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APRIL 02, 2026

THE CONTROLLER OF PATENT
THE PATENT OFFICE
DELHI

RE: OPPOSITION U/S 25(2) OF THE PATENT ACT – BY LOW COST STANDARD THERAPEUTICS AGAINST INDIAN PATENT NO. 564721 (FORMERLY PATENT APPLICATION NUMBER 201617031899) DATED 19/09/2016
PATENTEE: MERCK SHARPE & DOHME LLC

Respected Sir,

We are filing this representation by way of Post-Grant Opposition along with annexures u/s 25 (2) of the Patents Act, 1970 and Rule 55 of the Patent Rules, 2003 in Form 7.

The Learned Controller is requested to take said opposition along with annexures on record and proceed further in the matter and keep the Opponent advised of each and every step taken in the matter.

We crave the leave of the Learned Controller to submit additional documents and/or evidence to support any of the averments in the representation as may be necessitated during the future proceeding.

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

Thanking you,

Yours faithfully,



MARIAM SADAF (IN /PA – 5231)
OF RAJESHWARI AND ASSOCIATES
AGENT FOR THE OPPONENT

Encl.: As stated

C.C.: ANAND AND ANAND
EMAIL: EMAIL@ANANDANDANAND.COM;

BEFORE THE CONTROLLER OF PATENTS, DELHI

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. 564721

(Formerly Indian Patent Application No. 201617031899)

IN THE MATTER OF:

LOW COST STANDARD THERAPEUTICS …….. OPPONENT

VS.

MERCK SHARPE & DOHME LLC ……..PATENTEE

POST-GRANT OPPOSITION BY LOW COST STANDARD THERAPEUTICS

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10.	**Annexure – 8:** Copy of Article Krawczyk et al "*Synthesis and Antiproliferative and Antiviral Activity of 2'-Deoxy-2'-fluoroarabinofuranosyl Analogs of the Nucleoside Antibiotics Toyocamycin and Sangivamycin*" *J. Med. Chem. 1996,38,* 4106-4114	302 to 310
11.	**Annexure – 9:** Copy of duly notarized expert affidavit of Debkumar Nandi along with CV.	311 to 338
12.	Copy of duly stamped Power of Attorney	Will follow

Dated this 02nd day of April, 2026

MARIAM SADAF (IN /PA – 5231)
OF RAJESHWARI AND ASSOCIATES
AGENT FOR THE OPPONENT

TO
THE CONTROLLER OF PATENTS
THE PATENT OFFICE, DELHI

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,** an Indian Company of I Floor, Premananda Sahitya Bhavan, Opposite Lakadipul, Dandia Bazar, Vadodara, 390 001, Gujarat, India, hereby give Notice of opposition to the grant of patent in respect of Indian Patent No. 564721 (formerly patent application number 201617031899) dated 19/09/2016 made by **MERCK SHARPE & DOHME LLC** on the grounds:

(a) Section 25(2)(b): Lack of Novelty

(b) Section 25(2)(e): Lack of inventive step

(c) Section 25(2)(f): Invention is not patentable under section 3(d) and 3(e)

(d) Section 25(2)(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(2)(h): Failure to disclose the information required by section 8 of the Patents Act.

(Detailed grounds are set out in the Opposition)

Our address for service in India is:

RAJESHWARI & ASSOCIATES, S – 357, FIRST FLOOR, NEAR HDFC BANK, PANCHSHEEL PARK, NEW DELHI – 110017, INDIA, TEL: + 91-11-41038911, MOBILE NO. 9765843590, EMAIL: SADAF@RALEGAL.CO.IN;

Dated this 02nd day of April, 2026

MARIAM SADAF (IN /PA – 5231)
OF RAJESHWARI AND ASSOCIATES
AGENT FOR THE OPPONENT

TO,
THE CONTROLLER OF PATENTS
THE PATENT OFFICE, DELHI

BEFORE THE CONTROLLER OF PATENTS, THE PATENT OFFICE, DELHI

In the matter of Section 25(2) of The Patents Act, 1970 as amended by The Patents (Amendment) Act 2005;

And

In the matter of Rule 55 of The Patents Rules 2003 as amended by the Patent (Amendment) Rules, 2024

And

In the matter of Indian Patent number 564721 (formerly patent application number 201617031899) dated 19/09/2016, in the name of MERCK SHARPE & DOHME LLC

REPRESENTATION BY:

LOW COST STANDARD THERAPEUTICS .....OPPONENT

VS.

MERCK SHARPE & DOHME LLC .....PATENTEE

REPRESENTATION BY WAY OF POST-GRANT OPPOSITION UNDER SECTION 25(2) OF THE PATENTS ACT, 1970

We, LOW COST STANDARD THERAPEUTICS, having our registered office at having its registered office at I Floor, Premananda Sahitya Bhavan, Opposite Lakadipul, Dandia Bazar, Vadodara, 390 001, Gujarat, India,, hereby submit our representation by way of opposition to the patent 564721 (formerly patent application number 201617031899) dated 19/09/2016 in the name of MERCK SHARPE & DOHME LLC titled "4-Substituted Nucleoside Derivatives as HIV Reverse Transcriptase Inhibitors"

STATEMENT OF CASE OF OPPONENT

The Opponent herein respectfully submits as under:

1. A post-grant opposition under Section 25(2) of the Patents Act, 1970, is being submitted by the Opponent against Indian Patent No. 564721 (hereinafter referred to as the "impugned patent") which has been granted in favour of MERCK SHARPE & DOHME LLC (hereinafter referred to as the "Applicant/Patentee").

2. The Opponent, LOW COST STANDARD THERAPEUTICS (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.

3. 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.

4. 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). LOW 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 and the sales are directed at institutions and individuals that work on a not-for profit basis or cater to the poorest of people.

5. 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. 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.

6. 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).

7. The Opponent has an interest in filing the present post-grant opposition as it is affected by the continuance of the impugned patent on the register – as it would result in denial of access to important medicines to patients suffering from HIV. The Petitioner-Opponent believes that the patent is granted in error and is accordingly filing the present post-grant opposition to oppose the patent.

8. The present opposition is against Indian Patent No. 564721 which was originally filed as Indian Patent Application No. 201617031899 on 19/09/2016. The said application was granted on 31/03/2025 with patent number 564721 (hereinafter referred as the "Impugned Patent"). The Impugned patent is titled "4-SUBSTITUTED NUCLEOSIDE DERIVATIVES AS HIV REVERSE TRANSCRIPTASE INHIBITORS". The priority date of the impugned patent is 28/03/2014. The impugned patent application was published in the official journal of the patent office on 13/01/2017. The impugned patent is derived from the national phase application of PCT/US2015/022621 and draws its priority from application PCT/CN2014/074294 dated 28 March 2014.

BACKGROUND ON HIV AND ITS THERAPEUTIC LANDSCAPE

9. The global burden of HIV is estimated to be 40.8 million, and an estimated 1.3 million people acquired this disease in 2024, thus making it one of the most significant public health issues of our time. Although there is no cure for HIV infection. However, with access to effective HIV prevention, diagnosis, treatment and care, including for opportunistic infections, HIV infection has become a manageable chronic health condition, enabling people living with HIV to lead long and healthy lives. (Source: World Health Organization).

