raney Ni

10b **10c**

756.PF

5 A solution of **10b** (100 mg) in ethanol (about 10 ml) is treated with Raney Ni (about 500 mg) that is neutralized by washing with H_2O. The mixture is then heated to about 35 to about 80 °C until the reaction is complete. The catalyst is removed by filtration and the solution is concentrated *in vacuo*. The residue is purified by chromatography to give **10c**.

10

BBr$_3$, DCM

10c → **10**

Compound 10c may be treated with BBr_3 in a manner similar to that described in preparation of compound **2** to give **Compound 10**.

15

Compound 11

1. NaOMe
2. MCPBA

10a → **11a**

20 **10a** is treated with about one to ten mole equivalents of an alkali metal salt of methanol in a suitable solvent such as dioxane for about one to 48 hours. The mixture may also be heated from about 60 to about 110 °C for about one to 24 hours to complete the reaction. The mixture is neutralized with a strong acid and the intermediate is

5 isolated by extraction and chromatography. The intermediate is dissolved in DCM and treated with about two to about four mole equivalents of MCPBA for about one to about 24 hours. The mixture is treated with saturated $NaHCO_3$ and the solution is extracted with EtOAc. The organic layer is washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. The solvent is removed in vacuo and the mixture is purified by
10 chromatography to give **11a**.

11a → (1. NH_3) → 11b

A solution of **11a** in a suitable solvent such as methanol or THF is treated with about five to ten mole equivalents of NH_3 in methanol or THF. The reaction is followed by TLC. After about one to 48 hours, the solvent is evaporated and **11b** is isolated by
15 chromatography. Alternatively, the mixture of **11a** and NH_3 is heated in a sealed glass tube or Parr bomb to about 60 to about 120 °C for about one to about 48 hours and subsequently isolated in the same manner as described.

11b → (BBr_3) → 11

20

11b in DCM is cooled to about −78 °C and treated with about four to 10 mole equivalents of BBr_3 for about one to about 24 hours. The mixture is treated with about 4:1 MeOH–pyridine and the solution was warmed to room temperature. The solvent is

5 removed in vacuo and the mixture is treated with concentrated NH_4OH followed by removal of solvent (×3). The mixture is purified by reverse phase HPLC to give **11**.

Compound 12

DMSO, Ac_2O

10 **12a** **12b**

Compound **12a** (prepared according to *J. Org. Chem.*, **1961**, *26*, 4605; 10.0 g, 23.8 mmol) was dissolved in anhydrous DMSO (30 mL) and placed under nitrogen. Acetic anhydride (20 mL) was added, and the mixture was stirred for 48 h at room
15 temperature. When the reaction was complete by LC/MS, it was poured onto 500 mL ice water and stirred for 20 min. The aqueous layer was extracted with ethyl acetate (3 x 200 mL). The organic extracts were combined and washed with water (3 x 200 mL). The aqueous layers were discarded and the organic was dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was taken up in DCM and loaded onto a silica
20 gel column. The final product **12b** was purified by elution with 25% EtOAc / hexanes; 96% yield. ^{1}H-NMR (CD_3CN): δ 3.63-3.75 (m, 2H), 4.27 (d, 1H), 4.50-4.57 (m, 3H), 4.65 (s, 3H), 4.69-4.80 (m, 2H), 7.25 (d, 2H), 7.39 (m, 13H).

BuLi, TMSCl, THF

12b **12c**

25

756.PF

5 7-Bromo-pyrrolo[2,1-f][1,2,4]triazin-4-ylamine (prepared according to WO2007056170, 0.5 g, 2.4 mmol) was suspended in anhydrous THF (10 mL). Under nitrogen with stirring, TMSCl (0.668 mL, 5.28 mml) was added and the mixture was stirred for 20 min. at room temperature. The reaction was then cooled to -78 °C and a solution of BuLi (6.0 mL, 1.6 N in hexanes) was added slowly. The reaction was stirred
10 for 10 min. at -78 °C and then the lactone **12b** was added via syringe. When the reaction was complete by LC/MS, acetic acid was added to quench. Solvents were removed by rotary evaporation and the residue was taken up in a mixture of 50:50 dichloromethane / water (100 mL). The organic layer was collected and washed with 50 mL additional water, dried over anhydrous $MgSO_4$ and filtered. Evaporation and purification by
15 column chromatography (0 -50% EtOAc: hexanes) provided a 1:1 mixture of anomers **12c**; 25% yield. LC/MS (m/z: 553, M + H^+).

NH2 NH2 N N Bn O O N N Bn O O N N OH CH3OH AcOH O O O O O Bn Bn Bn Bn

12c **12d**

20 Compound **12c** (0.4 g, 0.725 mmol) was stirred in a 1:1 mixture of acetic acid and methanol (10 mL) for 12 h. When the reaction was complete by LC/MS, solvents were removed by high vacuum. The residue was taken up in dichloromethane and loaded onto a silica gel column. A mixture of anomers was eluted using a gradient of 0-75% ethyl acetate and hexanes; 51.4% yield of compound **12d**. ^{1}H-NMR (CD_3CN):
25 δ 2.87 (s, 3H), 3.58-3.79 (dd, 2H), 4.11-4.19 (m, 1H), 4.23-4.33 (m, 1H), 4.39-4.42 (m, 1H), 4.49-4.60 (m, 3H), 4.68-4.73 (m, 2H), 6.22 (bs, 2H), 6.72 (d, 2H), 6.79 (d, 1H), 6.84 (d, 1H), 7.17 (m, 2H), 7.39 (m, 13H), 7.84 (s, 1H).

H_2, 10% Pd/C

CH_3OH / AcOH

12d **Compound 12**

Compound **12d** (0.150 g, 0.265 mmol) was dissolved in a 1:1 mixture of methanol and acetic acid (20 mL). 10% Pd/C (150 mg) was added and the reaction was flushed with nitrogen three times. With stirring, hydrogen gas was introduced. The reaction was stirred under hydrogen for 16 h. When the reaction was complete by LC/MS, the catalyst was filtered off and solvents removed under vacuum. The residue was re-dissolved in a mixture of water and TEA (to keep pH at ~10), and both anomers were purified by prep HPLC under neutral conditions; a total of 51% yield. ^{1}H-NMR of **compound 12** (D_2O): δ 3.16 (s, 3H), 3.69-3.84 (dd, 2H), 4.07-4.10 (m, 1H), 4.22-4.24 (m, 1H), 6.74 (d, 1H), 6.78 (d, 1H), 7.70 (s, 1H). ^{1}H-NMR of the other alpha-anomer (D_2O): δ 2.87 (s, 3H), 3.58-3.84 (dd, 2H), 3.99-4.09 (m 1H), 4.30-4.38 (m, 1H), 4.49 (d, 1H), 6.75 (d, 1H), 6.82 (d, 1H), 7.69 (s, 1H).

Compound 13

TMSCN, BF_3-Et_2O

DCM

12c **13a**

Compound **12c** (0.28 g, 0.51mmol) was dissolved in anhydrous dichloromethane and placed under nitrogen. Trimethylsilyl cyanide (0.35 mL) was added and the mixture

was cooled to 0 °C. After stirring for 10 min., boron trifluoride etherate (50 ul) was added and the reaction was allowed to warm to room temperature. When the reaction was complete by LC/MS, triethylamine was added to quench and solvents were removed by rotary evaporation. The residue was taken up in dichloromethane and loaded onto a silica gel column. A mixture of anomers was eluted using a gradient of 0-75% ethyl acetate and hexanes; 37% yield of **13a**. ^{1}H-NMR (CD_3CN): δ 3.61-3.90 (m, 2H), 4.09-4.19 (m, 2H), 4.30-4.88 (m, 7H), 4.96 (d, 0.5H), 5.10 (d, 0.5H), 6.41 (bs, 2H), 6.73-6.78 (m, 1H), 6.81-6.88 (m, 1H), 7.17 (m, 2H), 7.39 (m, 13H), 7.86 (s, 0.5H), 7.93 (s, 0.5H).

NH_2 | BCl_3 / DCM | NH_2

13a → **Compound 13**

Compound **13a** (0.70 mg, 0.124 mmol) was dissolved in anhydrous dichloromethane (2 ml), placed under nitrogen, and cooled to -78 °C. A solution of 1 N boron trichloride in dichloromethane (0.506 ml) was added and the reaction stirred for 1 h at -78 °C. When the reaction was complete by LC/MS, methanol was added to quench. The reaction was allowed to rise to room temperature and solvents were removed by rotary evaporation. The product anomers were purified by prep-HPLC; a total of 74% yield. ^{1}H-NMR of **Compound 13** (D_2O): δ 3.65-3.75 (dd, 2H), 4.12 (t, 1H), 4.29 (q, 1H), 4.80 (d, 1H), 6.97 (d, 1H), 7.14 (d, 1H), 7.93 (s, 1H). ^{1}H-NMR of the other alpha-anomer (D_2O): δ 3.72-3.93 (dd, 2H), 4.16-4.19 (m, 1H), 4.60-4.62 (m 1H), 5.01 (d, 1H), 6.95 (d, 1H), 7.28 (d, 1H) 7.96 (s, 1H).

Compound 14

756.PF

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Compound 14 may be obtained from **12c** in a manner similar to the method used to synthesize **Compound 4**.

Compound 15

10

Compound 15 may be obtained from **12c** in a manner similar to that described in preparation of **Compound 13** except using $TMSN_3$ instead of TMSCN.

756.PF

5 **Compound 16**

Compound 16 may be obtained from 12c in a manner similar to that described in preparation of Compound 13 except using TMS-acetylene instead of TMSCN.

Compound 17

A mixture of about 0.05 mmol of **Compound 5** and about 0.5 mL of trimethylphosphate is sealed in a container for about one to about 48 hours. The mixture is cooled to about -10 to about 10 ^{0}C and about 0.075 mmol of phosphorous oxychloride is added. After about one to about 24 hours, the reaction is quenched with about 0.5 mL of 1M tetraethylammonium bicarbonate and the desired fraction are isolated by anion exchange chromatography. The appropriate fractions are then desalted by reverse-phase chromatography to give **Compound 17**.

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Compound 18

18

Compound 17 (about 1.19 mmol) is dried over phosphorous pentoxide in a
10 vacuum for about overnight. The dried material is suspended in about 4 mL of anhydrous DMF and about 4.92 mmol DIPEA. About 7.34 mmol of *iso*-propyl chloromethyl carbonate (*Antiviral Chemistry & Chemotherapy* 8:557 (1997)) is added and the mixture is heated to about 25 to about 60 ^{0}C for about 30 min to about 24 hours. Heating is removed for about one to about 48 hours and the reaction filtered. The filtrate
15 is diluted with water, **Compound 18** is partitioned into CH_2Cl_2, the organic solution is dried and evaporated, and the residue is purified by reverse-phase HPLC to isolate **Compound 18.**

20 **Mono Phosphoramidate Prodrugs**

Non-limiting examples of mono-phosphoramidate prodrugs comprising the instant invention may be prepared according to general Scheme 1.

25 **Scheme 1**

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19a 19b 19c

19d 19e

5

The general procedure comprises the reaction of an amino acid ester salt **19b**, e.g., HCl salt, with an aryl dichlorophosphate **19a** in the presence of about two to ten equivalents of a suitable base to give the phosphoramidate **19c**. Suitable bases include,
10 but are not limited to, imidazoles, pyridines such as lutidine and DMAP, tertiary amines such as triethylamine and DABCO, and substituted amidines such as DBN and DBU. Tertiary amines are particularly preferred. Preferably, the product of each step is used directly in the subsequent steps without recrystallization or chromatography. Specific, but non-limiting, examples of **19a**, **19b**, and **19c** can be found in WO 2006/121820 that
15 is hereby incorporated by reference in its entirety. A nucleoside base **19d** reacts with the phosphoramidate **19c** in the presence of a suitable base. Suitable bases include, but are not limited to, imidazoles, pyridines such as lutidine and DMAP, tertiary amines such as triethylamine and DABCO, and substituted amidines such as DBN and DBU. The product **19e** may be isolated by recrystallization and/or chromatography.

20

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5 **Compound 20**

20

About 3.1 mmol of phenyl methoxyalaninyl phosphorochloridate (prepared
10 according to McGuigan et al, *J. Med. Chem.* **1993**, *36*, 1048–1052) in about 3 mL of THF is added to a mixture of of about 0.5 mmol of **Compound 11** and about 3.8 mmol of N-methylimidazole in about 3 mL THF. The reaction is stirred for about 24 hours and the solvent is removed under reduced pressure. The residue is purified by reverse-phase HPLC to give **Compound 20**.

15

Compound 21

21

20 About 3.1 mmol of 4-chlorophenyl 2-propyloxyalaninyl phosphorochloridate (prepared according to McGuigan et al, *J. Med. Chem.* **1993**, *36*, 1048–1052) in about 3 mL of THF is added to a mixture of of about 0.5 mmol of **Compound 5** and about 3.8 mmol of N-methylimidazole in about 3 mL THF. The reaction is stirred for about 24 hours and the solvent is removed under reduced pressure. The residue is purified by
25 reverse-phase HPLC to give **Compound 21**.

Compound 22

756.PF

5

22

A mixture of about 0.52 mmol of **Compound 13** and about 12 mL dry acetone, about 0.7 mL of 2,2,-dimethoxypropane and about 1.28 mmol of di-*p*-nitrophenylphosphoric acid is stirred for about 24 hours to about seven days. The
10 reaction mixture is neutralized with about 20 mL of 0.1 N $NaHCO_3$ and the acetone is evaporated. The desired material is partitioned into chloroform, the chloroform solution is dried, and the solvent is evaporated. **Compound 22** is purified from the residue by conventional means.

15 **Compound 23**

23

A solution of about 0.53 mmol of **Compound 22** in about 5 mL of DMF is treated with about 1 mL of a 1 M solution of *t*-butylmagnesium chloride in THF. After
20 about 30 min to about 5 hours, a solution of about 0.65 mmol of *trans*-4-[(*S*)-pyridin-4-yl]-2-(4-nitrophenoxy)-2-oxo-1,3,2-dioxaphosphorinane (Reddy, *Tetrahedron Letters* **2005**, 4321-4324) is added and the reaction is stirred for about one to about 24 hours. The solution is concentrated in a vacuum and the residue is purified by chromatography to give **Compound 23.**

25

Compound 24

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24

A solution of about 70% aqueous trifluoroacetic acid is cooled to 0 ^{0}C and is treated with about 0.32 mmol of **Compound 23** for about one to 24 hours. The solution is concentrated and the residue is purified by chromatography to give **Compound 24**.

10

Compound 25

25

15 A solution of about 1.56 mmol of **Compound 24** in about 15 mL of THF is treated with about 4.32 mmol of CDI. After about one to about 24 hours, the solvent is evaporated and the residue is purified by chromatography to give **Compound 25**.

Compound 26

20

26

756.PF

5 About 3.1 mmol of 4-chlorophenyl 2-ethoxyalaninyl phosphorochloridate (prepared according to McGuigan et al, *J. Med. Chem.* **1993**, *36*, 1048–1052) in about 3 mL of THF is added to a mixture of about 0.5 mmol of **Compound 4** and about 3.8 mmol of N-methylimidazole in about 3 mL THF. The reaction is stirred for about 24 hours and the solvent is removed under reduced pressure. The residue is purified by 10 reverse-phase HPLC to give **Compound 26**.

Compound 27

27

15 A solution of **Compound 26** in DMSO is treated with about 3 mole equivalents of potassium *t*-butoxide for about 15 min to 24 hours. The reaction is quenched with 1N HCl and **Compound 27** is isolated by reverse-phase HPLC.

Compound 28

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TMSCN

BF_3-Et_2O

10c

28a

BCl_3

5 28

Compound 28 is prepared in the same manner as **Compound 5** but using **Compound 10c** as a starting material.

10 **Compound 29**

29

Compound 29 is prepared in the same manner as **Compound 17** using **Compound 28** as a starting material.

15

Compound 30

5

30

Compound 30 is prepared by treating **Compound 29** with about one to about five equivalents of DCC in pyridine and heating the reaction to reflux for about one to about 24 hours. **Compound 30** is isolated by conventional ion exchange and reverse-
10 phase HPLC.

Compound 31

31

15 A solution of about 0.4 mmol of **Compound 30** in about 10 mL of DMF is treated with about 0.8 mmol of DIPEA and about 0.8 mmol of chloromethyl isopropyl carbonate (WO2007/027248). The reaction is heated to about 25 to about 80 ^{0}C for about 15 min to about 24 hours. The solvent is removed under vacuum and the residue is purified by HPLC to give **Compound 31**.

20

Compound 32

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NH2 / SMe / BnO / OH / OBn / 10b → MCPBA → SO2Me / 32a → NH3 → NH2 / 32b → BBr3 → HO / OH / 32

5

Compound 10b is dissolved in DCM and treated with about two to about four mole equivalents of MCPBA for about one to about 24 hours. The mixture is treated with saturated $NaHCO_3$ and the solution is extracted with EtOAc. The organic layer is washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. The solvent is removed in

10 vacuo and the mixture is purified by chromatography to give **32a**. Compound **32a** is transferred to a steel bomb reactor, and is cooled at -78 °C. Liquid ammonia is collected at -78 °C and is added to the bomb reactor. The bomb reactor is tightly sealed and is warmed up to room temperature. The mixture is heated at about 50 °C for about 24 h. The gas is vented and **32b** is isolated by chromatography. **Compound 32b** is converted

15 to **Compound 32** in the same manner as for the conversion of **Compound 2a** to **Compound 2**.

Compound 33

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5

Compound 32b is converted to **Compound 33** in the same manner as the conversion of **Compound 2a** to **Compound 5**.

Compound 34

10

Compound **33** (about 0.22 mmmol) is dissolved in anhydrous pyridine (about 2 mL) and chlorotrimethylsilane (about 0.17 mL) is added. The mixture is stirred at about
15 0 to about 25 ^{0}C for about one to about 24 hours. Additional chlorotrimethylsilane (about 0.1 mL) is added and the reaction is stirred for about one to about 24 hours. 4.4'-Dimethoxytrityl chloride (about 0.66 mmol) and DMAP (about 0.11 to about 0.22 mmol) is sequentially added. The mixture is stirred for about one to about 24 hours. A solution of TBAF (1.0 M, about 0.22 mL) in THF is added and the reaction is stirred for about

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one to about 24 hours. The mixture is partitioned between ethyl acetate and water. The ethyl acetate layer is dried and concentrated. The residue is purified chromatography to afford **Compound 34** which may be a mixture of mono- and di-dimethoxytritylated compounds.

Compound 35

35

A mixture of about 1.25 mmol of **Compound 34** and about 1.9 mmol of triethylammonium 2-(2,2-dimethyl-3-(trityloxy)propanoylthio)ethyl phosphonate (WO2008082601) is dissolved in anhydrous pyridine (about 19 mL). Pivaloyl chloride (about 2.5 mmol) is added dropwise at about -30 to about 0 ^{0}C and the solution is stirred at for about 30 min to about 24 hours. The reaction is diluted with methylene chloride and is neutralized with agueous ammonium chloride (about 0.5M). The methylene chloride phase is evaporated and the residue is dried and is purified by chromatography to give **Compound 35** which may be a mixture of mono- and di-dimethoxytritylated compounds.

Compound 36

36

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5 To a solution of about 0.49 mmol of **Compound 35** in anhydrous carbon tetrachloride (about 5 mL) is added dropwise benzylamine (about 2.45 mmol). The reaction mixture is stirred for about one to about 24 hours. The solvent is evaporated and the residue is purified by chromatography to give **Compound 36** which may be a mixture of mono- and di-dimethoxytritylated compounds.

10

Compound 37

37

15 A solution of about 2 mmol of **Compound 36** in methylene chloride (about 10 mL) is treated with an aqueous solution of trifluoroacetic acid (90%, about 10 mL). The reaction mixture is stirred at about 25 to about 60 ^{0}C for about one to about 24 hours. The reaction mixture is diluted with ethanol, the volatiles are evaporated and the residue is purified by chromatography to give **Compound 37**.

20

Compound 38

38

25 About 90 mM **Compound 14** in THF is cooled to about -78 ^{0}C and about 2.2 to about 4.4 equivalents of *t*-butylmagneisum chloride (about 1 M in THF) is added. The

5 mixture is warmed to about 0 ^{0}C for about 30 min and is again cooled to about -78 ^{0}C. A solution of *(2S)*-2-{[chloro(1-phenoxy)phosphoryl]amino}propyl pivaloate (WO2008085508) (1 M in THF, about 2 equivalents) is added dropwise. The cooling is removed and the reaction is stirred for about one to about 24 hours. The reaction is quenched with water and the mixture is extracted with ethyl acetate. The extracts are
10 dried and evaporated and the residue purified by chromatography to give **Compound 38.**

Compound 39

dibromohydantoin

39a → 39

A solution of about one part **Compound 39a** (Patil, et al. ; *Journal of*
15 *Heterocyclic Chemistry* **1994**, *31(4)*, 781-6) in anhydrous DMF is cooled to about -20 °C and about 0.5 parts of 1,3-diromo-5,5-dimethylhydantoin is added in portions. After about one to about 24 hours, a saturate aqueous sodium bisulfite solution is added and the solids are collected by filtration. The solids are partitioned between ethyl acetate and dilute agueous sodium carbonate. The organic phase is washed with dilute sodium
20 carbonate then dried and concentrated to give **Compound 39.**

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Compound 40

1f

39

~4 eq. TMSCl
~5 eq. BuLi

40a

A solution of about one part of **39** and about four parts of trimethylsilylchoride

10 in THF is stirred at about 20 to about 60 ^{0}C for about 30 min to about six hours. The solution is cooled to about -70 to about -100 °C and a solution of about five parts of butyllithium in hexanes is added. After about 30 min. to about three hours, the reaction is allowed to warm to about 0 °C over about three hours. The reaction is quenched with saturated $NaHCO_3$ and the mixture is extracted with ether. The ether extracts are washed

15 with brine, dried, and the solvent evaporated to give **40a** which may be further purified by chromatography.

A solution of one part of **40a** in dichloromethane is cooled to about -100 to about -70 °C. A 1.0 M solution of BCl_3 in dichloromethane (about 10 to 20 parts) is added and the reaction is stirred for about 30 min. to about 3 hours. A mixture of pyridine and methanol (about 1:2) is then added to quench the reaction. The resulting mixture is slowly warmed to room temperature and concentrated. The residue is suspended in about 27% ammonium hydroxide and concentrated. This process is repeated twice. The residue is re-dissolved in methanol and concentrated. This process is repeated once. The residue is purified by RP-HPLC to give **40**.

Compound 41

Compound 41 may be prepared from **Compound 40a** in the same manner as **Compound 5** was prepared from **Compound 2a**.

Compound 42

756.PF

42

dibromohydantoin

42a 42b

A solution of about one part **Compound 42a** (Patil, et al. ; *Journal of Heterocyclic Chemistry* **1994**, *31(4)*, 781-6) in anhydrous DMF is cooled to about -20 °C and about 0.5 parts of 1,3-diromo-5,5-dimethylhydantoin is added in portions. After about one to about 24 hours, a saturate aqueous sodium bisulfite solution is added and the solids are collected by filtration. The solids are partitioned between ethyl acetate and dilute agueous sodium carbonate. The organic phase is washed with dilute sodium carbonate then dried and concentrated to give **Compound 42b**.

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1f

42b

~4 eq. TMSCl
~5 eq. BuLi

42c

5

A solution of about one part of **42b** and about four parts of trimethylsilylchoride in THF is stirred at about 20 to about 60 ^{0}C for about 30 min to about six hours. The solution is cooled to about -70 to about -100 °C and a solution of about five parts of butyllithium in hexanes is added. After about 30 min. to about three hours, the reaction
10 is allowed to warm to about 0 °C over about three hours. The reaction is quenched with saturated $NaHCO_3$ and the mixture is extracted with ether. The ether extracts are washed with brine, dried, and the solvent evaporated to give **42c** which may be further purified by chromatography.

42c

1. TMSCN
BF_3-Et_2O
2. BCl_3

42

15

Compound 42 may be prepared from **Compound 42a** in the same manner as **Compound 5** was prepared from **Compound 2a**.

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5

Compound 43

A solution of one part of **Compoud 2a** in CH_2Cl_2 is treated with about two parts of BF_3OEt_2 at about -78 °C under an argon atmosphere and about three parts of

10 $(CH_2{=}CH\text{-})_2SnBu_2$. The reaction temperature is gradually raised to rt during about one to four hours. Usual extractive workup followed by purification by chromatography will give **Compound 43a**. **Compound 43a** is dissolved in methanol and dichloromethane and cooled to about -78 °C. Ozone is bubbled into the stirred solution for about 1.5 hours at -78 °C. The solution is then flushed with nitrogen to remove the ozone. Sodium

15 borohydride (about 8 equivalents) is then added in small portions over about 5 minutes at -78 °C. Methanol is added and the reaction is slowly warmed to about 0 °C. After about 1.5 hours, the reaction is quenched with saturated bicarbonate solution and extracted with CH_2Cl_2. The combined organics are washed with brine, dried, filtered and the solvent is removed in vacuo. The residue is purified by chromatography to give

20 **Compound 43b**. **Compound 43b** may be debenzylated in the same manner as **Compound 2a** to give **Compound 43** that may be further purified by chromatography.