10. The present subject matter concerns nucleoside analogues used as inhibitors of viral replication, particularly those that inhibit reverse transcriptase. At the relevant priority date, it was well established that nucleoside analogues constituted a major class of antiviral agents, especially in the treatment of human immunodeficiency virus (HIV) infection. These compounds function by mimicking natural nucleosides and, following intracellular phosphorylation to their active triphosphate forms, are incorporated into viral DNA by reverse transcriptase, thereby disrupting viral replication.

11. It was part of the common general knowledge that the biological activity of nucleoside analogues depends on three principal structural elements: the sugar moiety, the nitrogenous base, and the phosphate group in its active form. Modifications to each of these components were routinely investigated to influence antiviral potency, selectivity, metabolic stability, and toxicity. Medicinal chemistry in this field was driven by systematic structure–activity relationship (SAR) studies, through which these parameters were progressively optimised.

12. Over time, nucleoside research evolved from an early focus on nucleobase modifications to increasingly sophisticated approaches involving sugar modification. Both purine and pyrimidine nucleobases were extensively studied in combination with modified sugar frameworks to develop selective and potent inhibitors of viral polymerases. Early work in the 1960s and 1970s established that structural modifications to nucleosides could interfere with nucleic acid synthesis and viral replication, thereby laying the foundation for antiviral nucleoside design.

13. Within this framework, modification of the sugar moiety emerged as a central strategy in the design of antiviral nucleoside analogues. Alterations to the sugar component were known to influence key properties such as enzyme recognition, incorporation efficiency, and chain termination, and were therefore extensively explored in antiviral drug development. Over time, systematic investigations identified certain positions on the sugar ring as particularly sensitive to modification. In particular, the 2′ and 4′ positions were recognised as critical sites, where substitutions could significantly modulate antiviral activity and interactions with viral enzymes, such as reverse transcriptase. Studies such as those reported by Ohrui (2006) and Kodama et al. (2001) demonstrated that nucleosides bearing substituents at the 4′-position, including ethynyl groups, exhibit potent antiviral activity across a broad spectrum of HIV strains, thereby

underscoring the importance of these positions in nucleoside design. [Hiroshi Ohrui, *"2'-Deoxy-4'-C-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, is highly potent against all human immunodeficiency viruses type 1 and has low toxicity,"* The Chemical Record, Vol. 6, No. 3, 2006, pp. 133–143; Ei-ichi Kodama, Masahiro Kohgo, Hironori Kitano, et al., *"4'-Ethynyl nucleoside analogs: potent inhibitors of multidrug-resistant human immunodeficiency virus variants,"* Antimicrobial Agents and Chemotherapy, Vol. 45, No. 5, 2001, pp. 1539–1546.].

14. Earlier developments had already shown that modifications to the sugar component alone could dramatically alter biological activity. For example, nucleoside analogues with modified sugar configurations demonstrated the ability to interfere with DNA chain elongation, a principle subsequently extended to antiviral research. These findings reinforced the importance of sugar modification as a key strategy in the design of antiviral nucleosides.

15. At the same time, it was well understood that the nitrogenous base is a variable structural element that can be modified or replaced with structurally related heterocyclic systems. Variations in the base, including substitution on purine and pyrimidine scaffolds and the use of bioisosteric analogues, were routinely employed to modulate enzyme binding, cellular uptake, and metabolic stability. Such base modifications were commonly explored in conjunction with sugar modifications as part of standard medicinal chemistry practice.

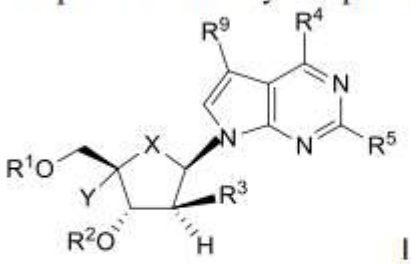
16. It was further recognised that combining known structural features—such as a sugar moiety capable of supporting antiviral activity and a nucleobase compatible with enzyme recognition—could yield active compounds. Accordingly, the development of nucleoside analogues proceeded through iterative modification of both the sugar and base components, with the expectation that suitable combinations of these features would yield compounds with antiviral activity.

17. In view of the above, a person skilled in the art at the relevant priority date would have been familiar with the established design principles underlying nucleoside-based antivirals, including the importance of sugar substitution, particularly at key positions such as 2′ and 4′, the flexibility of nucleobase selection, and the routine nature of modifying these components in order to generate further active compounds.

18. The Opponent by way of this present post-grant opposition submits that the claims currently on record, and granted, are not patentable under the provisions provided in this Act. The claims as granted and currently on record are annexed herewith as **Annexure-1** and reproduced herein below for ready reference:

1. A compound

or a pharmaceutically acceptable salt thereof, which has structural formula II:



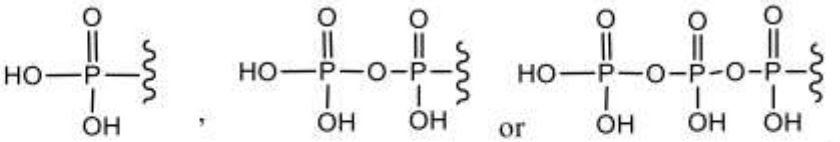
II

;

X is O;

Y is –C≡C-R^8;

R^1 is -H, -C(O)R^6, -C(O)OR^6, -C(O)N(R^6)$_2$,



or a pro-drug modification of the mono-, di- or triphosphate;

R^2 is -H, -C(O)R^{6a}, -C(O)OR^{6a} or -C(O)N(R^{6a})$_2$;

R^3 is –H;

R^4 is -N(R^X)$_2$, -NHC(O)OR^{6b},

-N(C(O)OR^{6b})$_2$, -NHC(O)N(R^{6b})$_2$, or -NHC(O)R^{6b};

R^5 is -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -C_3-C_7 cycloalkyl, halo,

-OR^X, -CN, -N(R^X)$_2$-NHC(O)OR^{6b}, -N(C(O)OR^{6b})$_2$, -NHC(O)N(R^{6b})$_2$, or -NHC(O)R^{6b};

R^6, **R^{6a}** and **R^{6b}** are each independently selected at each occurrence from -H, -C_1-C_6 alkyl, or -C_1-C_6 haloalkyl;

R^8 is -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -C_3-C_7 cycloalkyl;

R^9 is -H, halo, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -CN, -OR^Y or -N(R^Y)$_2$;

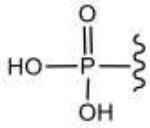
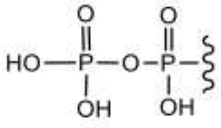
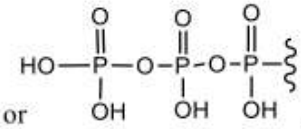
R^X is independently selected at each occurrence from -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, aryl, or 5- or 6- membered monocyclic heteroaryl;

or when either or both of **R^4** or **R^5** is -N(R^X)$_2$, each R^X may optionally be joined together with the nitrogen to which they are both attached to form a 5- or 6-membered monocyclic heteroaryl or 9- or 10-membered bicyclic heteroaryl; and

R^Y is -H, -C_1-C_6 alkyl or -C_1-C_6 haloalkyl.