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Compound 44

12c

$(CH_2{=}CH)_2SnBu_2$

BF_3-Et_2O

44a

1. O_3

2. $NaBH_4$

44b

BBr_3

44

Compound 44 may be obtained in the same manner as **Compound 43**, starting with **Compound 12c**.

Antiviral Activity

Another aspect of the invention relates to methods of inhibiting viral infections, comprising the step of treating a sample or subject suspected of needing such inhibition with a composition of the invention.

Within the context of the invention samples suspected of containing a virus include natural or man-made materials such as living organisms; tissue or cell cultures; biological samples such as biological material samples (blood, serum, urine, cerebrospinal fluid, tears, sputum, saliva, tissue samples, and the like); laboratory samples; food, water, or air samples; bioproduct samples such as extracts of cells,

5 particularly recombinant cells synthesizing a desired glycoprotein; and the like. Typically the sample will be suspected of containing an organism which induces a viral infection, frequently a pathogenic organism such as a tumor virus. Samples can be contained in any medium including water and organic solvent\water mixtures. Samples include living organisms such as humans, and man made materials such as cell cultures.

10 If desired, the anti-virus activity of a compound of the invention after application of the composition can be observed by any method including direct and indirect methods of detecting such activity. Quantitative, qualitative, and semiquantitative methods of determining such activity are all contemplated. Typically one of the screening methods described above are applied, however, any other method such as observation of the 15 physiological properties of a living organism are also applicable.

The antiviral activity of a compound of the invention can be measured using standard screening protocols that are known. For example, the antiviral activity of a compound can be measured using the following general protocols.

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5 **Cell-based Flavivirus Immunodetection assay**

BHK21 or A549 cells are trypsinized, counted and diluted to $2x10^5$ cells/mL in Hams F-12 media (A549 cells) or RPMI-1640 media (BHK21 cells) supplemented with 2% fetal bovine serum (FBS) and 1% penicillin/streptomycin. $2x10^4$ cells are dispensed in a clear 96-well tissue culture plates per well and palced at 37° C, 5% CO_2 overnight.
10 On the next day, the cells are infected with viruses at multiplicity of infection (MOI) of 0.3 in the presence of varied concentrations of test compounds for 1 hour at 37° C and 5% CO_2 for another 48 hours. The cells are washed once with PBS and fixed with cold methanol for 10 min. After washing twice with PBS, the fixed cells are blocked with PBS containing 1% FBS and 0.05% Tween-20 for 1 hour at room temperature. The
15 primary antibody solution (4G2) is then added at a concentration of 1:20 to 1:100 in PBS containing 1% FBS and 0.05% Tween-20 for 3 hours. The cells are then washed three times with PBS followed by one hour incubation with horseradish peroxidase(HRP)-conjugated anti-mouse IgG (Sigma, 1:2000 dilution). After washing three times with PBS, 50 microliters of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution (Sigma)
20 is added to each well for two minutes. The reaction is stopped by addition of 0.5 M sulfuric acid. The plates are read at 450 nm abosorbance for viral load quantification. After measurement, the cells are washed three times with PBS followed by incubation with propidium iodide for 5 min. The plate is read in a Tecan Safire™ reader (excitation 537 nm, emission 617 nm) for cell number quantification. Dose response curves are
25 plotted from the mean absorbance versus the log of the of the concentration of test compounds. The EC_{50} is calculated by non-linear regression analysis. A positive control such as N-nonyl-deoxynojirimycin may be used.

Cell-based Flavivirus cytopathic effect assay

For testing against West Nile virus or Japanese encephalitis virus, BHK21 cells
30 are trypsinized and diluted to a concentration of 4×10^5 cells/mL in RPMI-1640 media supplemented with 2% FBS and 1% penicillin/streptomycin. For testing against dengue virus, Huh7 cells are trypsinized and diluted to a concentration of 4×10^5 cells/mL in

5 DMEM media supplemented with 5% FBS and 1% penicillin/streptomycin. A 50 microliter of cell suspension (2 x 10^4 cells) is dispensed per well in a 96-well optical bottom PIT polymer-based plates (Nunc). Cells are grown overnight in culture medium at 37° C, 5% CO_2, and then infected with West Nile virus (e.g. B956 strain) or Japanese encephalitis virus (e.g. Nakayama strain) at MOI = 0.3, or with dengue virus (e.g. DEN-2
10 NGC strain) at MOI = 1, in the presence of different concentrations of test compounds. The plates containing the virus and the compounds are further incubated at 37°C, 5% CO_2 for 72 hours. At the end of incubation, 100 microliters of CellTiter-Glo™ reagent is added into each well. Contents are mixed for 2 minutes on an orbital shaker to induce cell lysis. The plates are incubated at room temperature for 10 minutes to stabilize
15 luminescent signal. Lumnescence reading is recorded using a plate reader. A positive control such as N-nonyl-deoxynojirimycin may be used.

Antiviral Activity in a Mouse Model of Dengue Infection.

Compounds are tested *in vivo* in a mouse model of dengue virus infection (Schul *et al.* J. Infectious Dis. 2007; 195:665-74). Six to ten week old AG129 mice (B&K
20 Universal Ltd, Hll, UK) are housed in individually ventilated cages. Mice are injected intraperitoneally with 0.4 mL TSV01 dengue virus 2 suspension. Blood samples are taken by retro orbital puncture under isoflurane anaesthesia. Blood samples are collected in tubes containing sodium citrate to a final concentration of 0.4%, and immediately centrifuged for 3 minutes at 6000g to obtain plasma. Plasma (20 microliters) is diluted
25 in 780 microliters RPMI-1640 medium and snap frozen in liquid nitrogen for plaque assay analysis. The remaining plasma is reserved for cytokine and NS1 protein level determination. Mice develop dengue viremia rising over several days, peaking on day 3 post-infection.

For testing of antiviral activity, a compound of the invention is dissolved in
30 vehicle fluid, e.g. 10% ethanol, 30% PEG 300 and 60% D5W (5% dextrose in water; or 6N HCl (1.5 eq):1N NaOH (pH adjusted to 3.5): 100 mM citrate buffer pH 3.5 (0.9% v/v:2.5% v/v: 96.6% v/v). Thirty six 6-10 week old AG129 mice are divided into six

5 groups of six mice each. All mice are infected with dengue virus as described above (day 0). Group 1 is dosed by oral gavage of 200 mL/mouse with 0.2 mg/kg of a compound of the invention twice a day (once early in the morning and once late in the afternoon) for three consecutive days starting on day 0 (first dose just before dengue infection). Groups 2, 3 and 4 are dosed the same way with 1 mg/kg, 5 mg/kg and 25
10 mg/kg of the compound, respectively. A positive control may be used, such as (2R,3R,4R,5R)-2-(2-amino-6-hydroxy-purin-9-yl)-5-hydroxymethyl-3-methyl-tetrahydro-furan-3,4-diol, dosed by oral gavage of 200 microliters/mouse the same way as the previous groups. A further group is treated with only vehicle fluid.

On day 3 post-infection approximately 100 microliter blood samples (anti-
15 coagulated with sodium citrate) are taken from the mice by retro-orbital puncture under isoflurane anaesthesia. Plasma is obtained from each blood sample by centrifugation and snap frozen in liquid nitrogen for plague assay analysis. The collected plasma samples are analyzed by plague assay as described in Schul *et al.* Cytokines are also analysed as as described by Schul. NS1 protein levels are analysed using a Platelia^{TM} kit (BioRad
20 Laboratories). An anti-viral effect is indicated by a reduction in cytokine levels and/or NS1 protein levels.

Typically, reductions in viremia of about 5-100 fold, more typically 10-60 fold, most typically 20-30 fold, are obtained with 5-50 mg/kg bid dosages of the compounds of the invention.

25

HCV IC_{50} Determination

Assay Protocol: NS5b polymerase assay (40 µL) was assembled by adding 28 µL polymerase mixture (final concentration: 50 mM Tris-HCl at pH 7.5, 10 mM KCL, 5
30 mM $MgCl_2$, 1 mM DTT, 10 mM EDTA, 4 ng/µL of RNA template, and 75 nM HCV Δ21 NS5b polymerase) to assay plates followed by 4 µL of compound dilution. The polymerase and compound were pre-incubated at 35 °C for 10 minute before the addition

5 of 8 μL of nucleotide substrate mixture (33P-α-labeled competing nucleotide at K_M and 0.5 mM of the remaining three nucleotides). The assay plates were covered and incubated at 35 °C for 90 min. Reactions were then filtered through 96-well DEAE-81 filter plates via vacuum. The filter plates were then washed under vacuum with multiple volumes of 0.125 M $NaHPO_4$, water, and ethanol to remove unincorporated label. Plates 10 were then counted on TopCount to assess the level of product synthesis over background controls. The IC50 value is determined using Prism fitting program.

Preferably, compounds described herein inhibited NS5b polymerase with an IC_{50}'s below 1000 μM, more preferably below 100 μM, and most preferably below 10 μM. For example, compound 17 has an IC_{50} below 1 μM.

15

HCV EC_{50} Determination

Replicon cells were seeded in 96-well plates at a density of 8 x 10^3 cells per well in 100 μL of culture medium, excluding Geneticin. Compound was serially diluted in 20 100% DMSO and then added to the cells at a 1:200 dilution, achieving a final concentration of 0.5% DMSO and a total volume of 200 μL. Plates were incubated at 37°C for 3 days, after which culture medium was removed and cells were lysed in lysis buffer provided by Promega's luciferase assay system. Following the manufacturer's instruction, 100 μL of luciferase substrate was added to the lysed cells and luciferase 25 activity was measured in a TopCount luminometer. Preferably, compounds described herein have EC50's below 1000 μM, more preferably below 100 μM, and most preferably below 10 μM.

Representative examples of the activity of the compounds Formula I-III are shown in the Table below wherein A represents an EC_{50} below 1 μM, B represents an 30 EC_{50} between 1 and 10 μM, and C represents an EC_{50} between 10 and 100 μM.

Example No.	EC_{50}, μM
2	C

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3	C
4	C
5	C
6	A
12	B
13	B

5

The cytotoxicity of a compound of the invention can be determined using the following general protocol.

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5

Cytotoxicity Cell Culture Assay (Determination of CC50):

The assay is based on the evaluation of cytotoxic effect of tested compounds using a metabolic substrate.

Assay protocol for determination of CC50:

10 1. Maintain MT-2 cells in RPMI-1640 medium supplemented with 5% fetal bovine serum and antibiotics.
2. Distribute the cells into a 96-well plate (20,000 cell in 100 µl media per well) and add various concentrations of the tested compound in triplicate (100 µl/well). Include untreated control.
15 3. Incubate the cells for 5 days at 37 °C.
4. Prepare XTT solution (6 ml per assay plate) in dark at a concentration of 2mg/ml in a phosphate-buffered saline pH 7.4. Heat the solution in a water-bath at 55°C for 5 min. Add 50 µl of N-methylphenazonium methasulfate (5 µg/ml) per 6 ml of XTT solution.
20 5. Remove 100 µl media from each well on the assay plate and add 100 µl of the XTT substrate solution per well. Incubate at 37 °C for 45 to 60 min in a CO_2 incubator.
6. Add 20 µl of 2% Triton X-100 per well to stop the metabolic conversion of XTT.
7. Read the absorbance at 450 nm with subtracting off the background at 650 nm.
8. Plot the percentage absorbance relative to untreated control and estimate the CC50
25 value as drug concentration resulting in a 50% inhibition of the cell growth. Consider the absorbance being directly proportional to the cell growth.

All publications, patents, and patent documents cited herein above are incorporated by reference herein, as though individually incorporated by reference.

30 The invention has been described with reference to various specific and preferred embodiments and techniques. However, one skilled in the art will understand that many variations and modifications may be made while remaining within the spirit and scope of the invention.

2009240642 01 Aug 2013

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A compound of Formula I:

Formula I

or a pharmaceutically acceptable salt, thereof;

wherein:

each R^1, R^2, R^3, R^4, or R^5 is independently H, OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, halogen, $(C_1$-$C_8)$alkyl, $(C_4$-$C_8)$carbocyclylalkyl, $(C_1$-$C_8)$substituted alkyl, $(C_2$-$C_8)$alkenyl, $(C_2$-$C_8)$substituted alkenyl, $(C_2$-$C_8)$alkynyl, $(C_2$-$C_8)$substituted alkynyl, or aryl$(C_1$-$C_8)$alkyl;

or any two R^1, R^2, R^3, R^4, or R^5 on adjacent carbon atoms when taken together are -O(CO)O- or when taken together with the ring carbon atoms to which they are attached form a double bond;

R^6 is OR^a, $N(R^a)_2$, N_3, CN, NO_2, $S(O)_nR^a$, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, halogen, $(C_1$-$C_8)$alkyl, $(C_4$-$C_8)$carbocyclylalkyl, $(C_1$-$C_8)$substituted alkyl, $(C_2$-$C_8)$alkenyl, $(C_2$-$C_8)$substituted alkenyl, $(C_2$-$C_8)$alkynyl, $(C_2$-$C_8)$substituted alkynyl, or aryl$(C_1$-$C_8)$alkyl or R^6 and either R^1 or R^2 when taken together are -O(CO)O-;

each n is independently 0, 1, or 2;

each R^a is independently H, $(C_1$-$C_8)$alkyl, $(C_2$-$C_8)$alkenyl, $(C_2$-$C_8)$alkynyl, aryl$(C_1$-$C_8)$alkyl, $(C_4$-$C_8)$carbocyclylalkyl, $-C(=O)R^{11}$, $-C(=O)OR^{11}$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, or $-S(O)_2NR^{11}R^{12}$;

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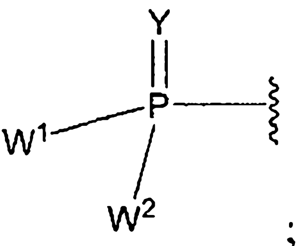
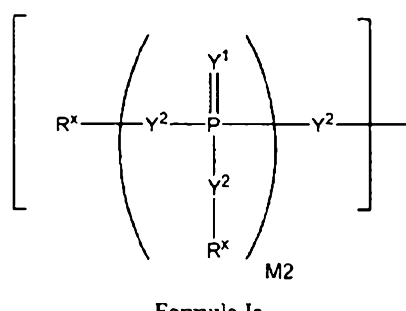
R^7 is H, $-C(=O)R^{11}$, $-C(=O)OR^{11})$, $-C(=O)NR^{11}R^{12}$, $-C(=O)SR^{11}$, $-S(O)R^{11}$, $-S(O)_2R^{11}$, $-S(O)(OR^{11})$, $-S(O)_2(OR^{11})$, $-SO_2NR^{11}R^{12}$, or



;

Y is O, S, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, or $N-NR_2$;

5 W^1 and W^2, when taken together, are $-Y^3(C(R^y)_2)_3Y^3-$; or one of W^1 orW^2 together with either R^3 or R^4 is $-Y^3-$ and the other of W^1 or W^2 is Formula Ia; or W^1 and W^2 are each, independently, a group of the Formula Ia:



Formula Ia

wherein:

10 each Y^1 is, independently, O, S, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, or $N-NR_2$;

each Y^2 is independently a bond, O, CR_2, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, $N-NR_2$, S, S-S, S(O), or $S(O)_2$;

each Y^3 is independently O, S, or NR;

M2 is 0, 1 or 2;

15 each R^x is a group of the formula:

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wherein:

each M1a, M1c, and M1d is independently 0 or 1;

M12c is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

each R^y is independently H, F, Cl, Br, I, OH, -CN, $-N_3$, $-NO_2$, -OR, $-C(=Y^1)R$, $C(Y^1)W^5$, $-C(=Y^1)OR$, $-C(=Y^1)N(R)_2$, $-N(R)_2$, $-^+N(R)_3$, -SR, -S(O)R, $-S(O)_2R$, $-SO_2W^5$, -S(O)(OR), $-S(O)_2(OR)$, $-OC(=Y^1)R$, $-OC(=Y^1)OR$, $-OC(=Y^1)(N(R)_2)$, $-SC(=Y^1)R$, $-SC(=Y^1)OR$, $-SC(=Y^1)(N(R)_2)$, $-N(R)C(=Y^1)R$, $-N(R)C(=Y^1)OR$, $-N(R)C(=Y^1)N(R)_2$, $-SO_2NR_2$, W^5, (C_1-C_8) alkyl, (C_1-C_8) substituted alkyl, (C_2-C_8) alkenyl, (C_2-C_8) substituted alkenyl, (C_2-C_8) alkynyl, (C_2-C_8) substituted alkynyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_2-C_{20} heterocyclyl, C_2-C_{20} substituted heterocyclyl, arylalkyl or substituted arylalkyl; wherein each alkyl, alkenyl, alkynyl, aryl, heterocyclyl, or arylalkyl is independently optionally substituted with one or more Z groups;

or when taken together, two R^y on the same carbon atom form a carbocyclic ring of 3 to 7 carbon atoms;

each W^5 is independently a carbocycle or a heterocycle optionally substituted with 1 to 3 R^z groups;

each R^z is independently F, Cl, Br, I, OH, -CN, $-N_3$, $-NO_2$, -OR, $-C(=Y^1)R$, $-C(=Y^1)OR$, $-C(=Y^1)N(R)_2$, $-N(R)_2$, $-^+N(R)_3$, -SR, -S(O)R, $-S(O)_2R$, -S(O)(OR), $-S(O)_2(OR)$, $-OC(=Y^1)R$, $-OC(=Y^1)OR$, $-OC(=Y^1)(N(R)_2)$, $-SC(=Y^1)R$, $-SC(=Y^1)OR$, $-SC(=Y^1)(N(R)_2)$, $-N(R)C(=Y^1)R$, $-N(R)C(=Y^1)OR$, $-N(R)C(=Y^1)N(R)_2$, $-SO_2NR_2$, (C_1-C_8) akyl, (C_1-C_8) substituted alkyl, (C_2-C_8) alkenyl, (C_2-C_8) substituted alkenyl, (C_2-C_8) alkynyl, (C_2-C_8) substituted alkynyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_2-C_{20} heterocyclyl, C_2-C_{20} substituted heterocyclyl, arylalkyl or substituted arylalkyl; wherein each alkyl, alkenyl, alkynyl, aryl, heterocyclyl, or arylalkyl is independently optionally substituted with one or more Z groups;

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each R is independently H, $(C_1\text{-}C_8)$ alkyl, $(C_1\text{-}C_8)$ substituted alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$ substituted alkenyl, $(C_2\text{-}C_8)$ alkynyl, $(C_2\text{-}C_8)$ substituted alkynyl, $C_6\text{-}C_{20}$ aryl, $C_6\text{-}C_{20}$ substituted aryl, $C_2\text{-}C_{20}$ heterocyclyl, $C_2\text{-}C_{20}$ substituted heterocyclyl, arylalkyl or substituted arylalkyl;

wherein each alkyl, alkenyl, alkynyl, aryl, heterocyclyl, or arylalkyl, is independently optionally substituted with one or more Z groups;

each X^1 or X^2 is independently C-R^{10} or N;

each R^8 is halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, N_3, NO, NO_2, CHO, CN, -CH(=NR^{11}), -CH=NHNR^{11}, -CH=N(OR^{11}), -CH$(OR^{11})_2$, -C(=O)$NR^{11}R^{12}$, -C(=S)$NR^{11}R^{12}$, -C(=O)OR^{11}, $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl, $(C_4\text{-}C_8)$carbocyclylalkyl, optionally substituted aryl, optionally substituted heteroaryl, -C(=O)$(C_1\text{-}C_8)$alkyl, -$S(O)_n(C_1\text{-}C_8)$alkyl, aryl$(C_1\text{-}C_8)$alkyl, OR^{11} or SR^{11};

wherein each aryl or heteroaryl is independently optionally substituted with one or more Z groups;

each R^9 or R^{10} is independently H, halogen, $NR^{11}R^{12}$, $N(R^{11})OR^{11}$, $NR^{11}NR^{11}R^{12}$, N_3, NO, NO_2, CHO, CN, -CH(=NR^{11}), -CH=NHNR^{11}, -CH=N(OR^{11}), -CH$(OR^{11})_2$, -C(=O)$NR^{11}R^{12}$, -C(=S)$NR^{11}R^{12}$, -C(=O)OR^{11}, R^{11}, OR^{11} or SR^{11};

each R^{11} or R^{12} is independently H, $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl, $(C_4\text{-}C_8)$carbocyclylalkyl, optionally substituted aryl, optionally substituted heteroaryl, -C(=O)$(C_1\text{-}C_8)$alkyl, -$S(O)_n(C_1\text{-}C_8)$alkyl or aryl$(C_1\text{-}C_8)$alkyl; wherein each aryl or heteroaryl is independently optionally substituted with one or more Z groups;

or R^{11} and R^{12} taken together with a nitrogen to which they are both attached from a 3 to 7 membered heterocyclic ring wherein any one carbon atom of said heterocyclic ring can optionally be replaced with -O-, -S- or -NR^a-;

each Z is independently halogen, -O^-, =O, -OR^b, -SR^b, -S^-, -$NR^b{}_2$, -$N^+R^b{}_3$, =NR^b, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, -NO_2, =N_2, -N_3, -NHC(=O)R^b, -OC(=O)R^b, -NHC(-=O)$NR^b{}_2$, -$S(=O)_2$-, -$S(=O)_2OH$, -$S(=O)_2R^b$, -$OS(=O)_2OR^b$, -$S(=O)_2NR^b{}_2$, -S(=O)R^b, -OP(=O)$(OR^b)_2$, -P(=O)$(OR^b)_2$, -P(=O)$(O^-)_2$, -P(=O)$(OH)_2$, -P(O)$(OR^b)(O^-)$, -C(=O)R^b, -C(=O)X,

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-C(S)R^b, -C(O)OR^b, -C(O)O$^-$, -C(S)OR^b, -C(O)SR^b, -C(S)SR^b, -C(O)NR$^b_2$, -C(S)NR$^b_2$, -C(=NR^b)NR$^b_2$, where each R^b is independently H, alkyl, aryl, arylalkyl, or heterocyclyl;

wherein each (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl or aryl(C_1-C_8)alkyl of each R^1, R^2, R^3, R^4, R^5, R^6, R^{11} or R^{12} is, independently, optionally substituted with one or more halo, hydroxy, CN, N_3, N(R^a)$_2$ or OR^a; and wherein one or more of the nonterminal carbon atoms of each said (C_1-C_8)alkyl is optionally replaced with -O-, -S- or -NR^a-.

2. A compound according to claim 1 represented by Formula II

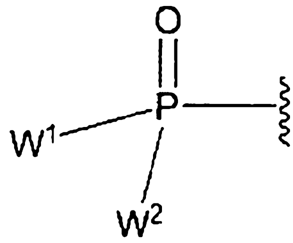
Formula II

wherein X^2 is C-R^{10} and each Y and Y^1 is O.

3. A compound according to claims 1 or 2 wherein R^8 is halogen, NR^{11}R^{12}, N(R^{11})OR^{11}, NR^{11}NR^{11}R^{12}, OR^{11} or SR^{11}.

4. A compound according to any one of claims 1 to 3 wherein R^9 is H or NR^{11}R^{12}.

5. A compound according to any one of claims 1 to 4 wherein R^7 is H or



6. A compound according to any one of claims 1 to 5 wherein R^6 is OR^a, N_3, halogen, CN, methyl, hydroxymethyl, substituted methyl, ethenyl, substituted ethenyl, ethynyl, or substituted ethynyl.

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7. A compound according to any one of claims 1 to 6 wherein X^2 is C-H and R^3 and R^5 are each H.

8. A compound according to any one of claims 1 to 7 wherein at least one of R^2 or R^4 is OR^a.

5 9. A compound according to any one of claims 1 to 8 wherein X^1 is N or C-R^{10} wherein R^{10} is H, halogen, CN or optionally substituted heteroaryl.

10. A compound according to any one of claims 1 to 9 wherein R^2 and R^4 are each OR^a.

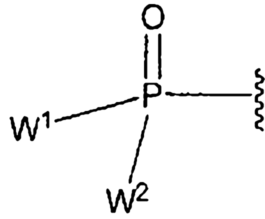
11. A compound according to any one of claims 1 to 10 wherein R^2 and R^4 are OH.

12. A compound according to any one of claims 1 to 11 wherein R^1 is H, methyl, CH_2OH,
10 CH_2F, ethenyl, or ethynyl.

13. A compound according to any one of claims 1 to 12 wherein X^1 is N.

14. A compound according to any one of claims 1 to 12 wherein X^1 is C-H.

15. A compound according to any one of claims 1 to 14 wherein



15 is selected from

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wherein Y^2 is, independently, a bond, O, or CR_2.

16. A compound according to any one of claims 1 to 15 wherein W^1 and W^2 are each, independently, a group of the Formula Ia.

5 17. A compound according to any one of claims 1 – 14 wherein R^7 is H.