2. The compound as claimed in claim 1, or or a pharmaceutically acceptable salt thereof, wherein:

R1 is –H,



or

; and

R4 is -N(R^X)$_2$.

3. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein **R2** is -H.

4. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof wherein **R4** is -N(R^X)$_2$.

5. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein **R5** is –H, halo, -C_1-C_6 alkyl, -OR^X, or -N(R^X)$_2$.

6. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein **R8** is–H.

7. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein **R9** is –H, halo, -C_1-C_3 alkyl, -C_1-C_3 haloalkyl, -CN, -OR^Y or -N(R^Y)$_2$.

8. The compound as claimed in claim 1 to 7 or a pharmaceutically acceptable salt thereof, wherein

R^X is -H, -C_1-C_6 alkyl or -C_1-C_6 haloalkyl.

9. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein:

R5 is –H, halo, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -CN, -OR^X, -N(R^X)$_2$, -NHC(O)OR^{6b}, -N(C(O)OR^{6b})$_2$, -NHC(O)N(R^{6b})$_2$, or -NHC(O)R^{6b}; and

R9 is –H, halo, -C_1-C_3 alkyl, -C_1-C_3 haloalkyl, -CN, -OR^Y or -N(R^Y)$_2$.

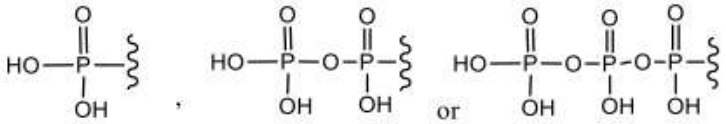
10. The compound as claimed in claim 9 or a pharmaceutically acceptable salt thereof wherein **R4** is -N(R^X)$_2$.

11. The compound as claimed in claim 9 or a pharmaceutically acceptable salt thereof wherein **R1** is –H and **R4** is -N(R^X)$_2$.

12. The compound as claimed in claim 1 or a pharmaceutically acceptable salt thereof, wherein:

Y is –C≡CH;

R1 is -H, -C(O)R6, -C(O)OR6, -C(O)N(R6)2,



;

R2 is H, -C(O)R6a, -C(O)OR6a or -C(O)N(R6a)2;

R3 is –H;

R4 is -N(RX)2, -NHC(O)OR6b or -NHC(O)N(R6b)2;

R5 is –H, halo, -C1-C6 alkyl, -C1-C6 haloalkyl, -CN, -ORX or -N(RX)2;

R6, **R6a**, and **R6b** are each independently selected at each occurrence from -H, -C1-C6 alkyl, or -C1-C6 haloalkyl;

R9 is –H, halo, -C1-C3 alkyl, -C1-C3 haloalkyl, -CN, -ORY, or -N(RY)2;

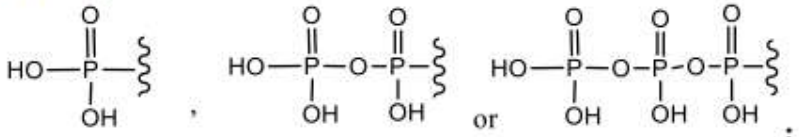
RX is independently selected at each occurrence from -H, -C1-C6 alkyl, or -C1-C6 haloalkyl; and

RY is independently selected at each occurrence from -H, -C1-C6 alkyl or -C1-C6 haloalkyl.

13. The compound as claimed in claim 1, or a pharmaceutically acceptable salt thereof, wherein:

Y is -C≡CH;

R1 is -H,



;

R2 is H;

R3 is -H;

R4 is NH_2;

R5 is –H, halo or -NH_2; and

R9 is –H, halo or -CH_3.

14. The compound as claimed in claims 1 or a pharmaceutically acceptable salt thereof, wherein one or more of R1, R2, R4 or R5 is selected as follows:

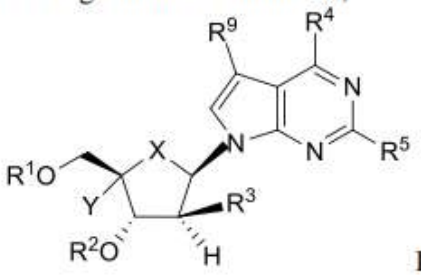
R1 is -C(O)R6, -C(O)OR6, -C(O)N(R6)2; and/or

R2 is -C(O)R6a, -C(O)OR6a or -C(O)N(R6a)2; and/or

R4 is -NHC(O)OR6b, -N(C(O)OR6b)2, -NHC(O)N(R6b)2, or -NHC(O)R6b; and/or

R5 is -NHC(O)OR6b, -N(C(O)OR6b)2, -NHC(O)N(R6b)2, or -NHC(O)R6b .

15. The compound as claimed in claim **1** or a pharmaceutically acceptable salt thereof, wherein the compound having structural Formula II,



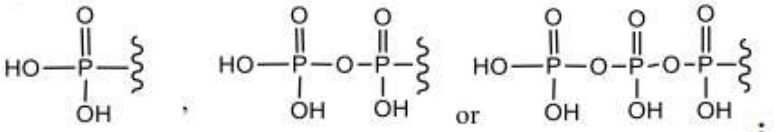
II,

or a pharmaceutically acceptable salt thereof, wherein:

X is O;

Y is –C≡CH;

R1 is –H,



;

R2 is –H;

R3 is –H;

R4 is –NH_2;

R5 is –H, halo or -NH_2; and

R9 is –H, halo or –CH_3.

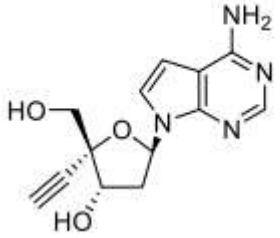
16. The compound as claimed in claim 1 that is:

1)	(2*R*,3*S*,5*R*)-5-(4-amino-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
2)	(2*R*,3*S*,5*R*)-5-(4-amino-5-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
3)	(2*R*,3*S*,5*R*)-5-(4-amino-5-methyl-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
4)	(2*R*,3*S*,5*R*)-5-(4-amino-2-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
5)	(2*R*,3*S*,5*R*)-5-(4-amino-5-bromo-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
6)	(2*R*,3*S*,5*R*)-5-(4-amino-5-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;

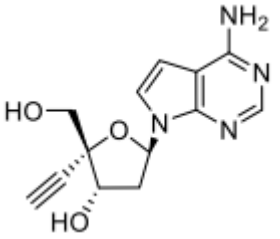
7)	(2*R*,3*S*,5*R*)-5-(4-amino-5-iodo-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
8)	(2*R*,3*S*,5*R*)-5-(2,4-diamino-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
9)	(2*R*,3*S*,5*R*)-5-(4-amino-2-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
10)	((2*R*,3*S*,5*R*)-5-(4-amino-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
11)	((2*R*,3*S*,5*R*)-5-(4-amino-5-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
12)	((2*R*,3*S*,5*R*)-5-(4-amino-5-methyl-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
13)	((2*R*,3*S*,5*R*)-5-(4-amino-2-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
14)	((2*R*,3*S*,5*R*)-5-(4-amino-5-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;

or a pharmaceutically acceptable salt thereof

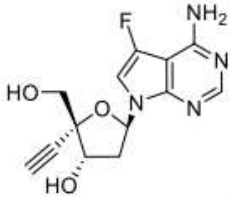
17. The compound as claimed in claim 1 that is:



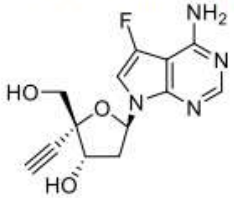
18. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



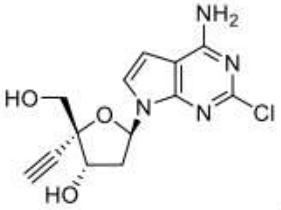
19. The compound as claimed in claim 1 that is:



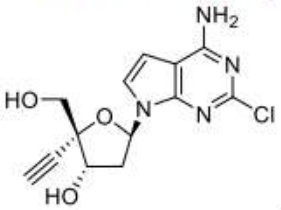
20. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



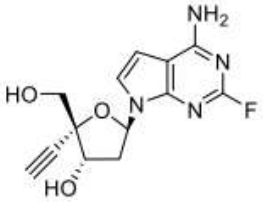
21. The compound as claimed in claim 1 that is:



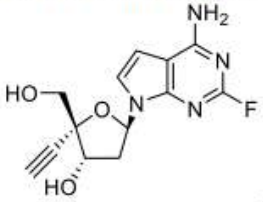
22. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



23. The compound as claimed in claim 1 that is:



24. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



25. A pharmaceutical composition comprising a compound as claimed in any one of claims 1 to 16, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier and optionally comprising at least one anti-HIV agent selected from an HIV antiviral agent, an immunomodulator, and an anti-infective agent such as herein described.

PRIOR ARTS: The opponent wishes to rely on the following prior arts as evidence in support of the grounds of opposition.

i. US20100249068 (US'068) published 30 Sep 2010 (**Annexed herewith as Annexure 2**)

ii. Hiroshi Ohrui, *"2'-Deoxy-4'-C-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, is highly potent against all human immunodeficiency viruses type 1 and has low toxicity,"* The Chemical Record, Vol. 6, No. 3, 2006, pp. 133–143 (**Annexed herewith as Annexure 3**)

iii. EP2177527B1 published 18 December 2013 (**Annexed herewith as Annexure 4**)

iv. CA2502109 published 23 February 2010 (**Annexed herewith as Annexure 5**)

v. Kawamoto et al. "*2'-Deoxy-4'-C-ethynyl-2-halo-adenosines active against drug-resistant human immunodeficiency virus type 1 variants*"; The International Journal of Biochemistry & Cell Biology 40 (2008) 2410–2420 (**Annexed herewith as Annexure 6**)

vi. Vittori et al. "*Antiviral Properties of Deazaadenine Nucleoside Derivatives*"; Current Medicinal Chemistry, 2006, 13, 3529-3552 (**Annexed herewith as Annexure 7**)

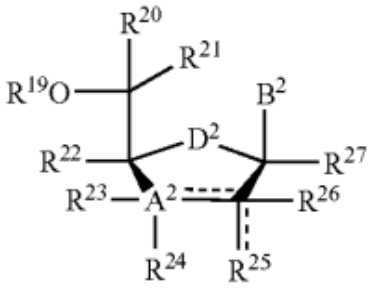
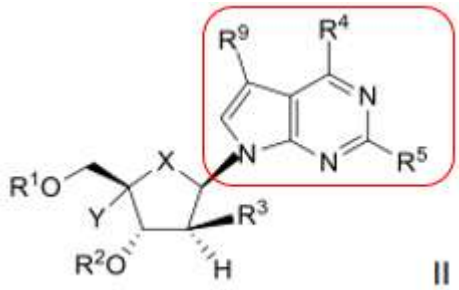
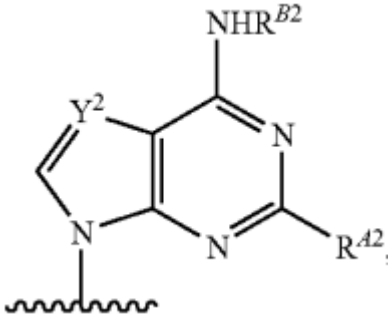
vii. Krawczyk et al "*Synthesis and Antiproliferative and Antiviral Activity of 2'-Deoxy-2'-fluoroarabinofuranosyl Analogs of the Nucleoside Antibiotics Toyocamycin and Sangivamycin*" *J. Med. Chem. 1996,38,* 4106-4114 (**Annexed herewith as Annexure 8**)

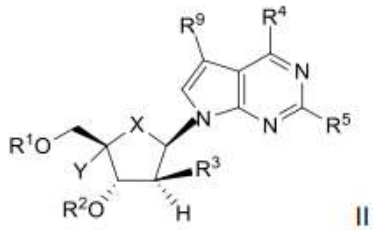
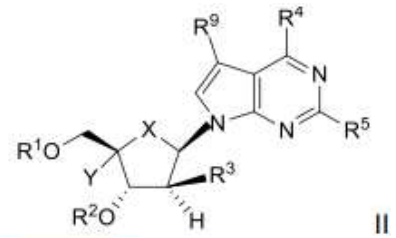
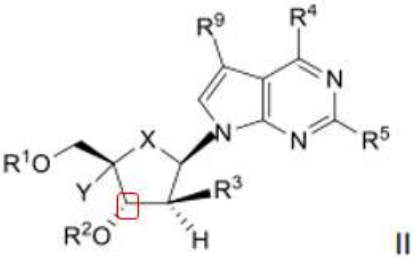
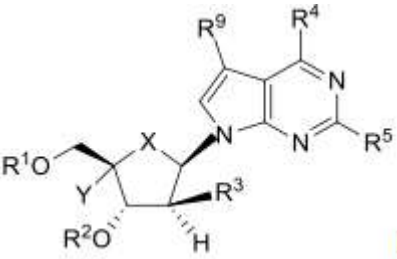
19. It is submitted that the claims of impugned patent application are liable to be refused on following grounds as below, which are without prejudice to each other:
    (a) Section 25(2)(b): Lack of Novelty
    (b) Section 25(2)(e): Lack of inventive step
    (c) Section 25(2)(f): Invention is not patentable under section 3(d) and 3(e)
    (d) Section 25(2)(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(2)(h): Failure to disclose the information required by section 8 of the Patents Act.