18. A compound that is

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NH2 HO OH HO OH N N N , NH2 HO O HO OH N N N ; NH2 HO HO OH N N N ,

NH2 HO CN HO OH N N N , O S O P O O S O O O CN HO OH NH2 N N N ,

HO-P-O-P-O-P-O OH OH OH CN HO OH NH2 N N N , NH2 HO N3 HO OH N N N ,

NH2 HO HO OH N N N , NH2 HO OH HO OH N N N ; O NH HO OH HO OH N N NH2 ,

NH2 HO O HO OH N N N , NH2 HO CN HO OH N N N , NH2 HO HO OH N N N ,

NH2 HO N3 HO OH N N N ; NH2 HO HO OH N N N , HO-P-O OH CN HO OH NH2 N N N ,

O O O O P O O O O CN HO OH NH2 N N N , O HN-P-O O O OH HO OH NH NH2 N N ,

O H N O P O O CN HO OH NH2 N N N Cl , N O O P O O CN HO OH NH2 N N N ,

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NH$_2$ N O O P O O O O N N N CN O O O , NH$_2$ O O H N P O O O O N N N HO OH Cl ,

NH$_2$ O O N N N O O H N P O O O OH , NH$_2$ N N HO O N N N CN HO OH ,

HO O P HO O O NH$_2$ N N N N CN HO OH , NH$_2$ N N O N N N CN O HO P O O OH O ,

NH$_2$ N O O O O N N N CN O P O O O OH , NH$_2$ N N HO O N N N NH$_2$ OH HO OH ,

NH$_2$ N N HO O N N N NH$_2$ CN HO OH ; HO O S O O P O NH NH$_2$ N O N N N NH$_2$ CN HO OH ,

NH$_2$ N HO O N N NH$_2$ OH HO OH , NH$_2$ N HO O N N NH$_2$ CN HO OH ,

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, , or ;

or a pharmaceutically acceptable salt thereof.

19. A pharmaceutical composition comprising a therapeutically effective amount of a compound as in any one of claims 1 to 18 and a pharmaceutically acceptable carrier.

5 20. The pharmaceutical composition of claim 19 further comprising at least one additional therapeutic agent selected from the group consisting of interferons, ribavirin analogs, NS3 protease inhibitors, NS5a inhibitors, NS5b polymerase inhibitors, alpha-glucosidase 1 inhibitors, cyclophilin inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV.

10 21. A method of inhibiting HCV polymerase comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1 to 18.

22. A method of treating a viral infection caused by a virus selected from the group consisting of dengue virus, yellow fever virus, West Nile virus, Japanese encephalitis virus, tick-borne encephalitis virus, Kunjin virus, Murray Valley encephalitis virus, St Louis encephalitis
15 virus, Omsk hemorrhagic fever virus, bovine viral diarrhea virus, Zika virus and Hepatitis C virus comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of any one of claims 1 to 18 or a pharmaceutical composition of claim 19 or 20.

23. The method of claim 22 wherein the viral infection is caused by Hepatitis C virus.

24. The method of claim 22 or 23 further comprising administering at least one additional
20 therapeutic agent selected from the group consisting of interferons, ribavirin analogs, NS3 protease inhibitors, NS5a inhibitors, NS5b polymerase inhibitors, alpha-glucosidase 1 inhibitors,

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cyclophilin inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV.

25. A compound as in any one of claims 1 to 18 used in the manufacture of a medicament for treating a viral infection caused by a virus selected from the group consisting of dengue virus, 5 yellow fever virus, West Nile virus, Japanese encephalitis virus, tick-borne encephalitis virus, Kunjin virus, Murray Valley encephalitis virus, St Louis encephalitis virus, Omsk hemorrhagic fever virus, bovine viral diarrhea virus, Zika virus and Hepatitis C virus.

26. A compound according to claim 1 substantially as hereinbefore described with reference to any one of the examples.

ANNEXURE 3

We Claim:

1. A compound of Formula I:

Formula I

or a pharmaceutically acceptable salt, thereof;

wherein:

R^1 is H or $(C_1–C_8)$alkyl;

R^2 and R^4 are each independently OR^a;

or R^2 and R^4 when taken together are –O(CO)O-;

R^3 and R^5 are each H;

R^6 is OR^a, N_3, CN, $(C_1–C_8)$alkyl, $(C_1–C_8)$substituted alkyl, $(C_2–C_8)$alkenyl, or $(C_2–C_8)$alkynyl;

each R^a is independently H or $(C_1$-$C_8)$alkyl;

R^7 is H, or

;

each Y or Y^1 is O;

W^1 and W^2, when taken together, are $–Y^3(C(R^y)_2)_3Y^3$-; or one of W^1 or W^2 together with either R^3 or R^4 is $–Y^3$- and the other of W^1 or W^2 is Formula Ia; or W^1 and W^2 are each, independently, a group of the Formula Ia:

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Formula Ia

wherein:

each Y^2 is independently O or NR;

each Y^3 is O;

M2 is 0, 1 or 2;

each R^x is independently R^y or the formula:

wherein:

each M1a, M1c, and M1d is independently 0 or 1;

M12c is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

each R^y is independently H, R, $-C(=Y^1)OR$, $-OC(=Y^1)R$, $-OC(=Y^1)OR$, or $-SC(=Y^1)R$;

each R is independently H, $(C_1\text{-}C_8)$ alkyl, $(C_1\text{-}C_8)$ substituted alkyl, $C_6\text{–}C_{20}$ aryl, $C_6\text{–}C_{20}$ substituted aryl, $C_2\text{–}C_{20}$ heterocyclyl, or arylalkyl;

X^1 is $C\text{-}R^{10}$ or N;

X^2 is $C\text{-}R^{10}$;

R^8 is $NR^{11}R^{12}$;

R^9 is H or $NR^{11}R^{12}$;

R^{10} is H;

each R^{11} or R^{12} is independently H, or $(C_1\text{-}C_8)$alkyl.

2. A compound as claimed in claim 1 wherein R^6 is OR^a, N_3, halogen, CN, methyl, hydroxymethyl, ethenyl, or ethynyl.

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3. A compound as claimed in claim 1 or 2 wherein R^2 and R^4 are OH.

4. A compound as claimed in any one of claims 1-3 wherein X^1 is N.

5. A compound as claimed in any one of claims 1-3 wherein X^1 is C-H.

6. A compound as claimed in any one of claims 1-4 wherein R^1 is H or methyl.

7. A compound as claimed in any one of claims 1-6 wherein W^1 and W^2 are each, independently, a group of the Formula Ia.

8. A compound as claimed in any one of claims 1-7 wherein R^7 is H.

9. A compound as claimed in claim 1 that is

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, , , , , , , , or ;

or a pharmaceutically acceptable salt thereof.

10. The compound as claimed in claim 9 is

,

or a pharmaceutically acceptable salt thereof.

11. A compound as claimed in claim 1 that is

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or ;

or a pharmaceutically acceptable salt thereof.

12. A compound as claimed in any one of claims 1-11 that is in a form of a racemate, enantiomer, diastereomer, tautomer, polymorph, pseudopolymorph or amorphous form.

13. The compound as claimed in any one of claims 1-11 for preparation of a composition wherein such composition optionally comprises a further therapeutic agent selected from group consisting of interferons, ribavirin analogs, NS3 protease inhibitors, NS5a inhibitors, NS5b polymerase inhibitors, alpha-glucosidase 1 inhibitors, cyclophilin inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV.

14. The compound as claimed in any one of claims 1-11 for inhibiting HCV polymerse or a viral infection wherein viral infection is caused by a virus selected from the group consisting of dengue virus, yellow fever virus, West Nile virus, Japanese encephalitis virus, tick-borne encephalitis virus, Kunjin virus, Murray Valley encephalitis virus, St. Louis encephalitis virus, Omsk hemorrhagic fever virus, bovine viral diarrhea virus, Zika virus and Hepatitis C virus.

Dated this the 06th day of October 2010

(Ritu Gandhi)
of SUBRAMANIAM & ASSOCIATES
Attorney for the applicants

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 ANNEXURE 4 205

Aryl Phosphoramidate Derivatives of d4T Have Improved Anti-HIV Efficacy in Tissue Culture and May Act by the Generation of a Novel Intracellular Metabolite

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Received August 14, 1995⊗

New phosphate derivatives of the anti-HIV nucleoside analogue d4T were prepared as potential membrane-soluble prodrugs of the bioactive free nucleotide. The enhanced antiviral potency and/or reduced cytotoxicity of the derivatives leads to an increase in selectivity relative to the parent nucleoside analogue. Moreover, the derivatives appear to bypass the dependence of the nucleoside on thymidine kinase-mediated activation, retaining full activity in thymidine kinase-deficient cells. This strongly suggests the successful intracellular delivery of free nucleotides by the masked phosphate triester prodrugs. This is further confirmed by studies using radiolabeled compound which clearly demonstrate the generation of d4T mono-, di- and triphosphates from the prodrug, even in thymidine kinase-deficient cells. Moreover, we herein report the generation of a new metabolite, a partially hydrolyzed phosphate diester, alaninyl d4T monophosphate. We suggest that at least part of the antiviral action of the prodrugs derives from the intracellular generation of such novel diesters which may add considerable weight to the suggested further preclinical development of the phosphate prodrugs.

Introduction

Several members of the 2′,3′-dideoxynucleoside (ddN) series are potent inhibitors of human immunodeficiency virus (HIV) in cell culture.[1−5] The 5′-triphosphates of these nucleoside analogues are potent inhibitors of HIV reverse transcriptase.[6−8] As a rule, the activation (phosphorylation) of these nucleosides is in most cases accomplished by cellular nucleoside and nucleotide kinases. Thus, in contrast to other antiviral agents (e.g. acyclovir) where (herpes simplex) virus-specific thymidine kinase mediates the first step of the conversion of the drug to the intracellular active species, the 2′,3′-dideoxynucleoside analogues depend on cellular nucleoside kinases for their phosphorylation. However, in many cases the ddN derivatives have a poor affinity for nucleoside kinases (i.e. 2′,3′-dideoxycytidine for deoxycytidine kinase, 2′,3′-didehydro-2′,3′-dideoxythymidine and 2′,3′-dideoxyuridine for thymidine kinase, 2′,3′-dideoxyadenosine for adenosine kinase and deoxycytidine kinase, and 2′,3′-dideoxyinosine for 5′-nucleotidase).[9−13] Moreover, the dependence on phosphorylation for activation of the particular nucleoside analogue may be a particular problem in cells where the nucleoside kinase activity is known to be low or even lacking (i.e. monocyte/macrophages).[14] Therefore, we have sought to overcome this dependence on nucleoside kinase activation by the development of a suitable nucleotide delivery strategy. The viability of such an approach is entirely based on the ability to suitably modify the phosphate structure of a membrane-soluble masked nucleotide to enable intracellular delivery and release of the free phosphate form. We have previously noted the success of this strategy with (aryloxy)phosphoramidates (**2**) derived from AZT (**1**).[15] These phosphoramidates retained good activity in thymidine kinase-deficient (TK$^-$) cells, by comparison to thymidine kinase-competent cells, indicating a (partial) independence of thymidine kinase activation. We were particularly keen to apply this technology to the 2′,3′-dideoxy-2′,3′-didehydro analogue of thymidine (d4T) (**3**) for several reasons. This nucleoside analogue has been noted to be a potent inhibitor of HIV, but displays reduced cytotoxicity in certain cells (e.g. bone marrow progenitor cells) compared to AZT.[3,16,17] Furthermore, it is known that the kinetics of the three phosphorylation steps from the nucleoside analogue to the (bioactive) triphosphate differ in the case of d4T, by comparison to AZT and other 3′-modified nucleoside analogues. In particular, the rate-limiting step for AZT appears to be the conversion of mono- to diphosphate, whereas the conversion of nucleoside to monophosphate may well be rate-limiting for d4T.[7] It could follow that the intracellular delivery of preformed d4T monophosphate (d4TMP) may be more useful than the delivery of AZT monophosphate. From a series of blocked phosphate esters of d4T we have noted significant and selective anti-HIV activity for a phosphoramidate derivative So324 which we herein describe. Particularly interesting is the observation that this material may act by an additional mechanism of action.

Results and Discussion

Chemistry. D4T (**3**) was prepared from thymidine essentially by the method of Horwitz et al.,[18] noting the later comments of Mansuri et al.[19] Then, phenyl methoxyalaninyl phosphorochloridate[20] was allowed to react with d4T using THF/*N*-methylimidazole to give compound **4a** in good yield (88). As anticipated, this material displayed two closely spaced signals in the ^{31}P NMR (δ_P ca. 3.3 ppm),[21] corresponding to the presence of diastereoisomers, resulting from mixed stereochemistry at the phosphate center. Similar diastereomeric splitting, and phosphorus coupling where appropriate,

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Table Aand Anti-HIV-2 Activity and Cytotoxicity of Test Compounds **1**, **3**, **4a**–**f**, **5a**–**e**, and **6a,b** *in vitro*[a]and Anti-HIV-2 Activity and Cytotoxicity of Test Compounds **1**, **3**, **4a**–**f**, **5a**–**e**, and **6a,b** *in vitro*[a]

	EC_{50} (μM)					CC50 (μM)	
compd	MT-4 HIV-1	MT-4 HIV-2	CEM/0 HIV-1	CEM/0 HIV-2	CEM/TK$^-$ HIV-1	MT4	CEM/0
1	0.002	0.003	0.003	0.004	>100	6	>500
3	0.651	0.770	0.800	0.775	33.3	19	174
4a	0.066	0.067	0.182	0.200	0.075	>100	100
4b	1.34	6.71	6	6	7	≥250	≥250
4c	3.58	11.2	12.5	12.5	4	>250	>250
4d	0.2	0.47	1.1	2.23	0.4	61.5	≥250
4e	0.19	0.38	0.8	1.35	0.33	34.1	216
4f	0.23	0.38	0.6	0.8	0.34	22	≥250
5a	ND	ND	0.04	0.055	0.025	ND	16
5b	0.046	0.11	0.15	0.15	0.11	9.75	29.8
5c	1.72	4.07	2.5	3.7	8.5	82.2	115
5d	0.037	0.12	0.11	0.15	0.11	10.5	26.9
5e	0.057	0.063	0.07	0.16	0.06	>100	60
6a	25.4	50.9	20	50	>250	>250	>250
6b	28.5	62.5	48	240	48	≥250	≥250

[a] EC_{50} is the 50% effective concentration or compound concentration required to protect MT-4 or CEM cells against the cytopathogenicity of HIV by 50%. Data are the mean of two to four independent experiments. CC_{50} is the 50% cytotoxic concentration or compound concentration required to reduce MT-4 or CEM cell viability by 50%.

were also noted in the H-decoupled ^{13}C spectrum. The presence of diastereoisomers was also apparent from ^1H NMR spectroscopy, and analytical HPLC studies on **4a**.

Similarly prepared were the analogues of **4a** with other amino acids: glycine **4b**, valine **4c**, leucine **4d**, phenylalanine **4e**, and methionine **4f**. Each of the analogues was isolated as a mixture of [phosphate] diastereoisomers, as evidenced from spectroscopic and chromatographic data.

We were also interested in studying the structure–activity relationships operating in the aryl region of the phosphoramidates. In particular, we had previously noted that, for diaryl phosphates of AZT, the introduction of electron-withdrawing groups within the aryl moieties leads to a substantial increase in antiviral activity.[22] This was taken as corresponding to the increased ability of such systems to undergo [intracellular] liberation of the aryl moiety and release of the phosphate diester *en route* to the free nucleotides. Thus, we prepared the 2,4-dibromo- (**5a**), 3-trifluoromethyl (**5b**), pentafluoro (**5c**), and 3,5-dichloro (**5d**) analogues, each with some degree of electron withdrawal. For purposes of comparison, we also prepared the 4-ethyl compound **5e** which lacks any electron-withdrawing nature.

Finally, we prepared the analogues lacking the aryl moiety entirely, and instead carrying a methyl group (**6a**) or an ethyl group (**6b**). These were designed to simply probe the necessity for antiviral action of an aryloxy group.

Antiviral Activity. The parent nucleoside d4T (**3**) and phosphoramidates (**4a**–**f, 5a**–**e,** and **6a,b**) were tested for their ability to inhibit the replication of HIV, as previously described,[23] and the results obtained using HIV-1- or HIV-2-infected CEM and MT4 cells are displayed in Table 1. The clinically used nucleoside analogue AZT (**1**) was included as reference material, and tests were also conducted in thymidine kinase-deficient CEM cells.

It is notable that the phosphoramidate derivative **4a** is approximately 4–10-fold more potent than d4T itself against HIV-1 and HIV-2 in MT-4 and CEM cells. Moreover, the >5-fold reduced cytotoxicity of **4a** by comparison to d4T **3** in MT-4 cells leads to a 50-fold improved selectivity index for the phosphoramidate. Furthermore, it is particularly striking that, whereas d4T is virtually inactive in thymidine kinase-deficient CEM cells, the phosphoramidate **4a** retains *full* activity, being ca. 400-fold more potent than d4T in these cells. Similarly, while AZT is inherently more potent than either d4T or the d4T phosphate in thymidine kinase-competent CEM cells, the phosphoramidate derivative **4a** is >1300 times more active than AZT in the kinase-deficient CEM cell line.

In terms of structure-activity relationships in operation, it is apparent that relatively small changes in the amino acid region lead to significant changes in activity. Thus, of the series **4a**–**f**, the alanine compound **4a** is the most potent, with leucine **4d**, phenylalanine **4e**, and methionine **4f** analogues being approximately 10-fold less active, depending on the virus and cell line in question. The glycine compound **4b** is less active still, and the valine analogue **4c** is the least active of the series, being approximately 100-fold less potent than the lead alanine compound **4a**. However, throughout this series, it is notable that full activity is always retained in TK$^-$ cells by comparison to the activity shown in TK$^+$ cells.

Similarly, there is some variation in activity with varying substitution in the aryloxy moiety, studying the series **4a**, and **5a**–**e**. The dibromophenyl analogue **5a** is perhaps slightly more active than the parent phenyl compound **4a**, although this is at the cost of some cytotoxicity in CEM cells. The (trifluoromethyl)phenyl (**5b**), dichlorophenyl (**5d**), and the ethylphenyl (**5e**) compounds are approximately equipotent with the parent phenyl system, while the pentafluorophenyl analogue **5c** is approximately 50-fold less potent. Again, unlike the parent phenyl compound, some of the substituted aryl analogues appear to exhibit significant cytotoxicity. As a result, the highest selectivity index is displayed by the parent phenyl compound **4a**.

Finally, the importance of an aryl moiety for antiviral activity is most evident noting the data for the methyl (**6a**) and ethyl (**6b**) phosphates. These show very little antiviral activity, being approximately 1000-fold less potent than the lead compound **4a**.

Data in TK$^-$ versus TK$^+$ cells clearly demonstrate that the antiviral activity of the phosphoramidates **4a**–**f** and **5a**–**e** is independent of thymidine kinase-mediated phosphorylation. The most obvious mechanism of action consistent with this observation is of intracellular delivery of the free nucleotide d4TMP from the phosphoramidates, and further phosphorylation to generate the active metabolite d4T triphosphate (d4TTP). In order to test this hypothesis, we incubated CEM cells with ^3H-labeled compound **4a** and subsequently studied the formation of radiolabeled intracellular metabolites by HPLC.[24] Markers of authentic d4T mono-, di-, and triphosphate were included, along with d4T (**3**) and blocked phosphoramidate **4a**. The study was compared with labeled d4T (**3**), and both experiments were also performed using thymidine kinase-deficient CEM cells. Measurable levels of d4T mono-, di-, and triphosphate were formed from either d4T incubation or incubation with **4a** using kinase-competent cells. However, with kinase-deficient cells there was no detectable phospho-

Figure 1.

rylation of d4T. On the other hand, levels of d4T triphosphate generated by **4a** incubation were entirely maintained in the thymidine kinase-deficient cells. Indeed, CEM (wild type) and CEM-TK$^-$ cells generate 0.25 and 0.48 nmol/10^9 cells of d4TTP respectively after 20 h of incubation with 0.2 μM **4a**. There was thus firm evidence that **4a** could give rise to significant levels of the bioactive metabolite d4T triphosphate, by a mechanism which was entirely independent of thymidine kinase, unlike d4T which could give similar levels of the triphosphate, but by an entirely thymidine kinase-dependent process. This is consistent with the suggested intracellular hydrolysis of phosphoramidates such as **4a** to give d4T monophosphate and the thymidylate kinase-mediated phosphorylation of this material to the higher phosphates. The alternative hydrolysis of **4a** to give d4T could not play a major role in the activation pathway, otherwise the generation of d4T triphosphate (and the antiviral activity) would have been significantly reduced in thymidine kinase-deficient cells.[24]

However, we noted in the HPLC chromatogram arising from incubation with radiolabeled **4a**, using either kinase-competent or kinase-deficient cells, the formation of high levels of a new metabolite, of apparent polarity intermediary between d4T mono- and diphosphate. The concentration of this material, as estimated from the radiolabel, was approximately 10−200 times the levels of d4T triphosphate generated, depending on the initial concentration of the phosphoramidate **4a**. At its EC_{50} (ca. 0.2 μM) **4a** generates levels of the new metabolite 10−15-fold higher than the levels of d4T triphosphate (d4TTP) generated from d4T administration to the cell cultures over a comparable incubation time (24 h). At longer incubation times (72 h) the new metabolite was still present at ca. 10-fold higher levels than those of d4TTP. Eventually we were able to prepare a synthetic material identical to this metabolite, on the basis of HPLC retention time in two different systems, by the regioselective base-catalyzed hydrolysis of compound **4a**. This product was then identified as the novel phosphate diester **7** arising from cleavage of both the phenyl and methyl ester groups. We noted that this material could also be generated from **4a** using hog liver esterase, incubated for 24 h at 37 °C. Further evidence for the structure of the metabolite, identified as **7**, came from the observation that compounds **4c, 4e,** and **4f** gave products with hog liver esterase with similar, but nonidentical HPLC retention times. This is entirely consistent with the suggestion that the amino acid moiety is retained in the metabolites. Furthermore, the metabolite and the synthetic material **7** were both stable to alkaline phosphatase, as anticipated for the proposed structure. We wondered whether the metabolite **7** could exert antiviral action via release of d4T (at least in TK$^+$ cells) or d4T monophosphate, and subsequent phosphorylation to the triphosphate act in an entirely novel way. Indeed, we suggest that the much greater concentrations of **7** generated, by comparison to the intracellular levels of d4T triphosphate,

indicates that **7** could contribute to the antiviral efficacy of phosphoramidates such as **4a** via intracellular release of d4TMP. However, it is likely that the contribution of each mechanism to the overall antiviral effect will vary with the exact structure of the phosphoramidate, the initial drug concentration, and the cell type studied. It is quite feasible that some of the structure–activity relationships we noted above, particularly involving variations in the aryl moiety, may relate to the efficiency of the intracellular generation of d4T monophosphate and/or the formation of the phosphate diester metabolite **7**. Alternatively, and particularly in the case of the amino acid variations, some of the evident preference for certain amino acids may also relate either to the relative efficiency of formation of the analogues of (**7**) or to the efficiency of their subsequent activation to d4TTP. This is currently under active investigation in our laboratory.

In conclusion, the phosphoramidate derivatives of d4T herein described show advantage over d4T itself, particularly in thymidine kinase-deficient cells. This leads to enhanced antiviral selectivity relative to the parent drug. Metabolic studies indicate that the phosphoramidates lead to the intracellular generation of bioactive d4T triphosphate by a mechanism which is entirely independent of thymidine kinase, with full retention of d4TTP levels in TK^- cells. We also note the novel discovery of a new metabolite, a phosphate diester retaining the amino acid. We thus report on a new mechanism of inhibition of HIV; the d4T phosphoramidates can act via a d4T or d4TMP depot form, which may be entirely independent of thymidine kinase. We suggest that this new discovery offers great promise for the development of new improved therapies for HIV infection and AIDS.

Experimental Section

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Triethylamine and dichloromethane were dried by heating under reflux over calcium hydride for several hours followed by distillation. Dichloromethane was further dried over activated 4 Å molecular sieves. Tetrahydrofuran was dried by heating under reflux over sodium and benzophenone followed by distillation. *N*-Methylimidazole was purified by distillation. Nucleosides were dried by storage at elevated temperature *in vacuo* over P2O5. Proton, carbon, and phosphorus Nuclear Magnetic Resonance (^{1}H, ^{13}C, ^{31}P NMR) spectra were recorded on a Bruker Avance DPX spectrometer operating at 300, 75.5, and 121.5 MHz, respectively, with ^{13}C and ^{31}P spectra being recorded proton-decoupled. All NMR spectra were recorded in $CDCl_3$ at room temperature (20 °C ± 3 °C). ^{1}H and ^{13}C chemical shifts are quoted in parts per million downfield from tetramethylsilane. *J* values refer to coupling constants, and signal splitting patterns are described as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), multiplet (m), or combinations thereof. ^{31}P chemical shifts 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 unless stated was fast atom bombardment (FAB) with MNOBA as matrix. Chromatography refers to flash column chromatography and was carried out using Merck silica gel 60 (40–60 μM) as stationary phase. Thin layer chromatography was performed using Alugram SIL G/UV254 aluminum-backed silica gel plates. HPLC was conducted on an ACS quaternary system, using an ODS5 column and an eluant of water/acetonitrile, with 82% water 0–10 min, then a linear gradient to 20% water at 30 min, with a flow rate of 1 mL/min and detection by UV at 265 nm. Final products showed purities exceeding 99% with undetectable levels (<0.02) of parent nucleosides in every case. (Aryloxy)phosphorodichloridates, and (aryloxy)aminophosphorochloridates were prepared entirely as previously noted.[15]

General Procedure. Phenyl methoxyalaninyl phosphorochoridate (250 mg, 0.9 mmol, 2.0 equiv) was added to a stirred solution of d4T (**3**) (100 mg, 0.45 mmol) and *N*-methylimidazole (143.5 μL, 1.8 mmol, 4 equiv) in tetrahydrofuran (THF) (2 mL) at ambient temperature. After 4 h, the solvent was removed under reduced pressure. The gum was dissolved in chloroform (10 mL) and washed with 1 M HCl (8 mL), sodium bicarbonate solution (10 mL), and water (15 mL). The organic phase was dried ($MgSO_4$), and the solvent was removed in *vacuo*. The residue was purified by column chromatography on silica with elution by chloroform–methanol (97:3). Pooling and evaporation of appropriate fractions gave the product as a white foam.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyalaninyl phosphate) (4a): yield 88%; δ_P 3.20, 3.86; δ_H 1.32, 1.34 (d, 3H, *J* = 6.8Hz, Ala-Me), 1.81, 1.84 (d, 3H, 5-Me), 3.69, 3.70 (s, 3H, OMe), 3.84–4.00 (m, 2H, Ala-CH, Ala-NH), 4.32 (m, 2H, H5′), 5.02 (m, 1H, H4′), 5.88 (m, 1H, H2′), 6.33 (m, 1H, H3′), 7.03 (m, 1H, H1′), 7.15–7.35 (m, 6H, Ph, H6), 9.22, 9.26 (bs, 1H, NH); δ_C 12.52 (5-Me), 21.02 (Ala-Me), 50.22–50.35 (Ala-CH), 52.74 (OMe), 66.62–67.29 (C5′), 84.80–84.88 (C4′), 89.69–89.93 (C1′), 111.44–111.57 (C5), 120.13–120.31 (Ph ortho), 125.30 (Ph para), 127.49–127.65 (C2′), 129.87–129.93 (Ph meta), 133.19-133.50 (C3′), 135.77–136.06 (C6), 150.51 (Ph ipso), 151.16 (C2), 164.14 (C4), 174.12 (Ala-CO); MS *m*/*e* FAB 466 (MH^+, 7), 340 (MH^+ − base), 200 (17), 136 (47), 89 (25), 81 (C_5H_5O, 100), HPLC t_R 22.48, 22.87 min (see experimental methods for HPLC conditions).

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyglycinyl phosphate) (4b): yield 90%; δ_P 4.89, 5.52; δ_H 1.79, 1.83 (s, 3H, 5-Me), 3.69 (s, 3H, OMe), 3.70–4.05 (m, 3H, Gly-CH_2, Gly-NH), 4.32 (m, 2H, H5′), 4.99 (m, 1H, H4′), 5.92 (m, 1H, H2′), 6.38 (m, 1H, H3′), 6.98 (m, 1H, H1′), 7.05–7.38 (m, Ph, H6), 9.44, 9.46 (s, 1H, NH); δ_C 12.75 (5-Me), 43.15 (Gly-CH_2), 52.94 (OMe), 66.78–67.52 (C5′), 84.98–85.10 (C4′), 89.68–90.16 (C1′), 111.69–111.80 (C5), 120.46–120.59 (Ph ortho), 125.66 (Ph para), 127.66–127.91 (C2′), 130.22 (Ph meta), 133.48–133.87 (C3′), 136.11–136.40 (C6), 150.65 (Ph ipso), 151.45 (C2), 164.46 (C4), 171.41–171.51 (Gly-CO); MS *m*/*e* FAB 452 (MH^+, 74), 474 (M + Na, 46), HPLC t_R 27.21 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyvalinyl phosphate) (4c): yield 86%; δ_P 4.85, 5.40; δ_H 0.92 (m, 6H, Val-Me), 1.82 (m, 1H, Val-iPrCH), 1.89, 1.91 (s, 3H, 5-Me), 3.76 (s, 3H, OMe), 3.82 (m, 2H, Val-CH, Val-NH), 4.30–4.48 (m, 2H, H5′), 5.07 (m, 1H, H4′), 5.96 (m, 1H, H2′), 6.38 (m, 1H, H3′), 7.10 (m, 1H, H1′), 7.18–7.35 (m, 6H, Ph, H6), 9.31 (s, 1H, NH); δ_C 12.80 (5-Me), 17.77–19.24 (Val-Me), 32.43–32.62 (Val-iPrCH), 52.67 (OMe), 60.32–60.38 (Val-CH), 66.92–67.65 (C5′), 85.04 (C4′), 89.98–90.24 (C1′), 111.76–111.87 (C5), 120.45–120.56 (Ph ortho), 125.54–125.59 (Ph para), 127.81–127.86 (C2′), 130.13–130.17 (Ph meta), 133.51–133.72 (C3′), 136.01–136.28 (C6), 150.83 (Ph ipso), 150.87–151.34 (C2), 164.30–164.37 (C4), 173.56–173.65 (Val-CO); MS *m*/*e* FAB 493.6 (MH^+, 100), HPLC t_R 28.50 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyleucinyl phosphate) (4d): yield 87%; δ_P 4.18, 4.83; δ_H 0.91 (m, 6H, Leu-Me), 1.42–1.70 (m, 3H, Leu-CH_2C*H*), 1.91, 1.93 (s, 3H, 5-Me), 3.73 (s, 3H, OMe), 3.76–3.98 (m, 2H, Leu-CHm Leu-NH), 4.28–4.46 (m, 2H, H5′), 5.08 (m, 1H, H4′), 5.96 (m, 1H, H2′), 6.36 (m, 1H, H3′), 7.09 (m, 1H, H1′), 7.18–7.35 (m, 6H, Ph, H6), 9.35 (s, 1H, NH); δ_C 12.76 (5-Me), 22.23–23.01 (Leu-Me), 24.75 (*C*H(CH_3)$_2$), 43.86–44.11 (CH_2CH), 52.75 (OMe), 53.42–53.60 (Leu-CH), 66.92–67.55 (C5′), 85.62 (C4′), 89.92-90.19 (C1′), 111.69–111.83 (C5), 120.37–120.62 (Ph ortho), 125.55–125.58 (Ph para), 127.79 (C2′), 130.12 (Ph meta), 133.51–133.70 (C3′), 136.00–136.36 (C6), 151.05 (Ph ipso), 151.38 (C2), 164.39–164.50 (C4), 174.55–174.88 (Leu-CO); MS *m*/*e* FAB 508 (MH^+, 62), 530 (M + Na, 59); HPLC t_R 30.17 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyphenylalaninyl phosphate) (4e): yield 89%; δ_P 3.96, 4.35; δ_H 1.89 (s, 3H, 5-Me), 3.00 (m, 2H, CH_2Ph), 3.74 (s, 3H, OMe), 3.80−4.28 (m, 4H, Phe-CH, Phe-NH, H5′), 4.94 (m, 1H, H4′), 5.91 (m, 1H, H2′), 6.21−6.30 (m, 1H, H3′), 7.04−7.32 (m, 12H, Ph, H1′, H6), 9.35 (s, 1H, NH); δ_C 12.54 (5-Me), 40.55 (CH_2Ph), 52.63 (OMe), 55.72−56.01 (Phe-CH) 66.50−67.10 (C5′), 84.78 (C4′), 89.71−89.95 (C1′), 111.53−111.64 (C5), 120.28 (OPh ortho), 125.40 (OPh para), 127.52 (C2′), 128.86, 129.65, 129.98 (CH_2*Ph*), 129.86−129.92 (OPh meta), 133.18−133.50 (C3′), 135.72 (CH_2*Ph* ipso), 135.79−136.06 (C6), 150.46 (OPh ipso), 151.13−151.17 (C2), 164.12−164.18 (C4), 173.00 (Phe-CO); MS *m/e* FAB 542 (MH^+, 77), 564 (M + Na, 29); HPLC t_R 29.88 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxymethioninyl phosphate) (4f): yield 81%; δ_P 4.09, 4.86; δ_H 1.74, 1.79 (s, 3H, MeS), 1.94, 1.97 (s, 3H, 5-Me), 1.80−2.40 (m, 5H, C*H*CH_2CH_2S), 3.72, 3.74 (s, 3H, OMe), 3.98−4.32 (m, 4H, H5′, Met-CH, Met-NH), 4.96 (m, 1H, H4′), 5.84 (m, 1H, H2′), 6.26 (m, 1H, H3′), 6.96 (m, 1H, H1′), 7.05−7.25 (m, 6H, Ph, H6), 9.58 (bs, 1H, NH); δ_C 12.80 (5-Me), 15.68 (CH_3S), 29.95 (CH_2SCH_3), 33.73−33.85 (CH_2CH_2S), 53.06 (OMe), 53.81−54.07 (Met-CH), 67.05−67.70 (C5′), 84.90−85.03 (C4′), 89.98−90.23 (C1′), 111.66−111.86 (C5), 120.39−120.66 (Ph ortho), 125.63 (Ph para), 127.81−127.91 (C2′), 130.18 (Ph meta), 133.44−133.69 (C3′), 136.00−136.38 (C6), 150.72−150.80 (Ph ipso), 151.41 (C2), 164.52 (C4), 173.61−173.94 (Met-CO); MS *m/e* FAB 526 (MH^+, 46), 548 (M + Na, 21); HPLC t_R 29.92 min.

2′,3′-Didehydro-2′,3′-dideoxythymidine 5′-(2,4-dibromophenyl methoxy alaninyl phosphate) (5a): yield 88%; δ_P 3.07, 3.62; δ_H 1.26, 1.28 (d, 3H, J = 6.8 Hz, Ala-Me), 1.75, 1.80 (s, 3H, 5-Me), 2.11 (s, 1H, NH), 3.64 (s, 3H, OMe), 3.92−4.30 (m, 3H, Ala-CH, H5′), 4.98 (m, 1H, H4′), 5.87 (m, 1H, H2′), 6.26 (m, 1H, H3′), 6.96 (m, 1H, H1′), 7.30−7.60 (m, 4H, Ph, H6), 9.41(bs, 1H, NH); δ_C 12.51 (5-Me), 21.00 (Ala-Me), 50.24 (Ala-CH), 52.80 (OMe), 67.37−67.83 (C5′), 84.49−84.61 (C4′), 89.80−89.92 (C1′), 111.60 (C5), 115.49 (Ph), 118.26 (Ph), 122.61−122.89 (Ph), 127.70 (C2′), 131.86 (Ph), 133.06−133.21 (C3′), 135.64 (Ph), 135.75−135.88 (C6), 147.01 (Ph), 151.07 (C2), 164.03 (C4), 173.71−173.82 (Ala-CO); MS *m/e* FAB 626 (MH^+, 2 × ^{81}Br, 3), 624 (MH^+, ^{81}Br, 6), 621.9507 (MH^+, $C_{20}H_{22}O_8N_3PBr_2$ requires 621.9516, 3), 500, 498, 496 (MH^+ − base, 5,9,5), 81 (100); HPLC t_R 41.17, 41.30 min.

2′,3′-Didehydro-2′,3′-dideoxythymidine 5′-(3-trifluoromethylphenyl methoxyalaninyl phosphate) (5b): yield 80%; δ_P 2.49, 3.16; δ_H 1.36 (3H, m, Ala-Me), 1.80, 1.86 (3H, d, 5-Me), 3.70, 3.71 (3H, s, OMe) 3.97 (2H, m, Ala-NH, Ala-CH), 4.32 (2H, m, H-5′), 5.03 (1H, m, H-4′), 5.92 (1H, m, H-2′), 6.31 (1H, m, H-3′), 7.03 (1H, m, H-1′), 7.45 (5H, m, Ph, H-6), 9.06 (1H, s, NH); δ_C 12.55, 12.47 (5-Me,) 21.11, 20.99 (d, Ala-Me, J = 4.9 Hz), 50.32, 50.26 (d, Ala-CH, J = 4.8 Hz), 52.87 (OMe), 67.60, 66.89 (d, C-5′, J = 4.9 Hz), 84.61 (d, C-4′, J = 7.8 Hz), 90.04, 89.77 (C-1′), 111.61, 111.44 (C-5), 117.54 (d, Ph, J = 3.9 Hz), 122.14 (Ph), 123.98, 123.79 (Ph), 123.84 (q, CF3, J = 272.0 Hz), 127.84, 127.74 (C-2′), 130.66 (Ph), 132.00 (q, Ph, J = 32.0 Hz), 133.30, 133.02 (C-3′), 135.86, 135.66 (C-6), 150.71 (d, Ph, J = 5.9 Hz), 150.96 (C-2), 163.91, 163.86 (C-4), 174.06, 173.89 (d, Ala-CO, J = 6.8 Hz); MS *m/e* FAB 534.1201(MH^+, $C_{21}H_{24}N_3O_8PF_3$ requires 534.1253, 6), 408 (MH^+ − thymine, 8), 268 (10), 149 (10), 81 (C_5H_5O, 100); HPLC t_R 30.56 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(pentafluorophenyl methoxyalaninyl phosphate) (5c): yield 76%; δ_P 4.74, 5.66; δ_H 1.34, 1.36 (d, 3H, Ala-Me, J = 6.7Hz), 1.75, 1.81 (s, 3H, 5-Me), 3.69 (s, 3H, OMe), 3.92−4.40 (m, 4H, Ala-CH, Ala-NH, H5′), 4.97 (m, 1H, H4′), 5.85 (m, 1H, H2′), 6.29 (m, 1H, H3′), 6.93 (m, 1H, H1′), 7.19 (m, 1H, H6), 9.38 (bs, 1H, NH); δ_C 12.23−12.43 (5-Me), 20.83 (Ala-Me), 50.22−50.34 (Ala-CH), 52.99 (OMe), 67.75−68.37 (C5′), 84.42−84.52 (C4′), 89.87−90.17 (C1′), 111.75 (C5), 127.69−127.93 (C2′), 132.86−133.13 (C3′), 132−143 (m, Ph), 135.74−135.96 (C6), 151.11 (C2), 164.15 (C4), 173.64−173.76 (Ala-CO); MS *m/e* FAB 556 (MH^+, 31), 578 (M + Na, 100); HPLC t_R 35.90 min.

2′,3′-Didehydro-2′,3′-dideoxythymidine 5′-(3, 5-dichlorophenyl methoxyalaninyl phosphate) (5d): yield 70%; δ_P 2.83, 3.42; δ_H 1.48 (3H, m, Ala-Me), 1.92, 1.97 (3H, s, 5-Me), 3.84 (3H, s, OMe), 4.07 - 4.48 (4H, m, Ala-NH, Ala-CH, H-5′), 5.14 (1H, m, H-4′), 6.04 (1H, m, H-2′), 6.44 (1H, m, H-3′), 7.14 (1H, m, H-1′), 7.29 (3H, m, Ph), 7.40 (1H, s, H-6), 9.74 (1H, s, NH); δ_C 12.51 (5-Me), 20.93 (Ala-Me), 50.26 (Ala-CH), 52.85 (OMe), 66.98, 67.68 (C-5′), 84.60 (C-4′), 89.74, 90.03 (C-1′), 111.40, 111.54 (C-5), 119.40 (Ph), 125.69 (Ph), 127.83 (C-2′), 132.89, 133.14 (C-3′), 135.60 (C-6), 136.01 (Ph), 151.06 (C-2) 151.27 (Ph), 164.09 (C-4), 173.93 (Ala-CO); MS *m/e* FAB 534.0589 (MH^+, $C_{20}H_{23}N_3O_8PCl_2$ requires 534.0600, 8), 408 (MH^+ − thymine, 12), 391 (10), 149 (12), 127 (thymine H^+), 12), 81 (C_5H_5O, 100); HPLC t_R 32.19 min.

2′,3′-Didehydro-2′,3′-dideoxythymidine 5′-(4-ethylphenyl methoxyalaninyl phosphate) (5e): yield 79%; δ_P 3.43 ; δ_H 1.19 (3H, m, Ala-Me), 1.31 (3H, m, CH_2CH_3), 1.80, 1.84 (3H, d, 5-Me, J = 1.2 Hz), 2.60 (2H, q, CH_2CH_3, J = 7.5 Hz), 3.67, 3.70 (3H, s, OMe), 3.93 (2H, m, Ala-NH, Ala-CH), 4.38−4.25 (2H, m, H-5′), 5.00 (1H, m, H-4′), 5.88 (1H, m, H-2′), 6.28 (1H, m, H-3′), 7.00 - 7.14 (5H, m, Ph, H-1′), 7.33, 7.34 (1H, s, H-6), 9.23, 9.25 (1H, s, NH); δ_C 12.41, 12.45 (5-Me), 15.69 (CH_2CH_3), 20.90, 20.97 (d, Ala-Me, J = 4.9 Hz), 28.19 (Ph-CH_2), 50.13, 50.26 (Ala-CH), 52.65 (OMe), 66.48, 67.11 (d, C-5′, J = 4.9 Hz), 84.70, 84.88 (d, C-4′,), 111.40, 111.51 (C-5), 119.90, 120.08 (d, Ph, J = 3.9, 4.9 Hz), 127.36, 127.54 (C-2′), 129.05, 129.11 (Ph), 133.15, 133.50 (C-3′), 135.76, 136.06 (C-6), 141.19, 141.24 (Ph), 148.16, 148.29 (Ph), 151.12, 151.15 (C-2), 164.17, 164.22 (C-4), 174.12, 174.25 (Ala-CO); MS *m/e* FAB 494.1693 (MH^+, $C_{22}H_{29}N_3O_8P$ requires 494.1692, 5), 368 (MH^+ − thymine, 25), 228 (15), 81 (C_5H_5O, 100); HPLC t_R 27.23, 27.48 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(methyl methoxyalaninyl phosphate) (6a): yield 86%; δ_P 9.36, 9.70; δ_H 1.29, 1.31 (d, 3H, Ala-Me, J = 6.7Hz), 1.81, 1.83 (s, 3H, 5-Me) 3.61−3.68 (m, 6H, OMe), 3.84 (m, 2H, Ala-CH, Ala-NH), 4.16 (m, 2H, H5′), 4.95 (bs, 1H, H4′), 5.83 (bs, 1H, H2′), 6.26 (m, 1H, H3′), 6.97 (m, 1H, H1′), 7.26 (d, 1H, H6), 9.30 (bs, 1H, NH); δ_C 12.26−12.48 (5-Me), 21.11 (Ala-Me), 49.93−50.06 (Ala-CH), 52.74 (OMe), 53.19−53.54 (MeOP), 65.93−66.75 (C5′), 84.83−84.94 (C4′), 89.61−89.87 (C1′), 111.37−11.43 (C5), 127.41−127.63 (C2′), 133.22−133.64 (C3′), 135.90−136.21 (C6), 151.07 (C2), 164.10 (C4), 174.39 (Ala-CO); MS *m/e* FAB 404.1223 (MH^+, $C_{15}H_{22}O_8N_3P$ requires 404.1248, 18), 278 (MH^+ − base, 39); 81 (C_5H_5O, 100); HPLC t_R = 18.72, 22.19 min.

2′,3′-Didehydro2′,3′-dideoxythymidine 5′-(ethyl methoxyalaninyl phosphate) (6b): yield 74%; δ_P 7.66, 7.69 ; δ_H 1.26 (6H, m, Ala-Me, CH_2CH_3), 1.83, 1.85 (3H, d, 5-Me, J = 1.2 Hz), 3.67, 3.68 (3H, s, OMe), 3.82−4.16 (6H, m, Ala-NH, Ala-CH, CH_2OP, H-5′), 4.94 (1H, m, H-4′), 5.88 (1H, m, H-2′), 6.28 (1H, m, H-3′), 6.95 (1H, m, H-1′), 7.22, 7.31 (1H, d, H-6, J = 1.2, 1.3 Hz) 10.01 (1H, s, NH); δ_C 12.28, 12.41(5-Me), 16.18 (d, CH_2CH_3, J = 6.8 Hz), 20.84, 20.92 (d, Ala-Me, J = 5.2 Hz), 49.83, 49.90 (Ala-CH), 52.51 (OMe), 62.90, 63.07 (d, CH_2OP, J = 4.9 Hz), 66.63, 66.93 (C-5′), 84.85 (d, C-4′, J = 8.8 Hz), 89.48, 89.75 (C-1′), 111.25, 111.31 (C-5), 127.28, 127.39 (C-2′), 133.20, 133.51 (C-3), 135.80, 136.03 (C-6), 151.15, 151.19 (C-2), 164.30 (C-4), 174.26, 174.32 (d, Ala-CO, J = 6.9 Hz); HPLC t_R = 38.90, 40.82 min.

2′,3′-Didehydro-2′,3′-dideoxythymidine 5′-(Alaninyl phosphate) (7). Compound **4a** (0.116 g, 0.25 mmol) was dissolved in a 1:1 mixture of triethylamine and water (8 mL). After 3 h at room temperature the triethylamine phase was removed and the aqueous phase evaporated under high vacuum at ambient temperature. The resulting crude product was purified on silica using the chromatotron with the mixture $CHCl_3$/MeOH/H_2O/NH_4OH: 120/70/10/1 as eluent. Pooling and freeze-drying of appropriate fractions gave the pure compound: yield 0.051 g, 54); δ_P (D_2O) 7.63; δ_H 1.12 (d, 3H, J = 6.9Hz, Ala-Me), 1.73 (s, 3H, 5-Me), 3.42 (m, 1H, Ala-CH), 3.83 (m, 2H, H5′), 4.93 (m, 1H, H4′), 5.80 (m, 1H, H2′), 6.34 (m, 1H, H3′), 6.78 (m, 1H, H1′), 7.45 (s, 1H, H6); δ_C (D_2O) 11.80 (5-Me), 19.65 (Ala-Me), 50.21 (Ala-CH), 65.32 (C5′), 86.26 (C4′), 90.38 (C1′), 111.57 (C5), 125.19 (C2′), 134.86 (C3′), 138.67 (C6), 152.57 (C2), 166.92 (C4), 179.30 (Ala-CO_2H); MS *m/e* FAB 398 (MNa+, 17), 376 (MH^+, 6); HPLC t_R 3.15 min.

Antiretroviral Evaluation. HIV-1 (HTLV-IIIB) was obtained from persistently HIV-infected H9 cells as described previously.[25] Virus stocks were prepared from the supernatants of HIV-1 (IIIB)-infected MT4 cells. HIV-2 (ROD) was provided by Dr. L. Montagnier (Pasteur Institute, Paris, France). MT-4 cells were provided by Dr. N. Yamamoto (Tokyo

Medical and Dental University School of Medicine, Tokyo, Japan). CEM/0 cells were obtained from the American Tissue Culture Collection (Rockville, MD), and CEM/TK$^-$ cells were a gift from Prof. S. Eriksson and Dr. A. Karlsson (Karolinska Institute, Stockholm, Sweden).

MT-4 and CEM cells were infected with HIV-1 as previously described.[23] Briefly, 5×10^5 MT-4 or CEM cells/mL were infected with HIV-1 or HIV-2 at approximately 100 CCID50 (50% cell culture infective dose)/mL of cell suspension. Then, 100 μL of the infected cell suspension were transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of the test compounds. After 4 days, giant cell formation was recorded microscopically in the HIV-infected CEM cell cultures, and after 5 days, the number of viable cells was determined by trypan blue staining of the HIV-infected MT-4 cell cultures. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) were defined as the compound concentrations required to reduce by 50% the number of viable cells (MT-4) or giant cells (CEM) in the virus-infected and mock-infected cell cultures, respectively.

C3H/3T3 cells were seeded into Costar Tissue Culture Cluster plates (Costar Broadway, Cambridge, MA) at 20 000 cells/mL into 1 cm^2 wells and grown to confluency. Cell cultures were then infected by 75 foci-forming units of Moloney murine sarcoma virus (MSV) during 90 min, whereafter medium was replaced by 1 mL of fresh culture medium containing different concentrations of test compound. After 6 days the transformation of the test cultures was examined microscopically. The EC_{50} was defined as the compound concentration that is required to inhibit MSV-induced transformation by 50%.

Esterase Hydrolysis. Compound **4a** was incubated with hog liver esterase (20 units/μL) at pH 7.6 in 25 mM Tris·HCl containing 10 mM magnesium dichloride at 37 °C and samples were analyzed by HPLC at appropriate time points.

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Expert Opinion

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Prodrug approaches to improving the oral absorption of antiviral nucleotide analogues

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Nucleotide analogues have been well accepted as therapeutic agents active against a number of viruses. However, their use as antiviral agents is limited by the need for phosphorylation by endogenous enzymes, and if the analogue is orally administered, by low bioavailability due to the presence of an ionizable diacid group. To circumvent these limitations, a number of prodrug approaches have been proposed. The ideal prodrug achieves delivery of a parent drug by attachment of a non-toxic moiety that is stable during transport and delivery, but is readily cleaved to release the parent drug once at the target. Here, a brief overview of several promising prodrug strategies currently under development is given.