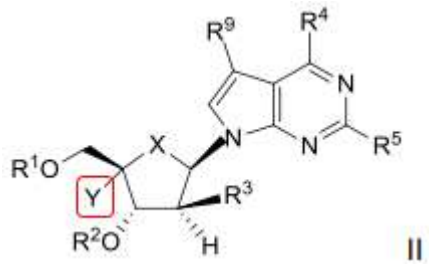
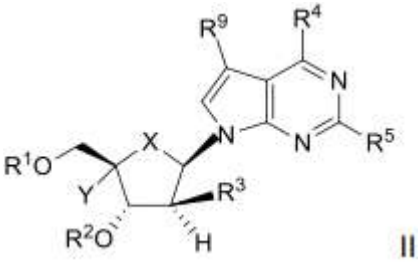
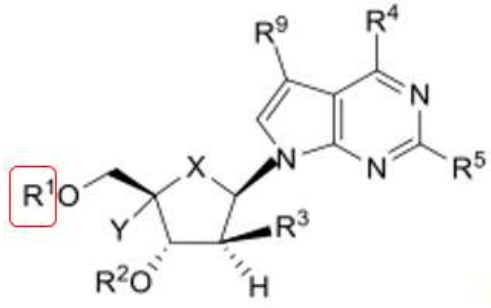
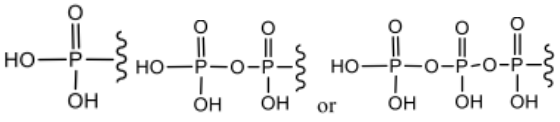
GROUND 1: Section 25(2)(b) - Lack of Novelty

20. It is submitted that the claims of the impugned patent lack novelty and are anticipated in view of US20100249068 (US'068), which was published on 30 Sep 2010. As detailed in the Affidavit of Dr. Debkumar Nandi, and as depicted and reproduced below, it can be seen that the compounds of the invention encompassed and represented in granted claim 1 lack novelty

US20100249068	**Claim 1**

	
B^2 is an optionally substituted **heterocyclic base** or a derivative thereof (para [0099] internal page 11)	 II
Various optionally substituted heterocyclic bases and optionally substituted heterocyclic base derivatives can be present in a compound of Formula (II). Examples of suitable optionally substituted heterocyclic bases (para[0108], internal page 15)  R^{A2} can be **hydrogen** or **halogen** R^{B2} can be **hydrogen** Y^2 can be CR^{F2}, wherein R^{F2} can be **hydrogen** (para [0108], internal page 16)	R^4 is -N(R^X)2, -NHC(O)OR^{6b}, -N(C(O)OR^{6b})2, -NHC(O)N(R^{6b})2, or -NHC(O)R^{6b} R^5 is -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -C_3-C_7 cycloalkyl, halo, -OR^X, -CN, -N(R^X)2-NHC(O)OR^{6b}, -N(C(O)OR^{6b})2, -NHC(O)N(R^{6b})2, or -NHC(O)R^{6b}; R^9 is -H, halo, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -CN, -OR^Y or -N(R^Y)2; R^X is independently selected at each occurrence from -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, aryl, or 5- or 6- membered monocyclic heteroaryl

D^2 is selected from the group consisting of C═CH_2, CH_2, **O (oxygen)**, S (sulfur), CHF, and CF_2; (para [0099] internal page 11)	 II ; **X** is O;
R^{27} can be **hydrogen** (para [0099] internal page 11) R^{26} and R^{25} can be **hydrogen** (para [0099] internal page 12)	 II **R3** is –H;
A^2 is selected from the group consisting of **C (carbon)**, O (oxygen) and S (sulfur) (para [0099] internal page 11)	 II
R^{23} and R^{24} can **independently** be **hydrogen** **—OR ^{a2}, —O—(C═O)R ^{a2}, —O—C(═O)OR ^{a2}, —O—C(═O)NR ^{a2}R ^{b2}** R ^{a2} and R ^{b2} can be **hydrogen** and can also be an optionally substituted **alkyl** (para [0099] internal page 12)	 II **R2** is -H, -C(O)R6a, -C(O)OR6a or -C(O)N(R6a)2; R^6, R^{6a} and R^{6b} are each independently selected at each occurrence from -H, -C_1-C_6 alkyl, or -C_1-C_6 haloalkyl

R^{22} can be an **optionally substituted C $_{2-6}$ alkynyl** (para [0099], internal page 11)	 II **Y** is $-C\equiv C-R^8$; R^8 is -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -C_3-C_7 cycloalkyl
R^{20} and R^{21} are independently selected from the group consisting of **hydrogen** (para [0099], internal page 11)	 II
R^{19} is selected from the group consisting of **hydrogen** (para [0099], internal page 11)  **diphosphate**, **triphosphate** or **derivatives** thereof; R^{28} and R^{29} can be **OH** (para [0100], internal page 12)	 II R^1 is -H, -C(O)R^6, -C(O)OR^6, -C(O)N(R^6)2,  or a pro-drug modification of the mono-, di- or triphosphate;

GROUND 2: Section 25(2)(e) - Lack of Inventive step

21. The Opponent submits that the claims of the impugned patent lack inventive step within the meaning of Section 2(1)(ja) read with Section 25(2)(e) of the Patents Act, 1970, in view of the prior art cited herein and the common general knowledge in the field of nucleoside medicinal chemistry prior to the earliest priority date of the impugned patent application i.e. prior to 28/03/2014.

22. At the relevant priority date, the development of nucleoside analogues as antiviral agents, particularly as inhibitors of HIV reverse transcriptase, was a well-established and mature field. It was understood that antiviral activity in such compounds arises from their ability to mimic natural nucleosides and interfere with viral DNA synthesis, and that this activity is governed by the structural interplay between the sugar moiety and the nucleobase. The optimisation of such compounds was routinely carried out through systematic structure–activity relationship (SAR) studies involving iterative modification of these structural components.

23. The prior art available at the priority date discloses nucleoside analogues bearing substitutions at the sugar moiety, including at the 4′-position, and demonstrates that such modifications can yield compounds with significant antiviral activity. The prior art further teaches that the nucleobase is a variable structural element that may be modified or replaced with structurally related heterocyclic systems in order to modulate biological properties. These teachings collectively provide a framework for the skilled person to explore combinations of known structural features to obtain additional antiviral compounds.

24. In this context, the skilled person seeking to develop additional nucleoside analogues with antiviral activity would consider retaining structural features known to confer activity and would explore modifications to other parts of the molecule using established medicinal chemistry strategies. Such an approach reflects routine and expected practice in the field and is guided by the teachings of the prior art and common general knowledge, rather than by any inventive insight.

25. The assessment of inventive step must therefore proceed by considering whether, in light of these teachings, the claimed subject matter represents more than the predictable

outcome of such routine modifications. The Opponent submits that, when properly analysed from this perspective, the claims do not involve any technical advance or non-obvious feature over the prior art and are therefore devoid of inventive step.