Keywords: antiviral agents, oral bioavailability, prodrugs, pronucleotides

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1. Introduction

Of the approximately 40 antiviral drugs formally licensed for use, half are nucleoside or nucleotide analogues [1]. Nucleoside drugs *per se* must usually be phosphorylated to the 5′-mono-, 5′-di-, and finally, 5′-triphosphate by intracellular or viral kinases [2] in order to inhibit their therapeutic targets. This requirement limits efficacy, as phosphorylation to the monophosphate by endogenous kinases is slow and typically is the rate-limiting step in human cells [3,4].

The administration of a nucleoside drug as its monophosphate (NMP) is a well-known approach to overcoming this obstacle [3,5]. However, this entails a penalty in the form of decreased membrane permeability. Nucleotide analogues contain an ionizable –O-P(O)$(OH)_2$ group that exists chiefly as a dianion at physiological pH, resulting in low oral bioavailability [5]. In addition, if a NMP succeeds in crossing the intestinal membrane, it then becomes a potential substrate for phosphohydrolases (phosphatases and 5′-nucleotidases), which remove the phosphate group [6]. The use of a nucleoside phosphonate $–CH_2$-P(O)$(OH)_2$ circumvents dephosphorylation, but decreased transport remains an obstacle.

Formulation strategies [7-11] to overcome these limitations are beyond the scope of this short review, which has as its focus an alternative approach: prodrug modification of nucleotide drugs. Promoieties can be attached at a number of positions on an NMP or nucleotide analogue [12,13]. However, the introduction of promoieties at the phosphorus (–[O,CH_2]-P(O)(X)(Y) where X,Y = OR, OR′, NHR″) directly addresses the problem of blocking P-OH ionization *in vivo*. The attachment of a well-designed promoiety increases delivery of the drug to its target, provided that its biochemical and physical properties – including lipophilicity, site-specificity and chemical stability – are conducive to this end [5,13,14].

A prodrug must be stable under delivery conditions [3,5], but it must be capable of conversion to its active parent drug *in vivo* [5], at a rate consistent with pharmacological efficacy. The prodrug and metabolized promoiety/promoieties

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should have low acute and chronic toxicity [5]. Control of these and other crucial properties, such as aqueous solubility and lipophilicity, remains a key challenge in the development of an effective prodrug.

Esterification with pivaloyloxymethyl (POM), *p*-acyloxybenzyl (PAOB), or isopropyloxy–carbonyloxymethyl (POC) groups has been reviewed extensively [3,5,6,13,15,16] and will not be addressed here. Also, of recent interest, but omitted from this discussion is the approach of Hostetler *et al.* to improve the oral bioavailability of certain antiviral phosphonate drugs by esterification with an ether lipid ester that mimics the natural lipid lysophosphatidylcholine, thus potentially delivering the prodrug within the cell intact [4,17,18]. Our review will examine the prodrug approaches represented by the structures in **Figure 1**.

2. Phosphoramidate 'ProTide' approach

McGuigan has introduced prodrugs ('ProTides') based on an amino acid ester promoiety, attached to the drug (as a aryl monophosphate or phosphonate) via a P-N bond, applying this approach to: 4′-azidouridine [19], 4′-azidoadenosine [20], 2′,3′-dideoxyuridine (ddU) [21], carbocyclic L-d4A (L-Cd4A) [22], stauvidine (d4T) [23], 9-[2-(phosphonomethoxy) ethyl]adenine (PMEA) [24], 3′-azidothymidine (AZT) [25], abacavir (ABC) [26] and tenofovir (PMPA, 9-[(*R*)-2-(phosphonomethoxy)propyl]adenine) [27].

The original approach involved preparation of simple alkyloxy phosphoramidates (**Figure 2A**(**1**)), but has evolved into aryloxy phosphoramide pronucleotides with distinct structure–activity relationship detail [28]. Phosphorodiamidates (**Figure 2A**(**2**)) were also prepared, but no biological benefit versus the phosphoramidates was observed and synthetic yields were lower [28]. Interestingly, analogues linked through an oxygen resulted in a significant decrease in antiviral activity [29], possibly because the nucleoside monophosphate was not released from the diester intermediate [28]. Diaryl pronucleotides (**Figure 2A**(**3**)) were not active in kinase-deficient cells [30], due to poor intracellular delivery of the NMP or possibly chemical instability of the diaryl masking groups. Overall, aryloxy phosphoramidates (**Figure 2A**(**4**)) appear to hold the most promise for delivery of the therapeutic agent.

Aryloxy phosphoramidates were designed to release the NMP intracellularly via both chemical and enzymatic mechanisms (**Figure 2B**). The first step in the activation process is cleavage of the amino acid ester by a carboxyesterase [28] to afford **6** (**Figure 2B**), although a thorough investigation by Venkatachalam and coworkers found that activation by a lipase or protease is also possible and that these enzymes have different specificities for the substituent on the aryl group, the amino acid and the stereochemistry at the phosphorus [31]. Subsequent nucleophilic attack at the phosphorus by the carboxyl group releases the aryloxy group, forming a transient cyclic diester, which is hydrolyzed to form the amino acyl metabolite (AAM, **Figure 2B**(**7**)) [28]. In the final step, the amino acid moiety is cleaved by a phosphoramidase to release the nucleoside monophosphate (**Figure 2B**(**8**)) and an amino acid [28].

McGuigan and coworkers have thoroughly studied the aryloxy phosphoramidates of d4T and have been able to gain extensive structure–activity relationship insight. In general, the methyl, ethyl and benzyl esters lead to potent activity, while bulkier esters (t-butyl and isopropyl) are significantly less active than the methyl ester [32], most likely due to the increased stability to enzymatic hydrolysis [20]. A quantitative structure–activity relationship (QSAR) study on the variation of amino acid esters further described the most potent esters as those with considerable lipophilicity slightly removed from the ester bond [33]. In a separate report, it was found that the conversion of AAM to NMP was inhibited when benzyl alcohol was released [34]. This inhibition was not observed when ethanol or methanol was released [34].

Although there are some exceptions depending on the nature of the drug used, the most successful pronucleotides contain L-alanine as the amino acid [34,35]. Exchange of L-alanine for either glycine or L-leucine reduced the antiviral activity 70- and 13-fold, respectively [36]. When L-valine was used, the antiviral activity was reduced 147-fold, and furthermore, 100% of the intact prodrug was recovered when it was exposed to pig liver carboxyesterases [34]. When D-alanine was substituted for L-alanine, the potency was decreased 35-fold [34]. The exact reason for the preference for alanine remains unknown. When the achiral amino acid analogue α,α-dimethylglycine was prepared, the antiviral activity was only reduced three-fold [36], which illustrates the fact that natural amino acids are not essential for activity. However, when such amino acids were used, a preference for α-amino acids was observed [34]. The β-amino acid phosphoramidates showed efficient ester cleavage, but no phenyl loss was detected, and the AAM was not observed [34]. This suggests a possible entropy barrier that increases with chain length.

Studies to determine the optimal aryloxy group were also performed [37]. The greatest activity was achieved when the aryl group had a *p*-Cl substituent, and generally for aryl groups that function as mildly electron-withdrawing, lipophilic substituents [37]. The potential for toxicity of the released phenol was not discussed. A naphthyl group was also reported to be an effective aryl moiety for delivering anticancer agents [38], and this activity is most likely transferable to antiviral agents.

To obtain successful intracellular delivery of NMP, the pronucleotides need to be resistant to hydrolysis during the absorption and distribution process. The chemical stability of the pronucleotides was studied, and all exhibited satisfying stability over the range of pHs studied (2.0 – 7.4) [39]. The aryloxy phosphoramidates were significantly less resistant to

Amino acid phosphoramidates

S-Acyl-2-thioethyl pronucleotides

*Cyclo*Sal triesters

Dipeptide pronucleotides

Figure 1. General structures exemplifying the antiviral prodrug approaches discussed in this review.

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decomposition in plasma or cell extracts, indicative of the need for enzymatic activation [39]. After overnight exposure to pig liver carboxyesterases, CEM cell extract, human serum and mouse serum, AAM was formed from a majority of the L-amino acid-containing pronucleotides [34]. When carbocyclic adenosine phosphoramidates were evaluated in intestinal and liver S9 homogenates, some of the most antivirally potent analogues exhibited complete decomposition over 1 h in intestinal homogenate [22], but isopropyl and t-butyl esters on the amino acid increased intestinal stability [22]. Similarly, D-alanine and glycine exhibited the highest intestinal stability [22], which highlights the complexity in obtaining a structure–activity relationship. Although the usage of this aryloxy phosphoramidate prodrug approach with nucleotide analogues containing a phosphonate may be more difficult due to decreased chemical stability [40], an example of successful application to tenofovir has been described [27].

The pharmacokinetics and oral bioavailability of aryloxy phosphoramidates, specifically abacavir phosphoramidates, were examined [35]. When the abacavir methyl alaninyl–phosphoramidate was administered intravenously, the pronucleotide was rapidly cleared from the plasma with a half-life of 7 min [35]. Similar results were observed following oral administration [35]. However, the major metabolite observed was the AAM [35]. Total exposure to the pronucleotide and its active metabolites was reported to approach that estimated for a similar dose of the parent drug, abacavir, resulting in an overall bioavailability of 50% [35]. The epithelial permeability of a series of d4T aryloxy phosphoramidates was evaluated in Caco-2 and MDCK monolayers [41]. The pronucleotides exhibited relatively low permeability, which may be partially explained by their susceptibility to first-pass metabolism in the intestinal epithelial cells and by being substrates of P-gp [41]. In general, this work exemplifies the difficulty in delivering the NMP to the target while avoiding significant metabolism during absorption and distribution. To obtain optimal antiviral activity of each pronucleotide, the fine tuning of each element (amino acid, ester, and aryl moiety) is required.

3. Monoester prodrugs

Amino acid phosphoramidate monoesters designed to release the NMP after a single activation by an endogenous phosphoramidase have been described by Wagner, who has applied this approach to AZT [42,43] and ddA [44], as well as anticancer drugs [45].

After the delivery of AZT monophosphate by a glycoslyated carrier attached through lysine was reported [46], Wagner and coworkers proposed that NMP could be efficiently delivered by non-polar amino acid phosphoramidate monoesters and that the aryl group was not necessary. Furthermore, these phosphoramidate monoesters were stable in cell culture and rat and human plasma [42]. A series of these compounds were synthesized containing an amino acid (tryptophan methyl ester [**Figure** 2C(9)] or phenylalanine methyl ester [**Figure** 2C(11)]) connected via a P-N bond to the NMP with the other P-OH left as a free acid or esterified to a

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A.

1

$R_1 = CH_3, CH_2CH_3, (CH_2)_2CH_3, CH_2Ph$
$R_2 = H, CH_3, CH(CH_3)_2, CH_2CH(CH_3)_2, CH_2Ph$

2

R_3 = alkyl group or $CHRCO_2CH_3$

3

Ar = Ph, p-XPh

4

$R_4 = CH_3, CH(CH_3)_2, CH_2Ph, CH_2CH(CH_3)_2$
$R_5 = CH_3, CH_2CH_3, CH_2Ph$
Ar = Ph, p-XPh

B.

5

Esterase step a

6

Hydrolysis step b

AAM, 7

Phosphoramidase step c

8

C.

9 X = O
10 X = N

11

Figure 2. A. General structures of pronucleotides investigated by McGuigan and coworkers. The alkyloxy phosphoramidates (**1**) were initially evaluated. Phosphorodiamidates (**2**) were also tested, but showed no biological advantage over the phosphoramidates. The diaryl triesters (**3**) lost antiviral activity in kinase-deficient cells. However, the aryloxy phosphoramidates (**4**) proved to be the most successful pronucleotides. **B. Activation pathway of the aryloxy phosphoramidate pronucleotides.** Step a involves cleavage of the ester group by an esterase to form intermediate **6**. Hydrolysis of the aryl group facilitated by intramolecular cyclization of the amino acid moiety followed by hydrolysis produces the amino acyl metabolite (AAM, **7**). Cleavage of the P-N bond by a phosphoramidase yields the 5′-nucleoside monophosphate **8** (step c). **C. Structures of the amino acid phosphoramidate monoesters:** tryptophan methyl ester (**9**), tryptophan methyl amide (**10**) and phenylalanine methyl ester (**11**). The L-Val, L-Leu and L-Ala methyl ester derivatives were also synthesized.

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simple alkyl group [47]. The tryptophan monoester (**Figure 2C**(**9**)) exhibited the best antiviral activity, with an eight-fold increase over AZT with no cytotoxicity observed at the levels tested [47]. Further studies have been done to investigate the activation pathway of these pronucleotides and optimize their structures.

The effect of changing the amino acid was studied in peripheral blood mononuclear cells (PBMC) [42]. The best antiviral activity was obtained with the L-alanine methyl ester [42], consistent with McGuigan's ProTides. Furthermore, enhanced activity was observed with the L-tryptophan derivative (**Figure 2C**(**9**)) compared with the L-phenylalanine (**Figure 2C**(**11**)), L-valine and L-leucine derivatives [42]. When evaluated in CEM cells, the L-alanine and L-phenylalanine derivatives exhibited antiviral activity comparable to AZT [42]. This suggests that a simple structure–activity relationship does not exist. In order to avoid the polar carboxylate formed after interaction of the pronucleotides with carboxyesterases, the amino acid methyl ester was substituted by a methyl amide [42]. The authors reported that this exchange had little effect on the antiviral activity of the tryptophan derivatives, while the phenylalanine methyl amide derivatives exhibited increased potency [42]. However, the methyl amide derivatives exhibited greater *in vitro* and *in vivo* stability [48]. The antiviral activity did not exhibit a strong dependence on the amino acid stereochemistry [42], but the inclusion of the D-isomer versus the L-isomer led to decreased volumes of distribution [48]. Overall, the L-tryptophan methyl amide derivative (**Figure 2C**(**10**)) was selected for further studies.

To better understand the differences in potency, Wagner and coworkers investigated the ability of the pronucleotide to deliver NMP intracellularly [42]. The antiviral activity is strongly related to the intracellular levels of nucleoside triphosphate. In both PBMCs and CEM cells, AZT was able to produce higher levels of AZT triphosphate than the pronucleotides [42]. However, when evaluated in CEM cells, the intracellular levels of the tryptophan methyl ester (**Figure 2C**(**9**)) and phenylalanine methyl ester (**Figure 2C**(**11**)) pronucleotides did not plateau [49]. Therefore, the differences in potency may be derived from the ability of a phosphoramidase to cleave the P-N bond and release the NMP.

The oral bioavailability, disposition and stability of the AZT phosphoramidate monoesters were evaluated in rats [50]. The phosphoramidate monoesters were stable in tissue homogenates, intestinal contents, and rat and human plasma [48,50]. The tryptophan methyl amide derivative (**Figure 2C**(**10**)) exhibited the best pharmacokinetic parameters. However, in simulated gastric fluids at pH 2.0, the pronucleotide exhibited a significantly reduced half-life of 5 h, but greater stability as the pH increased [50]. These results are consistent with greater chemical hydrolysis of P-N bonds at lower pH [51]. The pronucleotide was not detected in plasma or urine, which was confirmed in an *in situ* single pass perfusion study where little or no absorption of the pronucleotide in the 120 min perfusion period was detected [50]. AZT was observed in plasma and urine samples accounting for 29.5% of the dose, while 54.3% of the dose was recovered 4 h post-dosing (intravenously) as intact pronucleotide in the bile [50]. These results offer some possible explanations for the zero oral bioavailability of the pronucleotide. Not only will the pronucleotide exist as a charged species at physiological pH, but pre-systemic hydrolysis of the P-N bond would lead to a dianionic monophosphate. This fact, combined with its molecular weight, makes biliary excretion difficult to avoid [50].

The phosphoramidate monoester pronucleotide approach was applied to AZT to create a pronucleotide with increased antiviral activity and decreased cytotoxicity. Unfortunately, decomposition of the phosphoramidate monoester in simulated gastric fluid was observed, and the pronucleotide exhibited little or no bioavailability when evaluated in rats.

4. SATE pronucleotides

S-Acyl-2-thioethyl (SATE) protecting groups for nucleotide drugs have been introduced for the delivery of a number of NMP including d4T [52], PMEA [53], elvucitabine (β-L-FD4C) [54], acyclovir [55,56], AZT [57-59] and cytarabine (Ara-C) [60].

The SATE approach utilizes both enzymatic and chemical mechanisms to activate the pronucleotide and release the NMP (**Figure 3A**) [61]. Removal of the S-acyl-2-thioethyl protecting group and release of the monophosphate is initiated by esterase-mediated hydrolysis of the acyl group, which produces a reactive thiol group [61]. Nucleophilic attack on the α-carbon results in a reactive 2-mercaptoethyl ester that decomposes spontaneously to release the diester (**Figure 3A**(**14**)) and ethylene sulfide (episulfide) [61]. If the second ester is also a SATE group, the process is then repeated, resulting in release of the NMP [61]. It has been proposed that if SATE mixed pronucleotides are used, the aryl group in SATE phosphotriesters is cleaved by a type 1 phosphodiesterase and a phosphoramidase cleaves the protecting group bound via a P-N bond [61]. However, the cleavage of the aryl ester or phosphoramidate could be concomitant with cleavage of the SATE group [61].

Although the release of the NMP is essential, a further point to consider is promoiety toxicity. In the activation process, carboxylic acids and episulfide were released [61]. The body can metabolize the carboxylic acids, and no cytotoxicity was reported for the episulfide [61]. When the SATE promoiety and its metabolites were evaluated in various cell lines, no additional cytotoxicity was observed [61]. *In vivo* toxicity studies in cynomolgus monkeys showed neither clinical symptoms nor behavior problems indicative of toxicity [61].

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A.

Esterase step a

Decomposition step b

12 13 14

R = OSATE, OAr, $NHCHRCO_2Me$

B.

SATE

15 16

$R_1 = CH_3, C(CH_3)_3$

17 18

$R_1 = CH_3, C(CH_3)_3$

$R_1 = CH_3, C(CH_3)_3$
R_2 = alkyl groups

Figure 3. A. Activation of the SATE pronucleotides. The acyl group is cleaved to release the intermediate, which contains a reactive thiol group **13** (step a). Intramolecular cyclization to form an ethylene sulfide results in spontaneous decomposition to the diester or phosphoramidate **14** (step b). If the pronucleotide contains a second SATE moiety, the process is repeated until the 5´-nucleoside monophosphate is released. If the second group is an aryloxy group or a phosphoramidate, hydrolysis can either proceed sequentially or concomitantly to release the 5´-nucleoside monophosphate. **B. General structures of the SATE pronucleotides:** bis(SATE) triesters (**15**), bis(hydroxyl-tBuSATE) triesters (**16**), aryl SATE phosphotriesters (**17**), and SATE phosphoramidate diesters (**18**).

In all cases, application of the bis(SATE) phosphotriester (**Figure 3B(15)**) approach has led to increased *in vitro* antiviral activity compared to the parent nucleoside and efficient delivery of the NMP. Bis(MeSATE) d4T monophosphate was 10- to 17-fold more potent than d4T in wild-type CEM cells and PBMCs, and furthermore, antiviral activity was retained in thymidine kinase-deficient cells [52]. A slight increase in cytotoxicity was also observed for the pronucleotides [52]. Administration of the bis(tBuSATE) Ara-C phosphotriester resulted in significant antiviral activity in a resistant cell line due to lack of the appropriate kinase [60].

Stability and transport across a Caco-2 monolayer was used to determine the optimal R_1 group (**Figure 3B**) [53].

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Transport of the pronucleotides across Caco-2 monolayers resulted in intact pronucleotide in the basolateral compartment only when the bis(tBuSATE) phosphotriester was evaluated, whereas no intact pronucleotide or metabolites were observed for the bis(MeSATE) phosphotriester [53]. Although very low amounts of the intact pronucleotide were detected, the greatest uptake in the Caco-2 monolayer was observed for the bis(tBuSATE) phosphotriester, which could be due to the increased enzymatic stability or lipophilicity of the pivaloyl group [53]. The bis(tBuSATE) phosphotriester was extensively metabolized to the monoester during transport *in vivo*, resulting in no intact pronucleotide observed in the plasma [62]. Some bis(tBuSATE) phosphotriesters exhibit poor aqueous solubility and had to be administered with 5% dimethyl sulfoxide (DMSO), which affected the esterase activity and significantly increased the half-lifes of the pronucleotides [62].

In an attempt to obtain more favorable stability and bioavailability, a SATE group bearing a functionalized acyl moiety was investigated (**Figure 3B**(**16**)) [57,59,62]. The addition of one hydroxyl group led to antiviral activity comparable to the nucleoside and increased activity compared to the bis(tBuSATE) phosphotriester [62]. The addition of a hydroxyl group to the bis(tBuSATE) promoiety decreased the hydrolysis rate and increased the half-life and aqueous solubility of the pronucleotide [62]. Furthermore, intact pronucleotide was observed when the bis(hydroxyl-tBuSATE) phosphotriester was evaluated in a Caco-2 monolayer [62].

Several variations of the SATE pronucleotide approach have been described. Initial attempts at the intracellular delivery of NMP with SATE esters involved the use of two SATE groups [52,55,60,62]. Although these bis(SATE) pronucleotides (**Figure 3B**(**15** and **16**)) exhibited increased antiviral activity and decreased cytotoxicity, the removal of the second SATE group proved difficult and proceeded much more slowly due to the negative charge at the phosphate [61]. Therefore, Gosselin and Imbach evaluated two different kinds of SATE mixed esters: aryl SATE phosphotriesters (**Figure 3B**(**17**)) [64,65] and SATE phosphoramidate diesters (**Figure 3B**(**18**)) [64,67].

A phenyl (tBuSATE) mixed phosphotriester was synthesized, but the NMP was not released from the prodrug when studied in cell extract [63]. Several derivatives of L-tyrosine SATE phosphotriesters (**Figure 3B**(**17**)) were investigated for stability and antiviral activity [64,65]. Since the presence of the free carboxylic acid on the tyrosine residue resulted in decreased antiviral activity, most likely due to decreased membrane permeability, the moiety was modified to contain polar, but not anionic, functionalities [66]. The pronucleotide with the shortest half-life exhibited the best antiviral activity [64]. The resulting mixed SATE phosphotriesters exhibited enhanced antiviral activity in CEM kinase-deficient cells, illustrating the successful delivery of the NMP intracellularly [64].

Another solution to the slow activation of the bis(SATE) phosphotriesters was the SATE phosphoramidate diesters (**Figure 3B**(**18**)) modeled after the phosphoramidates of McGuigan and Wagner. Initial studies with various alkylamines illustrated that the rate limiting hydrolysis of the phosphoramidate was dependent on the basicity and bulk of the amine [65,67]. However, when the pKa of the amine was appropriate (approx. 5 – 11.2), the phosphoramidate diesters effectively delivered NMP intracellularly [67]. Phosphoramidate diesters of AZT were as potent as AZT in wild-type CEM cells and retained significant antiviral activity in kinase-deficient cells [58]. Interestingly, the isopropylamino derivative exhibited the greatest potency, which illustrates the flexibility in the amine moiety and demonstrates that an α-amino acid is not a structural requirement [58].

Périgaud and coworkers have recently investigated the use of aryl SATE phosphotriesters [57,59]. Building on the ability of (hydroxyl-tBu)SATE to increase the solubility and stability of the prodrug, functionalization of the acyl moiety with polar groups was applied to the phenyl SATE mixed phosphotriester analogues. Additionally, an analogue with a valine replacing the acyl group was studied, but the compound was unstable in cell extract and did not maintain antiviral activity in a thymidine kinase (TK) deficient cell line [57,59]. Introduction of one hydroxyl group on the acyl moiety resulted in greater stability in cell extracts compared to the tBuSATE analogue [57]. The derivative containing two hydroxyl groups showed decreased enzymatic stability compared to the (hydroxyl-tBu)SATE analogue, but it resulted in a significant loss in activity in a TK-deficient cell line [57]. The monohydroxylated prodrug showed anti-HIV activity in the micromolar range comparable to the tBuSATE analogue, and the solubility of the pronucleotide was greatly increased [57], which demonstrates the necessity of obtaining optimized pharmacokinetic properties to achieve effective prodrugs.

Application of the SATE pronucleotide approach to numerous nucleoside monophosphates and nucleotide analogues has resulted in increased antiviral activity *in vitro*. However, when evaluated for transport across Caco-2 monolayers and for bioavailability, no intact prodrug was observed illustrating premature hydrolysis. As pointed out by Gosselin, Imbach, and Périgaud, these facts illustrate the necessary balance that needs to be achieved between lipophilicity, solubility and enzymatic stability [61].