26. It is submitted that claims 1-25 of the impugned patent lack inventive step and are obvious in view of common general knowledge in art and combined with teachings of the following-

- Hiroshi Ohrui, *"2'-Deoxy-4'-C-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, is highly potent against all human immunodeficiency viruses type 1 and has low toxicity,"* The Chemical Record, Vol. 6, No. 3, 2006, pp. 133–143 (Annexure 3)
- EP2177527B1 published 18 December 2013 (Annexure 4)
- CA2502109 published 23 February 2010 (Annexure 5)
- Kawamoto et al. "*2'-Deoxy-4'-C-ethynyl-2-halo-adenosines active against drug-resistant human immunodeficiency virus type 1 variants*"; The International Journal of Biochemistry & Cell Biology 40 (2008) 2410–2420 (Annexure 6)
- Vittori et al. "*Antiviral Properties of Deazaadenine Nucleoside Derivatives*"; Current Medicinal Chemistry, 2006, 13, 3529-3552 (Annexure 7)
- Krawczyk et al "*Synthesis and Antiproliferative and Antiviral Activity of 2'-Deoxy-2'-fluoroarabinofuranosyl Analogs of the Nucleoside Antibiotics Toyocamycin and Sangivamycin*" *J. Med. Chem. 1996,38,* 4106-4114 (Annexure 8)

Hiroshi Ohrui, *"2'-Deoxy-4'-C-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, is highly potent against all human immunodeficiency viruses type 1 and has low toxicity,"* The Chemical Record, Vol. 6, No. 3, 2006, pp. 133–143

27. (Ohrui, 2006) discloses nucleoside analogues bearing substitution at the 4′-position of the sugar moiety, in particular 2′-deoxy-4′-C-ethynyl nucleosides, which are described as potent inhibitors of HIV reverse transcriptase. The compounds disclosed in A2 exhibit significant antiviral activity, including against resistant strains, and function by incorporating into viral DNA.

28. Ohrui is not limited to the disclosure of an isolated compound, but rather conveys a broader technical teaching, namely that substitution at the 4′-position of the sugar moiety can yield nucleoside analogues with substantial antiviral activity. This teaching situates the skilled person within a defined class of compounds—4′-substituted nucleosides—recognised as viable candidates for antiviral development.

29. At the relevant priority date, it was part of common general knowledge that the optimisation of nucleoside analogues proceeds through systematic variation of their structural components. Within this framework, the nucleobase was well understood as a variable element that could be modified or replaced with structurally related heterocyclic systems to influence biological properties. Such variations were routinely explored alongside modifications to the sugar moiety as part of standard medicinal chemistry practice.

30. Accordingly, when working within the class of nucleoside analogues disclosed therein, the skilled person would consider investigating structural variations within that class using established approaches. This includes examining different nucleobase structures in combination with sugar moieties already shown to support antiviral activity. The evaluation of such variations forms part of ordinary research activity in this field and does not depend on any specific direction beyond the general teachings of the prior art.

31. The assessment of inventive step in such circumstances does not turn on whether the prior art discloses each individual embodiment, but on whether the prior art provides a framework within which the skilled person would explore further compounds through routine modification. Where, as here, the prior art identifies a class of compounds exhibiting the desired activity and the common general knowledge provides established methods for varying their structural components, the development of further analogues within that class follows as a matter of routine investigation.

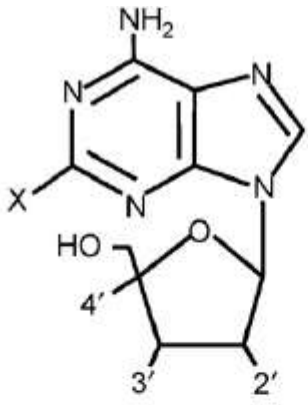
32. In this context, the presence of structural differences at the nucleobase level does not, by itself, establish inventiveness. The relevant inquiry is whether such variations would be contemplated by the skilled person in the ordinary course of research. Given the recognised flexibility of nucleobase selection in nucleoside chemistry and the demonstrated activity of compounds within the class disclosed in Annexure 2, the exploration of such variations falls within the expected scope of work in the field.

33. Further, in the absence of any evidence demonstrating that particular structural variations give rise to a technical effect beyond what would be expected from such routine investigation, no technical advance can be attributed to the claimed subject matter over the disclosure of Ohrui.

34. The teaching of Ohrui places the skilled person within a class of nucleoside analogues in which substitution at the 4′-position of the sugar moiety is associated with antiviral activity. The prior art, however, does not end with Ohrui. Additional disclosures available at the priority date further elaborate and reinforce the significance of this structural feature and the scope of variations that may be explored within this class. These documents confirm that the investigation of 4′-substituted nucleosides was not confined to isolated examples but formed part of a broader and actively developed area of medicinal chemistry.

Kawamoto et al. "*2'-Deoxy-4'-C-ethynyl-2-halo-adenosines active against drug-resistant human immunodeficiency virus type 1 variants*"; The International Journal of Biochemistry & Cell Biology 40 (2008) 2410–2420

35. Kawamoto et al. discloses that resistance (of HIV) to the drugs that form part of highly active antiretroviral therapy is a significant problem in treating this disease that necessitates development of newer compounds. To this end, the authors synthesized 4'-substituted adenosine analogs with halogen substitutions at their 2-position as shown below



Base X	Sugar 2′	Sugar 3′	4′	Compound abbreviation
-H	-H	-OH	-C ≡ CH	*E*dA
-F	-H	-OH	-C ≡ CH	2F-*E*dA
-F	-H	-H	-C ≡ CH	2F-*E*ddA
-F	C = C		-C ≡ CH	2F-*E*d4A
-F	-H	-OH	-C ≡ N	2F-CNdA
-Cl	-H	-OH	-C ≡ CH	2Cl-*E*dA

Fig. 1. Structures of 4′-substituted adenosine analogs. All nucleoside analogs discussed here have substitutions at the 4′-position of the sugar ring.

36. The results showed that the activity of 4'- and 2-substituted deoxyadenosine analogs against HIV-1 with the MTT assay using MT-4 cells was evaluated. It was reported that the 2'-deoxy-4'-C-ethynyl nucleoside with adenine as the base (EdA) exerted comparable activity to AZT.

37. Selectivity of EFdA and ECldA was much increased compared to parental EdA or AZT. However, EFdA was also relatively cytotoxic compared to other inhibitors of this series. The 2-chloro (Cl) substitution also provided enhanced activity but with a decreased toxicity.

38. Further modifications of the sugar ring from 2'-deoxyribose to 2',3'-dideoxy- or 2', 3'-didehydro-2',3'-dideoxy-ribose (EFddA or EFd4A) resulted in a drastic decrease of inhibitory potential. Substitution of the 4'-E group with a structurally similar 4'-cyano group also resulted in markedly decreased inhibitory activity. These results indicate that the 3'-OH and 4'-E moieties in the sugar ring are indispensable for high efficacy, and that antiviral activities are augmented by the modification with F- or Cl-moiety at the

adenine 2-position. Thus, a person skilled in the art would be motivated to retain the chloro-sbstituted analog due to its potency and the advantage of reduced toxicity and modify the adenine base as its activity is equivalent to AZT and there might be a scope for improvement by modifying the same.

39. The study shows that structural modifications are not confined to the sugar moiety alone, but may also be introduced at the nucleobase, including through the incorporation of substituents such as halogens. The resulting compounds retain antiviral activity, indicating that the nucleoside framework accommodates such variations while preserving biological function. This reflects an established approach in nucleoside medicinal chemistry, where both the sugar and base components are subject to modification in order to investigate and optimise activity.