5. *Cyclo*Sal prodrugs

Meier *et al.* have shown that salicyl alcohol is an effective bifunctional masking unit for nucleotides, that is cleaved by a pH-dependent mechanism to deliver the active drug [68]. They have illustrated the utility of this approach using various nucleoside and nucleotide analogues including acyclovir [69], 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, adefovir) [70],

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2′,3′-dideoxyadenosine (ddA) [71], 2′,3′-dideoxy-2′,3′-didehydro-adenosine (d4A) [71], 5-[(E)-2-bromovinyl]-2′-deoxyuridine (BVdU or brivudin) [72,73], 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T) [74-79] and carbocyclic 3′-azidothymidine analogues [80].

Their original goal in creating these pronucleotides was to find a masking unit that would deliver the nucleotide analogue exclusively by a chemical mechanism. Initial attempts using bis(alkyl), bis(phenyl), or bis(benzyl) nucleotide triesters proved unsuccessful at releasing the NMP by a purely chemical mechanism [6]. The charge formed once one ester was cleaved led to a stable compound resistant to further chemical hydrolysis [16]. However, Meier and coworkers found that they could successfully mask the phosphate with phenyl and benzyl esters of salicyl alcohol, while the nucleoside was attached by esterification via the 5′-hydroxyl group (**Figure 4A**(**19**)) [81]. These esters are distinct enough to achieve differentiated chemical hydrolysis independent of any enzymatic activity [82]. This principle was validated by comparing the half-lifes of the triesters in phosphate buffer at pH 7.3 and cell extracts with fetal calf serum, which showed similar half-lifes for the triesters in both media [83].

The successful *cyclo*Sal pronucleotide releases the nucleotide (**Figure 4B**(**23**)) and the salicyl alcohol (**Figure 4B**(**24**)) intracellularly [81]. The nucleotide is released through a cascade, which is initiated by cleavage of the phenyl ester via nucleophilic attack on the phosphorus atom by hydroxide to form the diester (**Figure 4B**(**22**)) (step a_1). The ring, now activated by the strong electron-donating hydroxyl group, allows for cleavage of the benzyl ester to yield the nucleotide analogue (**Figure 4B**(**23**)) and salicyl alcohol (**Figure 4B**(**24**)) via a S_N1-type reaction at the benzyl position (step a_2). It is also possible that hydrolysis of the benzyl ester can occur to first produce a charged intermediate (**Figure 4B**(**25**)) (step b_1), which then reacts with water to yield **26** (**Figure 2B**) (step b_2). Further hydrolysis of **26** (**Figure 2B**) does not occur, thus preventing the release of the nucleotide analogue. However, in hydrolysis studies, the major products were the NMP and salicyl alcohol. When evaluated for cytotoxicity in mice, salicyl alcohol showed no toxicity [82].

As the *cyclo*Sal pronucleotides were designed to release the active drug via a chemical cascade mechanism, the stability and hydrolysis pathways of these pronucleotides can be fine-tuned by varying the substituents on the aromatic ring. Acceptor substituents in the 5- or 6-position decrease stability, while donor substituents at the 3- or 5-position increase the stability of the triesters (**Figure 4A**(**19**)) [82]. Bulky substituents (*tert*-butyl groups) at the 3- and/or 5-position increase the amount of the phenyl phosphate diester (**Figure 4B**(**25**)) observed. When substitution was made at the benzyl position, the half-life decreased drastically compared to the unsubstituted analogue and the major product was the diester (**Figure 4B**(**25**)) in hydrolysis studies [82]. However, the addition of a donor substituent at the 6-position caused the major hydrolysis product to be the desired diester (**Figure 4B**(**22**)) [82].

Although there is a benefit – lack of dependence on enzyme expression differences in tissues, individuals and species – to the use of a pronucleotide activated by a chemical mechanism, the possibility of extracellular release of the active drug or efflux of the pronucleotide, due to the establishment of a concentration equilibrium across the cell membrane, cannot be ignored. To remedy these potential problems, Meier *et al.* designed a way to trap the pronucleotide inside the cell [77,79,84]. In theory, the attachment of a moiety to the aromatic ring that can be enzymatically activated to release a more polar group will prevent penetration of the cellular membrane by the compound and trap the pronucleotide [75]. The initial attempts included the use of an esterase to release an alcohol or carboxylic acid [85]. The released alcohol group was not polar enough to prevent efflux of the pronucleotide, and when a two, three, or four carbon linker was used to attach a methyl or benzyl ester to the aromatic ring, the compounds did not show good esterase affinity [78]. The feasibility of acetoxymethyl (AM) and pivaloyloxymethyl (POM) esters as enzyme-cleavable triggers was demonstrated by their significantly decreased stability in cell extracts versus plasma, illustrating selective activation of these compounds intracellularly [73]. Attempts at intracellular trapping of the pronucleotide with an amino acid ester trigger moiety resulted in a large differential between the buffer and cell extract stability and sustained antiviral activity in kinase-deficient cells [84]. Although a strongly polar group was required to trap the pronucleotide in the cell, Meier and coworkers found that the release of the NMP from the carboxylic acid intermediate was unsatisfactorily slow, and therefore, they sought a trigger to accelerate the release of the NMP [77].

They found that the incorporation of an aldehyde, a strong electron-withdrawing group, in the aromatic ring increased the release of the NMP [77,79]. When a diacetoxymethyl (di-AM) group was attached to the *cyclo*Sal ring at the 5-position, cleavage of the di-AM group resulted in the rapid formation of the benzyl phosphodiester intermediate and, subsequently, the release of the NMP, but the loss of antiviral activity in TK-deficient cells was 26-fold, illustrating only a partial delivery of the prodrug [74,75]. The attachment of 5-(1-acetoxyvinyl) to 3-alkyl-*cyclo*Sal-d4TMP showed the best potential for 'lock-in' of the pronucleotide, as these analogues showed increased chemical stability, the acetoxyvinyl groups were rapidly cleaved in cell extracts and the 5-(1-acetoxyvinyl)-3-alkyl-*cyclo*Sal-d4TMPs retained antiviral activity in thymidine kinase-deficient cells [77].

One potential limitation of the *cyclo*Sal approach is interaction between the pronucleotides and human acetylcholinesterase (AChE) or butyrylcholinesterase (BChE) [81,82]. None of the tested pronucleotides inhibit the essential

A.

19

R = H, CH_3

20

B.

S_NP reaction
step a_1

21

22

Hydrolysis
step a_2

S_N1-type reaction
step b_1

23

24

$+H_2O$
step b_2

Hydrolysis
step b_3

25

26

Figure 4. A. The triester pronucleotides studied by Meier and coworkers. The *cyclo*Sal pronucleotides (**19**) led to increased intracellular levels of NMP. The bis-*cyclo*Sal pronucleotides (**20**) are able to deliver two molecules of drug per biomolecule administered. **B. Activation pathway for the *cyclo*Sal pronucleotides.** Cleavage of the phenyl ester is initiated by nucleophilic attack at the phosphorus to produce the diester **22** (step a_1). Cleavage of the benzyl ester yields the nucleotide analogue **23** and salicyl alcohol **24** (step a_2). The benzyl ester of **21** can also first be hydrolyzed resulting in the charged intermediate **25** (step b_1), which then reacts with water to produce **26** (step b_2). Hydrolysis of **26** does not occur (step b_3), and the nucleotide is not released.

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human AChE [86], but inhibition of BChE has been observed and a structure–activity relationship has been established [87]. Studies have shown that inhibitory activity of the pronucleotide varies greatly with the NMP or nucleotide analogue used, the stereochemistry at the phosphorus and the substituents on the aromatic ring. Increasing the size of the pronucleotide by adding bulky substituents to the aromatic ring decreases the inhibitory activity of the triester. In one example, the IC_{50} value was decreased four-fold when a cyclohexyl group was added at the 5-position [88]. However, antiviral data was not reported for this compound and the fact that bulky substituents at that position increase the formation of the undesired diester (**Figure 4B(26)**) was not addressed. The best results (50-fold reduction in BChE inhibition) were observed when a bis-*cyclo*Sal moiety (**Figure 4A(20)**) was used [89].

Recently, the development of bis-*cyclo*Sal pronucleotides was reported (**Figure 4A(20)**) [89]. These pronucleotides deliver two molecules of active drug for each biomolecule that is delivered. The hydrolysis pathway of these pronucleotides was studied in detail. The bis-*cyclo*Sal pronucleotides were found to be more stable than the monomers. Interestingly, no hydrolysis of the benzyl ester was observed. Rather, the pronucleotides were hydrolyzed exclusively to the NMP [89]. These pronucleotides exhibited reduced BChE inhibition [88,89] and maintained antiviral activity comparable to that of the nucleoside in wild-type CEM cells while displaying significantly enhanced antiviral activity in kinase-deficient cells [89]. The pronucleotides displayed considerably higher cytotoxicity than the nucleoside.

The *cyclo*Sal approach has effectively achieved delivery of an NMP through a pH-dependent mechanism triggered by enzymatic release of the polar *cyclo*Sal phosphotriester intracellularly. In a majority of these cases, salicyl alcohol is successful in masking the phosphate and releasing the active drug in an enzyme-independent manner. These pronucleotides increase antiviral activity *in vitro* compared to the parent nucleoside, especially in kinase-deficient cells, while often demonstrating increased cytotoxicity, although Meier and coworkers reported that the salicyl alcohol is non-toxic. Modification of the salicyl alcohol aromatic ring substituents can increase the stability and decrease the BChE inhibitory activity of the pronucleotide. With the further refinement of their intracellular trapping strategy, Meier and coworkers have demonstrated a versatile method to deliver nucleoside monophosphates or nucleotide analogues.

6. New amino acid and peptide conjugates as pronucleotides

In the context of bioterrorism, the development of an orally available therapy [18] effective against such viral pathogens as variola virus (smallpox) is of urgent concern. Cidofovir is an FDA-approved, nucleoside monophosphonate derivative known to be effective against variola and related viruses, but has inadequate oral bioavailability and thus has become an important target of contemporary prodrug strategies.

McKenna and coworkers have recently studied the use of biologically benign amino acids and peptides conjugated to cidofovir or cyclic cidofovir via a phosphonate ester with the serine side chain hydroxyl group to increase oral bioavailability of the drugs [12,90-93].

Enhanced oral bioavailability resulting from conjugation of L-valine to acyclovir by esterification of the hydroxyl group [5,94] has stimulated interest in amino acids or dipeptides as promoieties. A dipeptide promoiety might interact with a nutritional peptide transporter, such as the human oligopeptide transporter 1 (hPEPT1), in the gastrointestinal tract [95]. In this approach, an amino acid or dipeptide was attached via an ethylene glycol (EG) linker (**Figure 5(27)**) or directly via esterification of a serine side chain hydroxyl group (**Figure 5(28 and 29)**). It was hypothesized that the use of a naturally occurring phosphoserine bond might allow for activation by a phosphoserine/phosphothreonine specific protein phosphatase or a dual-specificity protein phosphatase [96,97]. The other phosphonic acid -OH group was masked by intramolecular esterification to afford a cyclic cidofovir (cHPMPC) derivative (**Figure 5(29)**) or via esterification by an ethyl group (**Figure 5(28)**).

Pronucleotides containing an amino acid–EG–cHPMPC conjugate made by POH–HOC esterification were synthesized (**Figure 5(27)**) [90]. While the analogue was stable in moderately acidic and neutral buffers, it was rapidly metabolized to cHPMPC by liver, intestinal and cellular enzymes [90]. The L-valine pronucleotide exhibited a four-fold increase in antiviral activity compared to ganciclovir in a human cytomegalovirus (HCMV) plaque reduction assay and showed no cytotoxicity in KB cells [90]. However, the EG-linked amino acid conjugates did not exhibit increased bioavailability compared to the parent compound after direct injection into the gastrointestinal tract of rats [90].

Dipeptide diesters of acyclic cidofovir (HPMPC) were also synthesized (**Figure 5(28)**) [91], incorporating a dipeptide esterified to the phosphonate via the serine side chain hydroxyl group and an ethyl ester masking the other P-OH group. Although these conjugates exhibited enhanced bioavailability, the ethyl group was not cleaved in an *in vivo* rat model. The P-OEt monoester metabolite was detected in plasma, and this compound did not exhibit significant activity in an *in vitro* vaccinia plaque reduction assay [91].

Dipeptide monoesters of cHPMPC containing a variable N-terminal amino acid and serine esterified to cHPMPC via the side chain hydroxyl group of serine have been synthesized (**Figure 5(29)**) [12,92,98]. It should be noted that in addition to elegantly masking the P-OH by the internal esterification, the cHPMPC structure is associated with decreased nephrotoxicity compared to HPMPC [99]. When exposed to intracellular cyclic CMP phosphodiesterase,

27 $R_1 = CH(CH_3)_2, CH_2Ph$

28 $R_2 = CH_3, CH(CH_3)_2$

29 $R_3 = CH_3, CH(CH_3)_2, CH_2CH(CH_3)_2, CH_2Ph$
$R_4 = CH_3, H$

Figure 5. General structures of the ethylene glycol-linked amino acid (27) and serine phosphoester dipeptide pronucleotides (28 and 29).

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cHPMPC generates the parent drug [99]. Similar stability profiles were observed for the dipeptide pronucleotides and the EG-linked pronucleotides. An eight-fold increase in oral bioavailability compared to the parent drug was observed for the L-Val-L-Ser(OMe) cHPMPC pronucleotide in a rat model. Exchange of the L-valine for other hydrophobic amino acid residues, such as Ala, Phe and Leu, resulted in enhanced oral bioavailability compared to the parent compound, while the free carboxylic acid analogue, L-Val-L-Ser(OH) cHPMPC, exhibited the lowest bioavailability of all compounds tested [98]. To investigate the effect of varying the amino acid stereochemistry on bioavailability, a series of prodrugs were synthesized [93,98]. The use of D-amino acids has the potential to provide additional enzymatic stability without necessarily incurring toxicity [100]. The best bioavailability was achieved with the pronucleotides containing amino acids with D-stereochemistry in the N-terminal residue [98]. It was hypothesized that the increased enzymatic stability afforded by the D-isomers allows for more intact pronucleotide to be transported into the plasma. These modifications reveal that the observed enhanced transport might be due to a mechanism other than hPEPT1 [93,98].

Dipeptide conjugates of cHPMPC evaluated for *in vitro* antiviral activity in a plaque reduction assay demonstrated activity against HCMV in the submicromolar range, which was 10-fold lower than ganciclovir, the positive control [92]. These conjugates exhibited little to no cytotoxicity at concentrations up to 100 μM in human foreskin fibroblast (HFF) and KB cells [92]. *In vivo* antiviral experiments are needed to understand the full potential of these conjugates as prodrugs.

7. Conclusion

Nucleosides and nucleotide analogues represent an important tool in the treatment of viruses and cancer. However, their therapeutic use is hindered by several factors. The first phosphorylation step of nucleosides is often the rate-determining step in obtaining the active nucleoside triphosphate, and viruses can also develop resistance to nucleoside drugs through kinase deficiencies, as well as by modifying nucleoside transport. Nucleotide analogues usually exist as dianions at physiological pH, and, therefore, transverse of cellular membranes is often limited. Over the past decade, several creative prodrug strategies have been developed to overcome these deficiencies. Further optimization of the specifics to obtain a balance between transport, toxicity and activation should lead to the development of pronucleotides that can more effectively achieve oral absorption, bypass pre-systemic metabolism and deliver nucleotide analogues to exert their therapeutic action.

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8. Expert opinion

The treatment of viral infections by nucleotide analogues is well recognized. Although their value has been proven, their therapeutic utility is often limited by low bioavailability. As a result, the development of orally available prodrug modifications of such drugs is highly desirable. Several developing approaches aimed at improving the oral absorption of nucleotide drugs have been reviewed here. All of these approaches show promise at some level but, in our opinion, the efficient development of prodrug strategies in general is impeded by the lack of comprehensive, reliable models for predicting oral bioavailability, pharmacokinetics and toxicity based on the molecular structure of a prodrug.

The pronucleotide approaches discussed here have been developed using 'chemist's intuition' to optimize strategies for masking charge to achieve oral absorption and for activation *in vivo*. Absorption is traditionally estimated empirically by methods such as lipid/water solubility and membrane permeability in an animal model. Despite the rudimentary nature of these predictive tools, the application of chemical ingenuity has been remarkably successful in affording useful prodrug modifications. Yet the uncertainty of achieving a beneficial outcome in a given case points to a lack of sophistication in current models for prodrug behavior *in vivo*, and thus improvement of these models is a key long-term objective for drug delivery research.

When designing therapeutic agents with enhanced oral absorption, the fundamental processes of absorption, distribution, metabolism, excretion and transport can be addressed only individually and semi-empirically. For a prodrug to be successful, a complex series of conditions need to be met. The prodrug must have robust stability in the gut, be substantially transported intact into the plasma, and thence to the target cell. Preferably at the target, and certainly not prematurely, the prodrug must be converted to its active form at an efficacious rate. The prodrug and promoiety metabolites must not display excess toxicity. The ideal prodrug will satisfy all these criteria, and other desiderata. Unfortunately, today's state of the art does not allow us to optimize all of these properties in a rationalized, unified manner based on prediction from a particular molecular structure. Physiologically based computer models are now emerging to guide candidate drug selection prior to clinical studies [101]. However, it is clear that improved rational prodrug design will also depend on further advances in understanding transport at the molecular level, especially the expression of active transporters, their specificity and mechanisms of action, in addition to more predictable *in vivo* activation. Accurate *in silico* modeling of potential human toxicity remains a yet more difficult challenge.

In summary, although great strides in design have been made by combining simple pharmacological principles, intuition and empirical data, the ideal prodrug will only be obtained efficiently with the development and optimization of an integrated, comprehensive model for transport, activation and toxicity. In the meantime, progress in this field will continue to be driven by the heuristic *savoir-faire* and synthetic skills of medicinal chemists.

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Declaration of interest

LP and CM are co-inventors on a patent related to a portion of the work discussed in this review.

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Review

Advances in nucleoside monophosphate prodrugs as anti-HCV agents

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Nucleoside monophosphate prodrugs that are eventually bioconverted to the active nucleoside triphosphate (NTP) offer the potential to deliver increased intracellular NTP levels and/or organ-specific NTP enhancement. There are several classes of monophosphate prodrugs that have been applied to HCV drug discovery, and some of these approaches are currently being evaluated in humans. This review discusses recent advances in monophosphate prodrug approaches to improve oral absorption, stability and pharmacokinetic profile, including their advantages and potential pitfalls.

Introduction

HCV is a positive-stranded RNA virus, which was identified in 1989 as a member of the *Flaviviridae* family [1]. The resultant infection is a serious problem affecting approximately 3% of the worldwide population, according to the World Health Organization (WHO). The WHO also estimates that 4 million people contract HCV each year. Although the early stages of HCV are usually asymptomatic, and approximately 20% of infected individuals naturally clear the virus, the majority of infections progress to chronic infection. In addition, >20% of chronically infected individuals will eventually develop more serious liver problems, such as cirrhosis, hepatocellular carcinoma or liver failure requiring liver transplantation [2–4]. Indeed, in industrialized nations, HCV infection is the leading cause for liver transplants [5,6]. Unfortunately, reinfection often occurs post-transplantation [5]. The virus is transmitted parenterally by contaminated blood, usually from sharing contaminated needles, but also from improper sterilization of medical, dental, body piercing or tattoo equipment. Heterosexual transmission and vertical transmission (infected mother-to-child transmission during the birth process) of HCV can also occur, but are rare [7,8].

Presently, there is no vaccine for HCV, although early attempts have shown encouraging results [9]. Chimpanzees are currently the most useful animal model to study antiviral agents for HCV infections; however, ethical concerns, increased costs and other restrictions make large studies difficult. The current treatments that have been approved by the US Food and Drug Administration for chronic HCV are limited to pegylated interferon-α alone or in combination with ribavirin [10]. Unfortunately, these treatments have limited efficacy with response rates of only 40–50% for the genotype-1-infected population, which is the most prevalent genotype in the US and China [11,12]. This treatment also has major side effects, including depression, anaemia, central nervous system toxicity, fatigue, flu-like symptoms, mild alopecia, thyroid dysfunction and teratogenic effects [13–19]. The combination of pegylated interferon-α (given by injection) and ribavirin probably does not directly act on the virus and its mechanism is not well-characterized [20], but is thought to aid the immune system in the clearance of the virus from infected individuals. In addition, the drug combination is not well-tolerated in individuals coinfected with other viruses such as HIV [21].

There is an unmet medical need for safer and more effective treatments for HCV, and two major drug targets have been identified: the NS3/4A serine protease and NS5B RNA-dependent RNA polymerase (RdRp) [22,23]. Peptidomimetics comprise the majority of compounds studied for the NS3/4A serine protease target. Non-nucleoside and nucleoside analogue inhibitors are the two main categories of RdRp inhibitors, which differ

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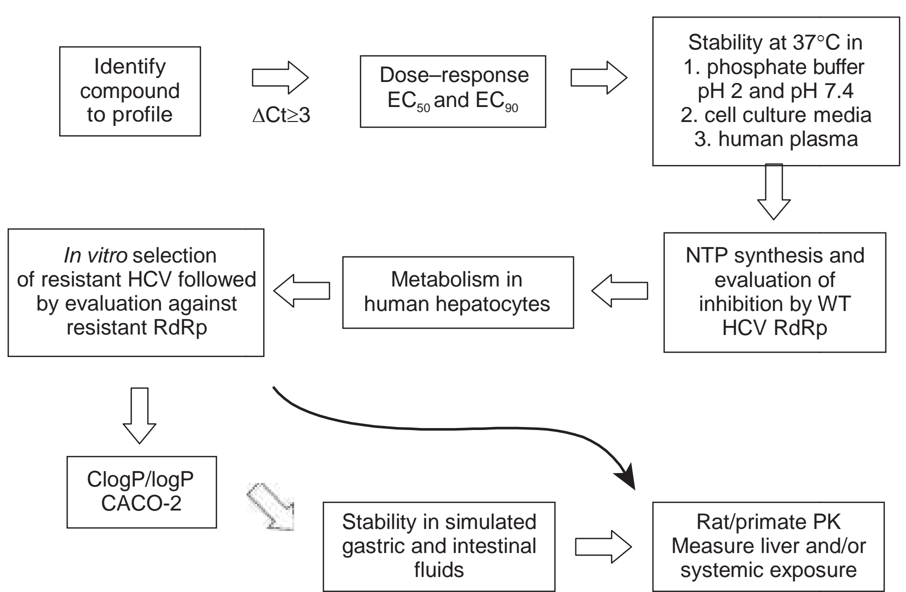
based on their chemical structure and mode of action. There have been several modified nucleosides reported that exhibit anti-HCV activity, most with modifications at the 2′ or 4′ positions of the sugar. However, potential problems for the use of these nucleoside analogues are poor cell permeability or unsuitability of the modified nucleoside as a substrate for cellular kinases, for which compatibility is needed in order to transform the nucleoside into a biologically active nucleoside triphosphate (NTP). Because HCV lacks virus-encoded nucleoside kinase expression, conversion to the biologically active NTP needs to be accomplished by cellular kinases [24]; however, poor turnover to the biologically active species is routinely seen. In many cases, it is the conversion to the nucleoside monophosphate (NMP) that is the rate-limiting step [20], but there are exceptions [25]. The anabolism and pharmacokinetic (PK) requirements of nucleoside and nucleotide analogue inhibitors of viral RNA are likely more stringent relative to DNA for polymerization in light of the one to two orders of magnitude higher intracellular levels of ribonucleoside triphosphates (TPs) that typically are in the mM range in the RNA [26]. Furthermore, the challenge of delivering sufficient concentrations of anti-HCV NTP analogues to the HCV polymerase provides an additional barrier to drug development. The NTP itself cannot be considered as a drug candidate because of degradation by intracellular phosphodiesterases and/or alkaline phosphatases and, in particular, because of poor cell permeability.

Prodrug strategies wherein a pharmacologically inactive compound must be converted to the biologically active species *in vivo* have been widely employed in the nucleoside area in an attempt to circumvent these problems. The development of monophosphorylated nucleoside prodrugs, thereby, bypassing the rate-limiting step of the initial phosphorylation, has been extensively explored [27–30].

Once the target compound is identified and tested in a cell-based assay, a dose–response curve (50% effective concentration [EC_{50}] and EC_{90}) is determined for promising compounds (Figure 1). Prodrugs that target the liver potentially offer an additional challenge of requiring liver enzymes for unmasking of the monophosphate (MP), which might not be present in the cell line used for antiviral activity evaluation [31]. The chemical stability of the prodrug in cell culture media, buffer and plasma will help eliminate prodrugs with no potential for oral delivery or with a short half-life ($t_{1/2}$). The NTP is synthesized and tested against the HCV RdRp to confirm chain termination. Metabolism studies in human hepatocytes follow, but can be done much earlier for prodrugs that are not unmasked in various cell-based assays. After *in vitro* selection of resistant HCV and further testing against these mutants, tests in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and human liver microsomes provide additional stability data prior to *in vivo* toxicity studies (there are instances where these steps can be skipped and compounds can be tested directly *in vivo*).

Figure 1. HCV prodrug evaluation strategy



CACO-2, human epithelial colorectal adenocarcinoma cells; ClogP/logP, partition coefficient; EC_{50}, 50% effective concentration; EC_{90}, 90% effective concentration; NTP, nucleoside triphosphate; PK, pharmacokinetics; RdRp, RNA-dependent RNA polymerase; WT, wild-type; ΔCt, change in cycle threshold.

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Finally, *in vivo* studies are performed in various animal models to determine PK profiles [32].

Currently, the most advanced MP prodrugs might bestow several problems, which include intracellular delivery of toxic agents, a racemic phosphorous centre, a weak nucleoside–MP phosphorous–oxygen bond that is prone to cleavage, and the preparation of many MP prodrugs that occur in low to very low yields. There are several classes of NMP prodrugs that have been successfully applied to HCV therapeutics; only the most significant from mid-2007 forward will be the subject of this review.

Aryloxy phosphoramidate

As discussed earlier, some nucleoside analogues have poor cell permeability and/or are not suitable substrates for cellular kinases, which transform it to the biologically active NTP. This problem can be alleviated by the use of a substituted MP prodrug strategy that would be converted, initially, to the NMP and then further to the NTP. It was suggested during the study of potential prodrugs for HIV in the 1990s that nucleosides with a 5′-phosphoramidate group might be able to bypass the problematic initial kinase-promoted phosphorylation step and eventually lead to the active triphosphorylated species [33]. Indeed, this was the case and these aryloxy phosphoramidate prodrugs, termed ProTides [34], have been incorporated in the study of many viral diseases, including recently to HCV. There have been many reports of modified nucleoside ProTides that show biological activity against HCV *in vitro* (subgenomic HCV replicon RNA) as well as *in vivo*. It has been shown that the TP of 2′-*C*-methylcytidine is quite potent against the isolated NS5B enzyme (50% inhibitory concentration [IC_{50}]=0.025 μM), but the parent nucleoside, 2′-*C*-methylcytidine, shows only modest activity (EC_{50}=2–7 μM) in a subgenomic cell-based replicon assay [35]. Recent studies indicate that conversion of 2′-*C*-methylcytidine to the biologically active NTP is a problematic pathway and, in particular, the conversion to the NMP is a rate-limiting step [36].

Merck & Co., Inc. [37] has recently reported a thorough evaluation of different phosphoramidate prodrugs of 2′-*C*-methylcytidine, which included NM-107 (Figure 2). The introduction of the ProTide group at the 5′-position shows promising activity. Structure–activity relationship studies were performed by varying both the alanine ester portion and the aryloxy portion of the phosphoramidate and representative examples are shown in Figure 2. Starting with the ethyl ester and 4-chlorophenyl phosphoramidate (**1**), an EC_{50} value of 1 μM was obtained with no apparent cytotoxicity (50% cell cytotoxicity [CC_{50}]>20 μM). Because the toxicity of 4-chlorophenol was less understood and less studied, the unsubstituted phenol substituent was also tested and a twofold increase in activity without any cytotoxicity was observed. Further substitution to a more lipophilic 1-napthyl (1-Nap) group resulted in comparable activity (EC_{50}=0.2 μM) and a slight increase in cytotoxicity (CC_{50} ranging from >20 μM for **2** to 15 μM for **3**). Using a more lipophilic ester moiety (butyl versus ethyl) resulted in a twofold increase in activity with an EC_{50} of 0.09 μM and use of the 2-propylpentyl ester gave even more potent activity with an EC_{50} of 0.04 μM, but with an increased cytotoxicity (CC_{50}=2 μM).

On the basis of anti-HCV data obtained in cell culture for the ProTides of 2′-*C*-methylcytidine, additional studies were performed with **1** in human hepatocytes [37]. High intracellular concentrations of the 2′-*C*-methylcytidine-TP were obtained after incubation at 10 μM for 4 h. When expressed as the area under the curve between 0 and 4 h, 517 μM•h of 2′-*C*-methylcytidine-TP was found: a 32-fold increase over NM-283 (valopicitabine, the valine prodrug of NM-107) under the same conditions. These promising results indicate successful intracellular delivery of the 2′-*C*-methylcytidine-MP and apparent benefit to bypassing the initial kinase-driven phosphorylation step. The stability of prodrugs **1** and **5** were tested in the plasma of various species. No significant degradation was seen in either dog or human plasma after 5 min for either prodrug; however, significant degradation in both rat and mouse plasma was observed. Further examination of prodrug **5** showed that it was moderately stable in hamster plasma after 5 min. Because of this stability in hamster plasma and *in vitro* potency, the PK profile of **5** was evaluated. Oral administration of **5** at 30 μmol/kg to hamsters resulted in low (1.1 μM) liver NTP levels at 6 h, indicating low bioavailability, but was twofold higher than NM-283. The stability was also tested in SGF and the compound was found to be stable with no degradation up to 3 h; thus, the low bioavailability could be caused by either poor absorption or degradation in the intestine. Intramuscular injection at a much lower dose of 1.5 μmol/kg resulted in liver NTP levels of 3.6 and 2.2 μM at 3 h and 6 h, respectively. Subcutaneous injection at the same dose resulted in even higher liver NTP levels of 5.6 and 10.1 μM at 3 h and 6 h, respectively. By contrast, oral dosing of NM-283 resulted in liver NTP levels of 0.48 μM and subcutaneous injection at a dose 5.5-fold higher than the dose used for **5** gave liver NTP levels below the lower limit of detection. Hence, MP prodrug **5** showed improved *in vivo* liver exposure when compared with the prodrug NM-283.

Merck & Co., Inc. [37] explored the removal of the aryloxy portion of the ProTide to eliminate the release of potentially toxic phenol resulting in hydroxy phosphoramidate prodrug **7** with the more lipophilic 2-propylpentyl

Figure 2. Antiviral activity and cytotoxicity of ProTide prodrugs of pyrimidine analogues

R=H; NM-107
R=valinyl; NM-283

1–14

PSI-7851, **15**, **16**

Compound	B	R_1	Ar	R_2	R_3	EC_{50}, μM[a]	CC_{50}, μM[b]	NTP, μM · h[c]	Reference
NM-107[d]	–	–	–	–	–	2–7	–	–	[35]
NM-283[d]	–	–	–	–	–	7.6	>100	16	[37]
1	C	H	4-ClPh	Et	Me	1	>20	517	[37]
2	C	H	Ph	Et	Me	0.45	>20	65	[37]
3	C	H	1-Nap	Et	Me	0.2	15	–	[37]
4	C	H	1-Nap	Bu	Me	0.09	9	–	[37]
5	C	H	Ph	2-Ethylbutyl	Me	0.22	7	460	[37]
6	C	H	Ph	2-Propylpentyl	Me	0.04	2	107	[37]
7	C	H	H	2-Propylpentyl	Me	8.2	>100	511	[37]
8	U[d]	N_3	–	–	H	>100	>100	–	[38]
9	U	N_3	Ph	Bn	H	0.61	>100	–	[38]
10	U	N_3	1-Nap	Bn	H	0.22	>100	–	[38]
11	C[d]	N_3	–	–	Me	>10	>10	–	[39]
12	C	N_3	Ph	Et	Me	4.9	>100	–	[39]
13	U[d]	N_3	–	–	Me	>10	>10	–	[39]
14	U	N_3	Ph	Et	Me	3	>100	–	[39]
PSI-7851	U	–	Ph	*i*-Pr	–	0.39[e]	–	–	[40]
15	U	–	4-ClPh	*i*-Pr	–	0.47[e]	–	–	[40]
16	U	–	Ph	Cyclohexyl	–	0.13[e]	–	–	[40]

[a]Effective concentration of compound required to reduce HCV replication by 50 % (EC_{50}). [b]Concentration of inhibitor reducing cell viability by 50 % (CC_{50}). [c]Intracellular nucleoside triphosphate (NTP) concentration after incubation at 10 μM with human hepatocytes. [d]Parent nucleoside. [e]Effective concentration of compound required to reduce HCV replication by 90 %. Bn, benzyl; Bu, butyl; C, cytosine; Et, ethyl; *i*-Pr, isopropyl; Me, methyl; Ph, phenyl; ProTide, aryloxy phosphoramidate; U, uracil; 1-Nap, 1-napthyl; 4-ClPh, 4-chlorophenyl.

ester. Although **7** was less active in the cell-based replicon assay (EC_{50}=8.2 μM), high NTP levels in human hepatocytes (511 μM•h) were observed. The hydroxy phosphoramidate prodrug **7** showed good stability in plasma from various species and a favourable metabolic stability profile in rat and human liver fractions. High NTP levels of 39 μM were obtained in hamster liver after subcutaneous injection of 1.5 μmol/kg; however, low bioavailability was seen when dosed orally at 30 μmol/kg.

McGuigan and colleagues [38] have also synthesized a series of ProTide prodrugs of 4′-azidouridine (**8**). The prodrugs that contained the more lipophilic Ar=1-Nap proved to be the most potent. Most of the prodrugs showed greater anti-HCV potency than the inactive parent nucleoside **8**. For example, an EC_{50} value of 0.61 μM for **9** (Ar=phenyl and R_2=benzyl) and 0.22 μM for **10** (Ar=1-Nap and R_2=benzyl; Figure 2) was observed. All of the prodrugs as well as the parent nucleoside **8** were non-toxic in the replicon assay (CC_{50}>100 μM); however, additional *in vivo* studies, such as oral bioavailability, were not reported. This study reinforces their earlier conclusion that a separate ProTide motif optimization process is required for each nucleoside analogue versus a given target.

Other recent phosphoramidate prodrug studies include the prodrugs of 2′-*C*-methyl-4′-azidocytidine (**11**) and 2′-*C*-methyl-4′-azidouridine (**13**), which were studied by Schinazi and colleagues [39]. Both parent nucleosides **11** and **13** had an EC_{50} value >10 μM, whereas the corresponding prodrugs **12** and **14** had

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EC_{50} values of 4.9 and 3 μM, respectively. Again, neither prodrug displayed any toxicity, even up to 100 μM; however, *in vivo* studies were not reported.

Pharmasset, Inc. [40] has also recently reported 2′-deoxy-2′-fluoro-2′-*C*-methyl-uridine ProTide prodrugs, PSI-7851, **15** and **16** (Figure 2). Submicromolar activity in the replicon assay was reported with impressive EC_{90} values as low as 0.13 μM for prodrug **16**. A monotherapy trial for PSI-7851 (40 treatment-naive HCV-infected patients allocated to 4 arms with 10 patients per arm, each arm with 8 active and 2 placebo patients receiving either 50, 100, 200 or 400 mg) showed a dose-dependent decrease in HCV RNA up to -1.95 log_{10} IU/ml from baseline at day 3 with 400 mg/day oral dosing [41]. However, the S282T mutation, common among 2′-*C*-methyl-substituted nucleoside analogues, was selected by PSI-7851 in an HCV subgenomic replicon in Huh7 cells [42]. Earlier work in mutated HCV replicons had shown that the S282T substitution in NS5B severely debilitates replication and no compensatory residues were encoded to increase the replication fitness [43]. The intracellular metabolism of PSI-7851 was assessed using both cellular and biochemical assays to determine the mechanism of PSI-7851 activation. The study indicated that five steps are required to reach PSI-7851-TP (PSI-7409): four enzyme-dependent steps and one non-enzymatic step [44]. Similar to the results found in earlier studies [34], the first step involves ester hydrolysis catalysed by either cathepsin A or carboxylesterase 1. The resulting carboxylic acid attacks the P-centre and displaces phenol. Subsequently, the histidine triad nucleotide binding protein 1 catalyses the removal of the amino acid to form the NMP. Final conversion to the NTP is accomplished first by uridine MP-cytidine MP kinase-1 catalysed conversion to the nucleoside diphosphate followed by final catalysis by nucleoside diphosphate kinase to form the NTP.

The ProTide strategy is not limited to pyrimidine nucleosides. Several purines and substituted purines have been converted to their corresponding 5′-phosphoramidate prodrugs and tested for anti- viral activity against HCV (Figure 3). Interestingly, 2′-*C*-methylguanosine (**17**) and 2′-*C*-methyladenine (**18**) have rather different activity profiles. Although the EC_{50} for nucleoside **17** is 10.1 μM against HCV in Huh 5-2 cells, the EC_{50} for nucleoside **18** is 0.25 μM, suggesting that guanosine analogue **17** might not be a suitable substrate for the cellular kinases responsible for the initial phosphorylation, whereas **18** is a viable substrate [6]. However, most phosphoramidate prodrugs of 2′-*C*-methylguanosine (**19–23** with the exception of the *tert*-butyl [*t*-Bu] ester **23**) show rather good antiviral activity in the cell-based replicon assay with an EC_{50} as low as 0.12 μM and no cytotoxicity for prodrug **22** (all CC_{50}>50 μM), suggesting that the 5′-MP prodrug is indeed delivered intracellularly and efficiently converted to the biologically active NTP by cellular kinases, at least at the *in vitro* level. Phosphoramidate prodrugs of 2′-*C*-methyladenine (**18**) showed no significant increase in antiviral activity in the replicon assay compared with the parent nucleoside (**18** versus **24** or **25**; Figure 3). These data demonstrate that the prodrugs **24** and **25** offer no advantage to intracellular transport versus parent nucleoside **18,** and that the initial kinase-driven phosphorylation of **18** is not a rate-limiting step towards the formation of 2′-*C*-methyladenine-TP. The *t*-Bu esters **23** and **26** were inactive in replicon assays, which might be related to the stability of tertiary esters to enzyme-mediated hydrolysis. Inhibitex, Inc. [45] recently reported preliminary results for INX-189, a ProTide of a 2′-*C*-methylguanosine analogue of undisclosed structure. In a cell-based HCV replicon assay, an EC_{50} value of 0.01 μM and an EC_{90} value of 0.04 μM were reported with no apparent mitochondrial toxicity up to 1 μM. PK studies in rats and non-human primates indicated that NTP levels exceeding the EC_{90} value were achieved for ≥24 h following 14-day oral dosing of a human equivalent dose of 100 mg. As seen with PSI-7851, the S282T mutant was also selected *in vitro* and was moderately resistant (≤10-fold increase in EC_{50}) to INX-189 [46].

McGuigan and colleagues [47] have shown that ProTide prodrugs of 4′-azidoadenosine (**27**) also provide an increase in antiviral potency compared with the parent nucleoside while showing no signs of cytotoxicity (CC_{50}>100 μM). When Ar=1-Nap, both the ethyl (**29**) and benzyl (**30**) esters show much improved activity with EC_{50} values of 0.59 μM and 0.22 μM, respectively, over the parent nucleoside (**27**), which was inactive in the replicon assay (EC_{50}>100 μM). However, as seen in previous examples with other nucleoside analogues (**23** and **26**), the *t*-Bu ester (**28**) was inactive in the replicon assay, which might be related to the stability of tertiary esters to enzyme-mediated hydrolysis [47].

In 2007, Valeant Pharmaceuticals International [48] reported that 6-hydrizinopurine-2′-*C*-methylnucleosides had poor stability and/or a poor selectivity index. This eventually led to the discovery of the corresponding ProTide prodrugs. Neither of the parent nucleosides displayed significant activity (**31** with EC_{50}=300 μM and **33** with EC_{50}=92 μM; Figure 3); however, prodrugs **32** and **34** showed antiviral activity with EC_{50} values of 0.68 μM (**32**; Ar=4-chlorophenyl, R_1=benzyl and R_2=H) and 0.11 μM (**34**; Ar=4-chlorophenyl, R_1=benzyl and R_2=H), respectively. The stability of these prodrugs was also tested in human plasma, human SGF and human SIF. Both prodrugs were stable in human plasma and SGF up to 1 h, but unstable in SIF. Utilizing α-methylalanine

Figure 3. Antiviral activity and cytotoxicity of ProTide prodrugs of purine analogues

17–36

AA: R=H
DAA: R=NH_2

Compound	B	Ar	R_1	R_2	R_3	EC_{50}, μM[a]	CC_{50}, μM[b]	Reference
17[c]	G	–	–	H	Me	10.1	>50	[6]
18[c]	A	–	–	H	Me	0.25	>50	[6]
19	G	1-Nap	Me	H	Me	0.23	>50	[6]
20	G	Ph	Me	H	Me	0.88	>50	[76]
21	G	1-Nap	Et	H	Me	0.28	>50	[6]
22	G	1-Nap	Bn	H	Me	0.12	>50	[6]
23	G	1-Nap	*t*-Bu	H	Me	27	>50	[6]
24	A	1-Nap	Et	H	Me	0.24	>50	[6]
25	A	1-Nap	Bn	H	Me	0.27	>50	[6]
26	A	1-Nap	*t*-Bu	H	Me	4.18	>50	[6]
27[c]	A	–	–	N_3	H	>100	>100	[47]
28	A	1-Nap	*t*-Bu	N_3	H	>100	>100	[47]
29	A	1-Nap	Et	N_3	H	0.59	>100	[47]
30	A	1-Nap	Bn	N_3	H	0.22	>100	[47]
31[c]	AA	–	–	H	Me	300	–	[48]
32	AA	4-ClPh	Bn	H	Me	0.68	–	[48]
33[c]	DAA	–	–	H	Me	92	–	[48]
34	DAA	4-ClPh	Bn	H	Me	0.11	–	[48]
35	AA	4-ClPh	Me	H	Me	1.4[d]	–	[48]
36	DAA	4-ClPh	Me	H	Me	0.26[d]	–	[48]

[a]Effective concentration of compound required to reduce HCV replication by 50% (EC_{50}). [b]Concentration of inhibitor reducing cell viability by 50% (CC_{50}). [c]Parent nucleoside. [d]α-Methyl alanine methyl ester used as an amino acid. A, adenine; Bn, benzyl; Et, ethyl; G, guanine; Me, methyl; Ph, phenyl; ProTide, aryloxy phosphoramidate; *t*-Bu, *tert*-butyl; 1-Nap, 1-napthyl; 4-ClPh, 4-chlorophenyl.

methyl ester as the amino acid portion of the phosphoramidate gave favourable antiviral activity (EC_{50}=1.4 μM for **35** and 0.26 μM for **36**), but more importantly, no degradation was observed in SIF up to 3 h. Additional *in vivo* studies are currently underway for these prodrugs. Acyloxyethylamino phosphoramidates have also been studied, but poor NTP levels in rat liver after oral dosing suggests low bioavailability [49].

To avoid the release of potentially toxic phenol and other potentially toxic aryloxy derivatives, cyclic phosphoramidate prodrugs have been developed by Meppen *et al*. [24] and Merck & Co., Inc. [50]. Only cyclic prodrug **37** was more active than NM-283, with an EC_{50} value of 4 μM. All of the other cyclic prodrugs (**38–41**; Figure 4) were less active than NM-283 (all EC_{50} values ≥10 μM), regardless of the ester substituent, in the cell-based replicon assay. However, conversion to the NTP was observed in human hepatocytes for all prodrugs with up to a 10-fold increase compared with the parent NM-283 (NTP concentration ranging from 6–186 μM•h compared with 16 μM•h for NM-107). Subcutaneous injection of prodrug **40** (1.5 μM) resulted in improved NTP formation as high as 2.6 nmol/g in hamster liver after 6 h. The process responsible for the intracellular conversion of the cyclic prodrugs to their corresponding MPs is not well understood.

Both the ProTide prodrugs and the cyclic phosphoramidate prodrugs have shown promising activity in cell-

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Figure 4. Antiviral activity of cyclic phosphoramidate prodrugs of 2′-*C*-methylcytosine

Compound[a]	R	EC_{50}, μM[b]	NTP, μM · h[c]
NM-283	–	7.6	16
37	Ethyl[d]	4	6
38	Ethyl[e]	>20	23
39	Heptyl	10	140
40	2-(Hexyloxy)ethyl	15	158
41	3-Cyclohexylpropyl	10	186

37–41

[a]From [24] and [50]. [b]Effective concentration of compound required to reduce HCV replication by 50% (EC_{50}). [c]Intracellular nucleoside triphosphate (NTP) after incubation at 10 μM with human hepatocytes. [d]Slow eluting phosphorous diastereomer by reverse phase (RP)-HPLC. [e]Fast eluting phosphorous diastereomer by RP-HPLC.

based replicon assays and, in many cases, have produced a significant boost in NTP formation *in vivo*. Overall as a class, ProTides provide limited systemic exposure upon oral dosing and pose significant potential for toxicity associated with the liberated aryl alcohol, which appears problematic for clinical applications, especially if the drug is to be given at high doses (>400 mg daily). However, the ProTide prodrugs have proven quite valuable in elucidating the anabolic pathway for a number of nucleoside analogues. Their utility in liver-directed clinical applications remains to be determined.

S-acyl-2-thioethyl prodrugs

Another prodrug strategy developed in an effort to increase the cell permeability characteristics of nucleosides for potential therapeutic use is the incorporation of the *S*-acyl-2-thioethyl (SATE) group into the 5′-MP. The MP is exposed by a multistep process involving an esterase-mediated cleavage of the thioester to reveal a thioethyl ether, which has been shown to decompose with release of ethylene sulfide (Figure 5) [51,52]. Although the toxicity risk associated with ethylene sulfide has limited the development of SATE-type prodrugs, many groups continue to explore modified SATE-type prodrugs of nucleoside analogues as potential anti-HCV agents.

Benzaria *et al*. [53] from Idenix Pharmaceuticals, Inc. have shown that bis(*t*-BuSATE) MP prodrugs of NM-107 and 2′-*C*-methyluridine (**42**) exhibit a large improvement in the antiviral activity: from EC_{50}=2.2 μM for NM-107 to 0.7 μM for prodrug **43** and from 46 μM for 2′-*C*-methyluridine (**42**) to 0.1 μM for prodrug **44**. However, an increase in cytotoxicity was also observed with both prodrugs exhibiting CC_{50} values ≤8.4 μM. Several SATE prodrugs of modified adenine derivatives (**46**, **48**, **50** and **52–54**; Figure 5) have also been synthesized by Gilead Sciences [54,55] and Valeant Pharmaceuticals International [48,56], and have been tested for anti-HCV activity. In all cases, the increased lipophilicity of the SATE prodrugs led to improved cell penetration capabilities and coincided with greater potent activity with EC_{50} values as low as 0.02 μM for prodrug **52**. However, these prodrugs showed poor stability in human plasma with $t_{1/2}$ in the order of min. Further investigation into cyclic SATE prodrugs was also attempted by Valeant Pharmaceuticals International [57] in order to obtain a better PK profile (Figure 6). Again, in all cases, the cyclic SATE prodrugs (**55–58**) displayed an enhancement in antiviral activity, with EC_{50} values as low as 0.01 μM for prodrug **58** and no significant cytotoxicity observed for any of the prodrugs (CC_{50}>50 μM). The stability of cyclic SATE prodrugs **55–58** was also observed in SIF and SGF with no significant degradation detected in 2 h. The ultimate goal was to deliver these cyclic MP prodrugs to hepatocytes; therefore, prodrug **58** was further tested for stability in rat plasma. After incubation in rat plasma for 1 h, only 4% of **58** remained unchanged with various metabolites observed (59% of cyclic MP, 19% of 5′-MP and 18% of **51**). However, prodrug **58** was stable in human and monkey plasma for 1 h suggesting that this class of prodrugs might be able to be administered by an intravenous route.

A hybrid prodrug that combined the lipophilicity of the SATE prodrugs with the stability of the ProTide phosphoramidates was recently disclosed from Idenix Pharmaceuticals, Inc. [58] (Figure 7). The mono-SATE *N*-benzyl phosphoramidate prodrugs **60** and **61** both displayed improved potency (EC_{50}<1 μM) compared with the parent nucleoside, 2′-*C*-methylguanosine

Figure 5. Metabolic activation and antiviral activity of SATE monophosphate prodrugs

Compound	B	EC_{50}, μM[a]	CC_{50}, μM[b]	Reference
NM-107	C	2.2	>75	[53]
42	U	46	>75	[53]
43	C	0.7	8.4	[53]
44	U	0.1	3.4	[53]
45	Nuc DA[c]	10–100	–	[54]
46	DA	<1	–	[54]
47	Nuc MA[c]	<10	–	[55]
48	MA	<10	–	[55]
49	Nuc SA[c]	300	–	[48]
50	SA	0.06	–	[48]
51	Nuc ASA[c]	92	–	[48]
52	ASA	0.02	–	[48]
53	6-Methoxypurine	0.1	300	[56]
54	6-Ethoxypurine	0.08	300	[56]

[a]Effective concentration of compound required to reduce HCV replication by 50% (EC_{50}). [b]Concentration of inhibitor reducing cell viability by 50% (CC_{50}). [c]Parent nucleoside (Nuc). C, cytosine; SATE, *S*-acyl-2-thioethyl; *t*-Bu, *tert*-butyl; U, uracil.

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Figure 6. Antiviral activity and cytotoxicity of cyclic mono-SATE prodrugs

49, 51, 55–58

Compound[a]	R_1	R_2	EC_{50}, μM[b]	CC_{50}, μM[c]
49[d]	H	Nucleoside[e]	300	–
55	H	H	0.15	>50
56	H	O*n*-Pr	0.04	>50
51[d]	NH_2	Nucleoside[e]	92	–
57	NH_2	H	0.02	>50
58	NH_2	O*n*-Pr	0.01	>50

[a]From reference [57]. [b]Effective concentration of compound required to reduce HCV replication by 50% (EC_{50}). [c]Concentration of inhibitor reducing cell viability by 50% (CC_{50}). [d]From reference [48]. [e]Parent nucleoside. O*n*-Pr, O*n*-propyl; SATE, *S*-acyl-2-thioethyl.