40. In this context, Kawamoto et al. provide further evidence that nucleoside analogues bearing a 4′-ethynyl-modified sugar constitute a class of compounds within which structural variation at the nucleobase, including halogen substitution, may be explored. The disclosure therefore supports the broader understanding that antiviral nucleoside design involves the combined variation of multiple structural elements within a recognised chemical framework.

EP2177527 (EP'527)

41. The Opponent further relies on EP2177527 annexed as Annexure A3 ("EP'527") as highly relevant prior art for the assessment of inventive step. EP'527 discloses a class of 2′-fluoro-4′-substituted nucleoside derivatives and their use as antiviral agents, including for the treatment of HIV, HBV and HCV infections. In particular, EP'527 teaches that substitution at the 4′-position of the sugar moiety, including with groups such as ethynyl (–C≡CH), cyano, methyl, and azido, yields compounds with significant antiviral activity.

42. EP'527, therefore, establishes that 4′-substitution of nucleosides is a well-developed and actively explored strategy in antiviral drug design. The disclosure is not limited to a single compound but encompasses a broad genus of nucleoside analogues with variation in both the sugar and nucleobase portions, as well as multiple substituent options at the 4′-position. Importantly, the presence of 4′-ethynyl substituents is

explicitly contemplated within this prior art. Thus, the structural feature relied upon in the claimed invention is already disclosed as part of a broader and established class of antiviral nucleosides.

43. In view of EP'527, the skilled person is taught that antiviral activity can be achieved and optimised through systematic modification of nucleosides, including variation of both the 4′-substituent and the nucleobase. The document, therefore, provides a clear motivation to explore alternative nucleoside analogues within this framework. When read in combination with A1 (Ohrui), which identifies 4′-ethynyl nucleosides as particularly potent HIV reverse transcriptase inhibitors, EP'527 reinforces the understanding that 4′-ethynyl substitution is a known and promising design feature, and that further modifications, including to the nucleobase, fall within routine medicinal chemistry optimisation.

44. Accordingly, starting from either A2 and with insights from EP'527, the skilled person would have been led to retain the 4′-substituted sugar moiety, particularly the 4′-ethynyl group, and to explore variations in the nucleobase to obtain compounds with improved or alternative pharmacological properties. Such modifications, including the use of purine analogues and fused heterocyclic systems, are well-known in the art and form part of standard structure–activity relationship studies. The claimed compounds, which differ primarily in the selection of the nucleobase while retaining a known active sugar framework, therefore represent predictable variations within an already established class of compounds.

45. In the absence of any demonstrated technical effect that would not be reasonably expected by a skilled person in comparison to the compounds disclosed in EP'527, the claimed subject matter cannot be regarded as involving an inventive step. Instead, it represents an obvious selection from a known and limited set of alternatives disclosed in the prior art.

CA2502109 (CA'109)

46. The opponents submit CA2502109 with priority of 24/03/2005 annexed as A4, which discloses a class of nucleoside analogues characterised by substitution at the 4′-position of the sugar moiety, including compounds bearing ethynyl and cyano substituents. The

document teaches that such compounds exhibit potent anti-HIV activity, including activity against strains resistant to multiple antiviral agents, while maintaining low cytotoxicity.

47. The disclosure further indicates that these 4′-substituted nucleosides retain biological activity across structural variations and may also show enhanced resistance to enzymatic degradation, including deactivation by adenosine deaminase. This establishes that substitution at the 4′-position is not only compatible with antiviral activity but may also contribute to favourable pharmacological properties.

48. Importantly, CA2502109 presents the 4′-substituted sugar moiety as a general structural feature associated with antiviral efficacy, rather than as a property confined to a single compound or narrowly defined embodiment. The teaching of the document, therefore, situates 4′-substitution within a broader framework of nucleoside design, where structural variations may be explored within the same class of compounds using established medicinal chemistry approaches.

49. In this context, CA2502109 reinforces the understanding that nucleoside analogues incorporating 4′-substituted sugars constitute a recognised and viable class of antiviral agents, and that investigation of structural variations within this class forms part of routine research activity in the field.

50. The prior art discussed above does not consist of isolated disclosures, but forms a coherent and mutually reinforcing body of technical teaching relating to nucleoside analogues bearing substitution at the 4′-position of the sugar moiety.

51. Ohrui establishes that nucleoside analogues incorporating substitution at the 4′-position, in particular 4′-ethynyl substitution, exhibit significant antiviral activity and define a class of compounds suitable for further investigation. This teaching places the skilled person within a defined framework of 4′-substituted nucleosides as viable antiviral agents. Kawamoto et al. build on this framework by demonstrating that compounds in this class remain active even when additional structural variation is introduced at the nucleobase, including halogen substitution. The study shows that such variations may influence potency and toxicity while preserving antiviral activity, thereby confirming that the nucleoside scaffold tolerates modifications at multiple positions. This

reinforces the understanding that investigation within this class is not limited to fixed structures but involves systematic variation of both sugar and nucleobase components.

52. EP2177527B1 expands on this teaching by disclosing nucleoside derivatives bearing substitutions at both the 2′ and 4′ positions, thereby confirming that these positions are important sites for structural modification in antiviral nucleoside design. EP2177527 further expands this teaching by disclosing a broad genus of nucleoside analogues bearing a variety of substituents at the 4′-position, including ethynyl, cyano, methyl and azido groups, and confirms that such compounds exhibit antiviral activity. The document demonstrates that 4′-substitution is not confined to a specific substituent or compound but represents a general and versatile strategy in antiviral nucleoside design. It also indicates that variation across both the sugar moiety and nucleobase forms part of routine optimisation within this class. CA2502109 provides additional confirmation that nucleoside analogues bearing 4′-substituted sugars, including ethynyl and cyano groups, exhibit potent antiviral activity, including against drug-resistant strains, and may display favourable pharmacological properties such as reduced cytotoxicity and improved metabolic stability. This disclosure reinforces the understanding that the 4′-substituted sugar moiety constitutes a reliable and functionally significant feature, and that structural variations within this class may be explored to obtain compounds with desirable properties.

53. When these teachings are considered together, they provide the skilled person with a consistent and unified framework in which:

    - nucleoside analogues bearing 4′-substituted sugars are recognised as a viable and productive class of antiviral compounds;
    - such compounds retain activity across variations in substituents and nucleobase structure; and
    - further compounds within this class may be investigated through systematic modification of known structural elements using established medicinal chemistry approaches.

54. In this context, the skilled person, working within the framework defined by A2 and further elaborated by Kawamoto et al. (EP2177527 and CA2502109), would undertake routine investigations of structural variations within this class. This includes exploring different nucleobase structures in combination with sugar moieties already known to support antiviral activity. Such work represents ordinary and expected research activity in the field and does not require the application of any inventive faculty.

55. The assessment of inventive step in such circumstances is not determined by whether each individual embodiment is explicitly disclosed in a single document, but by whether the prior art provides a clear direction and expectation that such embodiments would arise from routine investigation. The combined teachings of the cited documents establish that this is indeed the case.

56. Further, no evidence has been provided to demonstrate that the claimed compounds exhibit any technical effect beyond what would be expected from such routine variation within the known class of 4′-substituted nucleoside analogues. In the absence of such evidence, the claimed subject matter represents the predictable and systematic modification within a known chemical framework and therefore lacks inventive step.