(**17**; EC_{50}>10.1 μM). The cytosine prodrug **59** showed similar activity to NM-107 in replicon assays with both resulting in an EC_{50} value of 1–10 μM. However, intracellular NTP levels of liver extracts were much higher for **59** than for NM-107 (1,838 pmol/10^6 cells versus 10 pmol/10^6 cells in monkeys and 991 pmol/10^6 cells versus 19 pmol/10^6 cells in humans after 24 h incubation at 10 μM). It was also found that chimpanzee hepatic NTP levels were 10–50-fold higher for **59** than NM-107 after oral dosing of 50 mg/kg/day for 14 days. This mixed prodrug showed promising *in vivo* stability and activity against HCV. However, formation of the toxic alkylating agent ethylene sulfide might severely limit the clinical potential of this hybrid MP prodrug.

In December 2009, Idenix Pharmaceuticals, Inc. [59] disclosed results for IDX-184 (presumed structure is compound **60** based on their patent filing; Figure 7) from a 3-day Phase I proof-of-concept study. This double-blinded, placebo-controlled, monotherapy, dose-escalation study enrolled 41 treatment-naive HCV genotype-1-infected patients into four dosing cohorts (25 mg, 50 mg, 75 mg and 100 mg) [60,61]. Mean viral load decreases ranged from 0.47 $\log_{10}$ in the 25 mg group to 0.74 $\log_{10}$ in the 100 mg group after 3 days of treatment.

The use of either the bisSATE MP prodrug at the 5′-position or the 5′,3′-cyclic SATE MP prodrug has increased the lipophilicity of the nucleotide enabling the intracellular delivery of MPs resulting in potent anti-HCV activity in the cell-based replicon assays. There still remains a stability issue with these compounds that needs to be resolved if oral administration is to be realized. Improved oral bioavailability and a

Figure 7. Mixed mono-SATE ProTide prodrugs[a]

NM-107 B=C, parent nucleoside
59 B=C, R=H
60 B=G, R=H
61 B=G, R=

[a]From reference [58]. C, cytosine; G, guanosine; ProTide, aryloxy phosphoramidate; SATE, *S*-acyl-2-thioethyl.

favourable pharmacokinetic profile were found with the mixed mono-SATE ProTide prodrug. However, formation of the toxic ethylene sulfide, an alkylating agent, as a by-product could severely limit the clinical potential of this group of MP prodrugs. Hence, the dose selected for human studies should be low enough to avoid releasing large quantities of ethylene sulfide.

Phosphate/phosphonate prodrugs

Simple alkyl esters of nucleoside MP are metabolically stable and not suitable for use as prodrugs [27]; however, modified phosphate esters of nucleosides have been evaluated for the treatment of HCV. Both acyloxyalkyl esters and aryl-substituted cyclic phosphate esters (HepDirect prodrugs [62]) as well as acyclic phosphonates have led to some promising *in vitro* and *in vivo* results.

Acyloxyalkyl ester

One of the most commonly used prodrug for the phosphate group is the acyloxyalkyl ester. The carboxylate or carbonate ester is cleaved enzymatically by esterases generating a transient hydroxymethyl intermediate (**62**; Figure 8), which rapidly loses formaldehyde to generate the deprotected phosphate group (**63**) [27]. However, oral bioavailability of this type of prodrug can be limited as the esterases responsible for this cleavage are found in numerous tissues and at high levels [63]. Also, the intracellular generation of formaldehyde raises some toxicity concerns for prolonged treatment; however, there are antiviral phosphonate prodrugs of this type that have been approved by the US Food and Drug Administration currently on the market [27].

Valeant Pharmaceuticals International [57] recently disclosed a cyclic acyloxyalkyl ester prodrug of a

Figure 8. Metabolic activation and antiviral activity of cyclic monophosphate prodrugs of modified adenosines

Esterase

62

CH_2O

63

49, 51, 64–67

Compound[a]	R_1	R_2	EC_{50}, μM[b]	CC_{50}, μM[c]
49[d]	H	Nuc[e]	300	–
64	H	O*i*-Pr	0.46	>50
65	H	*t*-Bu	0.41	>50
51[d]	NH_2	Nuc[e]	92	–
66	NH_2	O*i*-Pr	0.16	>50
67	NH_2	*t*-Bu	0.09	>50

[a]From reference [57]. [b]Effective concentration of compound required to reduce HCV replication by 50% (EC_{50}). [c]Concentration of inhibitor reducing cell viability by 50 (CC_{50}). [d]From reference [48]. [e]Parent nucleoside (Nuc). O*i*-Pr, O-isopropyl; *t*-Bu, *tert*-butyl.

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modified adenine nucleoside that shows activity towards HCV in a cell-based replicon assay. Significant increases of activity were observed in comparison with the parent nucleoside (**49**; EC_{50}=300 μM) with both the isopropylcarbonate **64** prodrug (EC_{50}=0.46 μM) and the pivaloyloxymethyl prodrug (POM; **65**; EC_{50}=0.41 μM; Figure 8). There was no cytotoxicity observed up to 50 μM. Slightly improved potency was noted when R_1=NH_2 (**66** and **67**; Figure 8) with observed EC_{50} values as low as 0.09 μM and with no apparent cytotoxicity (CC_{50}>50 μM). Both the carbonate prodrug and the POM prodrug were found to be stable in SIF and SGF with no significant degradation detected after 2 h. The stability in SIF is in contrast to the 5′-phosphoramidate prodrug of the same nucleoside and could be further evaluated in advanced studies as a potential drug candidate.

HepDirect prodrugs

Because esterases are expressed throughout the gut, small intestine, plasma, muscle and various other tissues, activation of prodrugs by this route can often be problematic. After ester cleavage, the resulting ionic phosphate species have little to no membrane permeability, so the distribution is largely limited to the site of metabolism. This often leads to toxicity in non-target organs throughout the body [63]. A prodrug was designed by Erion *et al.* [64] from Metabasis Therapeutics, Inc. in 2004 in an effort to deliver NMP analogues in high concentration specifically to the liver for the treatment of HBV and HCV infections, and hepatocellular carcinoma. In order to achieve this goal, rapid activation of the prodrug (specifically in the liver) and good stability in aqueous solutions, such as blood, was necessary. Substituted arylpropanyl esters achieved both of these goals. Benzylic oxidation of the prodrug **68** by cytochrome P_{450} in the liver gives hydroxylated prodrug **69**, which, after rearrangement, undergoes β-elimination to give the activated drug (**71**) and an aryl enone by-product (Figure 9). The β-elimination is relatively slow, allowing for the hydroxylated prodrug to pass through the liver and be distributed to tissues. In this pathway, **69** would be in equilibrium with the ring-opened ketone **70** and the equilibrium should favour the ring closed **69**. Two anticancer drugs on the market (cyclophosphamide and ifosfamide) are activated in a similar manner.

Various HepDirect prodrugs of substituted nucleosides have been tested for NTP formation [65]. Activation of

Figure 9. Metabolic activation and antiviral activity of HepDirect prodrugs of modified adenosines

Compound[a]	R, Ar	Rat hepatocyte NTP, nmol/g[b]	Rat liver NTP, nmol/g[c]
18[d]	–	209	ND
72	H, Ar=(*S*)-3-ClPh	389	2.9
73	CO, Ar=(*S*)-3-ClPh	296	29.2
74	H, Ar=(*S*)-4-pyridyl	47	3.6
75	CO, Ar=(*S*)-4-pyridyl	185	28.3

[a]From reference [65]. [b]Concentration of nucleoside triphosphate (NTP) at 2 h after incubation at 25 μM. [c]Concentration of NTP at 3 h after a 50 mg/kg oral dose. [d]Parent nucleoside (Nuc). CYP_{450}, cytochrome P450; ND, not determined; (*S*)-3-ClPh, (*S*)-3-chlorophenyl.

Figure 10. Antiviral activity of HepDirect prodrugs of pyrimidines

76–77, 80–83

78, 79

Compound	B	R_1	R_2	R_3	Rat hepatocyte NTP, nmol/g[a]	Rat liver NTP, nmol/g[b]	Rat liver NTP, nmol/g[c]	Reference
76[d]	C	F	H	Me	<1	<0.04	–	[66]
77	C	F	H	Me	1	0.33	–	[66]
78[d]	U	F	H	Me	<2.5	0.73	1.71	[66]
79	U	F	H	Me	87.8	78.4	23.3	[66]
80[d]	C	OH	N_3	H	BLD	0.04[e]	0[f]	[67,68]
81	C	OH	N_3	H	36	0.04	0.19	[67,68]
82[d]	U	OH	N_3	H	BLD	0.1	0	[67,68]
83	U	OH	N_3	H	123.5	24.5[e]	1.58[f]	[67,68]

[a]Concentration of nucleoside triphosphate (NTP) at 2 h after incubation at 25 μM. [b]Concentration of NTP at 3 h after 5 mg/kg intraperitoneal injection. [c]Concentration of NTP at 3 h after an oral dose of 10 mg/kg. [d]Parent nucleoside. [e]4 mg/kg. [f]8 mg/kg. BLD, below limit of detection; C, cytosine; Me, methyl; Pr, propyl; U, uracil.

prodrugs **72–75** (Figure 9) to the NTP would require cleavage of the arylpropanyl group as well as two subsequent phosphorylations by cellular kinases. In some cases, NTP in rat hepatocytes was increased for the HepDirect prodrugs compared with the parent nucleoside (**72–75**; Figure 9); increased NTP was observed in only two out of four rats. Testing the oral bioavailability of prodrugs **72** and **74** led to disappointing low NTP levels in rat liver of approximately 3 nmol/g. However, decreasing the hydrogen bond donating capability of the nucleoside by the incorporation of a 2′,3′-carbonate protecting group resulted in good formation of the NTP at a dosing level of 50 mg/kg (NTP values were 29.2 nmol/g for **73** and 28.3 nmol/g for **75**). Prodrug **75** was stable with a $t_{1/2}$ of 13 h in pH 7 buffer at 25°C and was efficiently converted to **74** in rat plasma at 37°C without any further degradation.

Modifications to both the base and the sugar of the HepDirect prodrugs have led to some promising results. Prodrugs **77**, **79**, **81** and **83** all showed increased levels of NTP in rat hepatocytes compared with the parent nucleoside (Figure 10) [66–68]. Administration of the prodrugs intraperitoneally by injection at 5 mg/kg resulted in high NTP for prodrugs **79** and **83** compared with the parent nucleoside (NTP from 0.73 nmol/g for **78** to 78.4 nmol/g for **79** and from 0.1 nmol/g for **82** to 24.5 nmol/g for **83**). Unfortunately, oral dosing only resulted in good NTP levels in rat liver for prodrug **79** (NTP=23.3 nmol/g), but at a lower dose of only 10 mg/kg compared with the previous study of 50 mg/kg.

HepDirect prodrugs show promise by delivering the monophosphorylated nucleoside prodrug to the liver. Once in the liver, metabolic activation by cytochrome P_{450} releases the active compound. High levels of NTP can be observed via intraperatonial injection or even with oral administration of the prodrug. Despite this promise, to date, no HepDirect prodrugs have been approved for human use. Furthermore, formation of potentially reactive by-products, such as aryl vinyl ketone, will require further evaluation.

Phosphonate prodrugs

Another way to bypass the initial phosphorylation of nucleosides is with the phosphonate group. Acyclic nucleoside phosphonates have been used to treat a variety of viral diseases, including HIV and HBV. Hostetler and colleagues [69] have developed alkoxyalkyl esters of acyclic adenines that show activity in an HCV replicon system. Both the *R*- and *S*-enantiomers of the octadecyloxyethyl ester and hexadecyloxy ester of 9-[3-hydroxyl-2-(phosphonomethoxy)-

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Figure 11. Acyclic nucleoside phosphonates[a]

84–87

Compound	Stereochemistry of carbon[b]	*n*	Genotype 1b EC_{50}, µM[c]	Genotype 2a EC_{50}, µM[c]	CC_{50}, µM[d]
84	*S*	15	2.0	6.5	85.3
85	*S*	17	1.3	0.7	35.6
86	*R*	15	6.7	41	>100
87	*R*	17	7.0	26.7	>100

[a]From reference [69]. [b]At the position indicated with an asterisk. [c]Effective concentration of compound required to reduce HCV replication by 50% (EC_{50}). [d]Concentration of inhibitor reducing cell viability by 50% (CC_{50}).

propyl]adenine (**84–87**; Figure 11) show activity for both genotypes 1B and 2A. The *S*-enantiomers were more active than the *R*-enantiomers with **85** being the most potent (EC_{50}=1.3 µM for genotype 1B and 0.7 µM for genotype 2A). The octadecyloxyethyl esters were more potent than the hexadecyloxy esters (**85** and **87** versus **84** and **86**; Figure 11), but the hexadecyloxy esters were less cytotoxic. The inclusion of the ester increased the bioavailability of **84** and it has been estimated at 74% compared with intraperitoneal injection [70]. These nucleotide analogues were also found to be active against a number of double-stranded DNA viruses, including vaccinia virus, cowpox virus, human and murine cytomegalovirus and adenovirus [70]. Further studies on these compounds are currently underway.

Conclusions

HCV continues to be a serious problem throughout the world. With >3% of the worldwide population chronically infected and only two approved drugs for treatment with limited efficacy, improved treatment modalities utilizing drugs with a long intracellular $t_{1/2}$, such as nucleosides, are needed. In addition, nucleoside analogues are effective against all HCV genotypes and maintain a high genetic barrier to HCV resistance [12,61,71–74]. They are currently the best in any class because of these properties and we anticipate that they will be widely used in the future as a backbone to anti-HCV treatment similar to HIV and lead towards a cure for this disease because HCV does not establish latency [75]. Although modified nucleosides exhibit promising activity against viral diseases such as HCV in cell-based assays, their highly polar nature might complicate their development as an orally available medication. Conversion to the biologically active NTP can be problematic with initial kinase-driven conversion to the MP often rate-limiting. ProTide prodrugs (aryloxy phosphoramidate approach) and cyclic phosphoramidate prodrugs have shown promising activity *in vitro*; however, toxicity concerns continue to slow development. The increased lipophilicity of the SATE prodrugs has enabled the intracellular delivery of the nucleoside MP in cell-based *in vitro* assays, but poor stability and potential toxicity remains an issue with this class of compounds. Acyloxyalkyl ester prodrugs of cyclic phosphates show some promise, eliminating the potentially toxic phenolic by-product of the ProTide approach that could be delivered intracellularly. However, formaldehyde is a by-product of this class and thus long-term exposure could be problematic. The HepDirect class of prodrugs is capable of bypassing the first phosphorylation step and selective activation in the liver could reduce systemic toxicity exposure. The presence of cytochrome P450 isoenzymes in the gastrointestinal tract might be problematic and the formation of a potentially toxic aryl vinyl ketone by-product necessitates further studies. Acyclic nucleoside phosphonate prodrugs also show some promising results by bypassing the initial conversion to the NMP and further studies are currently underway.

It is apparent that many of these approaches are quite attractive because of the intracellular increase of NTP levels in the liver and low levels of the parent drug in the plasma. Progress towards this goal has been recently achieved in a clinical trial with PSI-7851, the first ProTide, specifically designed for HCV that produced a 2 $\log_{10}$ decrease in viral load after 3 days of oral treatment at 400 mg once daily. Nevertheless, much more work is warranted to make MP prodrugs a safe and non-toxic clinical reality.

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Disclosure statement

RFS is the principal founder and a Director of RFS Pharma, LLC. He is also a founder and major shareholder of Idenix Pharmaceuticals and Pharmasset, Inc. All his conflicts of interest were reviewed and are managed by Emory University School of Medicine, and Veterans Affairs Medical Center. All other authors declare no competing interests.

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BEFORE THE CONTROLLER OF PATENTS, DELHI OFFICE

IN THE MATTER OF:

THE PATENT ACT 1970 (as amended by the Patents (Amendment) Act, 2005) and Rule 55 of The Patent Rules 2003 as amended by the Patents (Amendment) Rules 2019 ("the Rules")

IN THE MATTER OF:

Post grant opposition under section 25(2) of the Patents Act against Indian Patent No. 319927 dated: 22.07.2011

IN THE MATTER OF:

Low Cost Standard Therapeutics ... Opponent

Versus

Gilead Sciences INC ... Patentee

[A] **INTRODUCTION:**

1. I, Dr. Umesh Rewaji Zope, aged about 58 years, residing at 145B, Kanchanjunga Apartment, NOIDA UP 201301, India, do hereby solemnly affirm and declare as under:

2. I have been approached by **Low Cost Standard Therapeutics**(hereinafter referred as the "the Opponent"). I am informed by the Opponent that they are filing a post grant opposition against an Indian patent **319927 (formerly Indian application 1328/CHENP/2013)** (herein

after referred as “impugned patent”) in the name of **GILEAD SCIENCES INC** (hereinafter referred as “the Patentee”). The impugned Patent claims **COMPOUNDS FOR TREATING PARAMYXOVIRIDAE VIRUS INFECTIONS**, which has the INN name Remdesivir and has the following structure:

Remdesivir

The claim of the impugned patent reads as:

We Claim:

1. A compound that is

or a pharmaceutically acceptable salt thereof.

3. I have been requested to independently review impugned patent and express my opinion on the claimed matter made therein against the prior-art publications.

4. My opinion is purely based on my education, knowledge, training and experiences in the relevant scientific field. While, I am being compensated but neither my compensation is related to outcome of this proceedings nor I have personal interest therein.

5. Further, I am familiar with basic concepts of patent law including novelty and inventive step which shall be assessed on the priority date of impugned patent or patent application by a person having ordinary skilled in the art (herein after referred as "PHOSITA"). I am also aware that hindsight approach is not permissible in these deliberations. In addition to above all, I firmly believe that the inventions filed without merits shall loudly be opposed.

6. As per Indian Patents Act, 1970 (herein after referred as "the Patents Act") that to be patentable; it must be novel, inventive/non-obvious and have industrial applicability. More importantly, the invention shall also cross the barrier of non-patentable subject matters those are described in Section-3 and Section-4 of the Patents Act.

7. As per the Patents Act new invention means any invention or technology which has not been anticipated by publication in any document or used in the country or elsewhere in the world before the date of filing of patent application with complete specification, i.e. the subject matter has not fallen in public domain or that it does not form part of the state of the art.

8. I am quite acquainted with the definition of inventive step which is also known as non-obviousness that it is a feature of an invention that involves technical advance as compared to the existing knowledge or having economic significance or both and that makes the invention not obvious to a person having ordinary skill in the art (PHOSITA).

[B] **EDUCATIONAL AND PROFESSIONAL BACKGROUND:**

9. My educational qualifications, work experience, associations are more particularly stated in Exhibit-1, hereto. I have expertise in the field of pharmaceutical chemistry.

[C] **<u>TECHNICAL:</u>**

10. I have gone through various prior arts and find that the compound as claimed in claim 1 of the impugned patent is inherently anticipated by AU2009240642.

11. The patent in question claims a compound or its pharmaceutically active salts.AU2009240642 (hereinafter referred to as AU'642) discloses 1' -substituted carba-nucleoside analogs for antiviral treatment. There is disclosed as general formula II

12. I note that when the substituents disclosed in the markush are applied to the above formula they disclose the compound claimed in the impugned patent

Where;

$R^{1,}$ R^3, R^5 can be H; (Page 9)

R^2 and R^4 represents OR^a;(Page 9)

R^a represents H (Page 10) means R^2 and R^4 are OH

R^6 represents CN (Page 10)

R^8 represents $NR^{11}R^{12}$;(Page 12), R11and R^{12} represents H

(Page 12) means R^8 isNH_2, R9 represents H (Page 12)

X^1 and X^2 represents C-R^{10} (Page 11)

R^{10} represents H (Page 12) means X^1 and X^2 represents -CH

R^7 represents (Page 10)

Y represents O (Page 10)

W^1 represents (Page 10)

Y^2 is O (Page 11), M2 is 0 (Page 11)

R^x represents R^y and R^y is R and R can C_6-C_{20} aryl- that is R^x is phenyl (Page 11).

W^2 represents (Page 10)

Y^2 is NR-where R can be H, So Y^2 is NH (page 11).

In Formula II; Rx represents Ry or formula

M1a is Zero, M1c is zero, M12c is one (Page 11)

Each R^y in above formula can be independent.

So (from the left) R^y can be H, R^y can be R and R can be C1-C8 alkyl such as CH_3 (methyl); (Page 11), Y1 is O (page 10) and Y^2 is O (page 11).

R^y can be R and R can be substituted alkyl. (Page 11).In substituted alkyl, substituted represents R^b and Rb represents alkyl means ethyl-butyl.

13. I understand that the Markush structure as forming part of claim 2 of AU’642 encompasses compound claimed in the impugned patent for antiviral activity.

14. On studying the literature in the field, prior to the priority of the impugned patent, I found documents which are extremely relevant to the subject matter claimed in impugned patent. My understanding of disclosure of said documents is elaborated in following paragraphs.

15. I note that **AU2009240642** published in 2009 and hereinafter referred to as AU’642discloses 1’-substituted carba-nucleoside analogs for antiviral treatment. Bare perusal of the compounds disclosed in this document reveal certain common structural features which have been incorporated in all the compounds disclosed therein. These common structural features give rise to a compound of following structure:

16. Furthermore, following the results of the EC50 values stated in AU'642, it is evident to a person skill in the art that phosphorylating at 5' position is preferable, such as in Compound 17 of the document, and forming a further derivative of this 5' phosphate group with ester is more preferred, such as in Compound 6 of the document.

17. On perusal of many other documents I noted that at the time of the invention it was general understanding of a person skilled in the art that formation of a phosphoramidate prodrug of nucleoside antiviral compounds increases their efficacy. McGuigan and his group did significant work in this direction and worked on development and optimization of phosphoramidate prodrugs of many nucleoside antivirals

18. In a 1996 **McGuigan *et al.*** publication, the authors discuss aboutphosphoramidate derivatives of various nucleoside

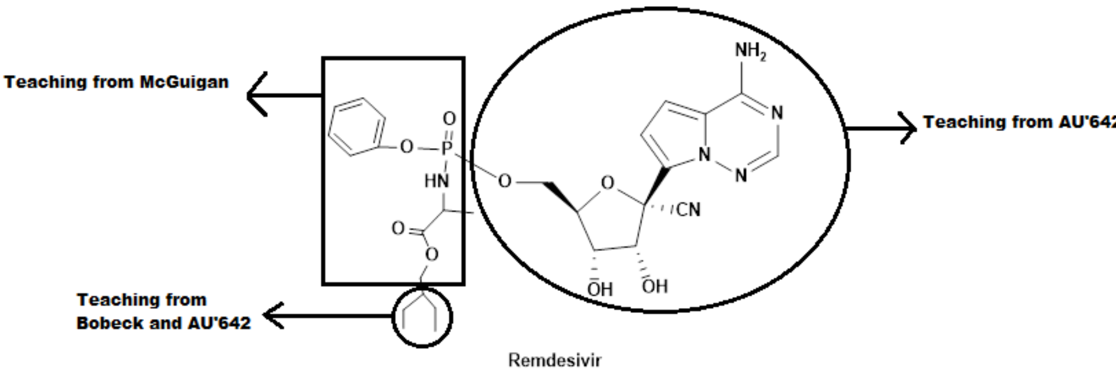
antivirals such as AZT and d4T and concluded that among these the alanine containing ester derivative of the phosphoramidate nucleoside was most effective, such asin Compound 4a. The structure of compound 4a is shown below:

(4a) R = Me **(4d)** R = CH_2iPr
(4b) R = H **(4e)** R = CH_2Ph
(4c) R = iPr **(4f)** R = CH_2CH_2SMe

19. Over time many other groups continued to work on further optimizing the phosphoramidate group since it was found that change in the distal alkyl group of the ester part of phosphoramidate resulted in modulation of efficacy. One such work was by Bobeck et al. which was published in April 2010, wherein they found that in phosphoramidate group where there is a phenyl ring, presence of a distal 2-ethylbutyl or 2-propylpentyl as the alkyl group in the ester part gave better efficacy.

20. Since in the compounds of AU'642, tertiary butyl had already been applied and the resultant compound demonstrated good efficacy, a person skilled in the art is motivated to apply 2-ethylbutyl group from the disclosure of Bobeck as the distal alkyl group of the ester part in the phosphoramidate portion.

21. In view of the aforesaid disclosure of prior art documents and common general knowledge in art, the claim of impugned patent lacks novelty and is obvious in following manner:



22. The statements made above are true and correct and based on records.

DEPONENT

VERIFICATION

I, Dr. Umesh Rewaji Zope, the Deponent do hereby verify that the contents of my affidavit at para 1 to 40 are true and correct to the best of my knowledge, my experience and records. No part of it is false and nothing material has been concealed therefrom.

Verified at New Delhi on this 30th day of June, 2020.

DEPONENT