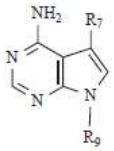
Vittori et al. "*Antiviral Properties of Deazaadenine Nucleoside Derivatives*"; Current Medicinal Chemistry, 2006, 13, 3529-3552

57. Vittori et al. discloses research conducted on deazaadenine antiviral analogues and derivatives. These were synthesized and tested on different viruses belonging to diverse virus families, although it is reported that most of the reported data have been produced in antiHIV, antiHCMV, and antiHSV biological testing.

58. It is disclosed that deaza analogues of natural purine nucleosides have been quite extensively investigated, such as 1-deazapurine, 3-deazapurine, 7-deazapurine, 1,3-dideazapurine, 1,7-dideazapurine, and 3,7-dideazapurine.

59. Vittori et al. provide several examples of 7-Deazaadenosine Analogues and their IC_{50} values which were tested on a plethora of viruses (Table 9-12, pages 3536-3542)

Table 9. Biological Properties of 7-Deazaadenosine Analogues; nt = Not Tested [77-79]



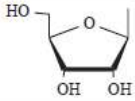
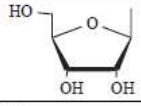
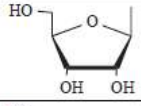
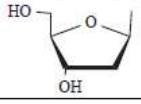
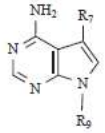
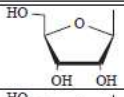
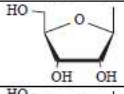
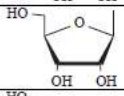
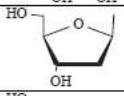
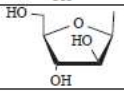
Cp	R_7	R_9	Virus inhib. (IC_{50}, μM)[a]		Ref.
			HCMV	HSV-1	
77 (Tubercidin)	H		0.5	nt	77
78 (Toyocamycin)	CN		0.05	nt	77
79 (Sangivamycin)			0.3	nt	77
80	CN		35	4.5[b]	78

Table 10. Biological Properties of 7-Deazaadenosine Analogues [80, 81]



Cp	R_7	R_9	Virus inhib. (MIC_{50}, μg/ml)[a]					
			HSV-1[b]	HSV-2[b]	VV[b]	VSV[b]	B-4[c]	polio-1[c]
77 (Tubercidin)	H		0.1	0.1	0.07	0.02	0.07	0.02
78 (Toyocamycin)	CN		>0.04	>0.04	≥0.04	>0.04	≥0.4	≥0.4
79 (Sangivamycin)			0.07	0.07	0.02	0.02	>0.4	0.07
110 (2'-De-tubercidin)	H		50	40	10	125	>400	150
111 (Ara-tubercidin)	H		125	40	40	>400	>400	>400

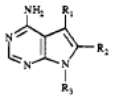
60. Thus, Vittori et al. teaches that 7-deazaadenosine is a very well-explored nucleoside base in antiviral research, and a person skilled in the art would not be dissuaded from substituting the same to get better antiviral activity.

Krawczyk et al "*Synthesis and Antiproliferative and Antiviral Activity of 2'-Deoxy-2'-fluoroarabinofuranosyl Analogs of the Nucleoside Antibiotics Toyocamycin and Sangivamycin*" *J. Med. Chem. 1996, 38,* 4106-4114

61. Krawczyk et al. discloses that work on sugar-modified analogues of the naturally-occurring compounds toyocamycin and sangivamycin by the authors as well as others' work on synthesizing analogues of these compounds for potential antiviral activity led to the present research. The authors synthesized 2'-deoxy-2'-substituted arabinofuranosyl analogs of toyocamycin and sangivamycin for evaluation of their antiviral activities.

62. The antiviral evaluations of these compounds showed that the 2'-deoxy and arabinofuranosyl derivatives of sangivamycin were the most active against human cytomegalovirus. The antiviral activity of the synthesized 2'-deoxy-2'-substituted arabinofuranosyl analogs is shown in Table 2 and Table 3

Table 2. Antiviral Activity and Cytotoxicity of 2′-Fluoroarabinosylpyrrolo[2,3-*d*]pyrimidine Analogs



				50% or 90% inhibitory concentration (μM)					
				antiviral activity[a]					
				HCMV		HIV-1[c]	HSV-1[b]	cytotoxicity[d]	
compd	R_1	R_2	R_3	plaque	yield	RT	ELISA	visual	growth
4a	CN	Br	α-D-2-F-arabinosyl	3.2	>100	32	>100[e]	55	>150
4b	CN	Br	β-D-2-F-arabinosyl	12	7	12	>100	21	144
9a	CN	H	α-D-2-F-arabinosyl	16	100	>100	46	100	>100
9b	CN	H	β-D-2-F-arabinosyl	1.0	1.1	12	6.5	6.8	8.8
10	$CONH_2$	H	β-D-2-F-arabinosyl	0.025	0.075	0.02	0.47	5.5	1.9
11	$CSNH_2$	H	β-D-2-F-arabinosyl	0.22	1.5	0.07	8.7	3.1	3
zidovudine[f]						0.011 ± 0.0008		>100	
ganciclovir[g]				7.4 ± 6.5	1.6 ± 1.2		3.5 ± 2.1	>100	>100

Table 3. Antiviral Activity of 2′-Deoxy-2′-fluoroarabinosylsangivamycin (**10**)

virus and cell line	50% inhibitory concentration[a] (μM)	
	antiviral	cytotoxicity
Antiviral Activity		
HSV-1	0.03	
HSV-2	0.03	
VZV	0.003	
HIV-1[b]	0.0037 ± 0.0017	
Cytotoxicity		
human foreskin fibroblasts		
stationary cells, neutral red uptake		6.7
stationary cells, MTT assay		0.13
proliferating cells, MTT assay		0.16
CEM-SS cells		
visual inspection		0.4
[^{3}H]dThd uptake[c]		0.193 ± 0.074

63. Thus, it is apparent that compounds that have 7-deazaadenosine base may represent a viable route to development of antiviral (including anti-HIV) compounds. The disclosure and teaching in Vittori et al. and Krawczyk et al. shows that this base can be substituted for bases in compounds such as those disclosed in Kawamoto et al. without expecting detriment to the their activity and might serve to enhance the same.

64. Vittori et al. report comprehensive research on deazaadenine nucleoside analogues, including 7-deaza derivatives, which have been extensively studied for antiviral activity across different virus families, including HIV. The study shows that replacing the purine base with related deaza-analogues is a well-established and widely investigated strategy in nucleoside chemistry, and such modifications are compatible with antiviral activity.

65. The breadth of compounds disclosed by Vittori et al., including multiple deazapurine variants, further indicates that the nucleobase is not a fixed structural element, but a flexible component subject to systematic variation. The reported biological data confirm that such modifications are routinely evaluated and may yield compounds with desirable antiviral properties.

66. Similarly, Krawczyk et al. disclose sugar-modified nucleoside analogues incorporating modified nucleobases, including toyocamycin and sangivamycin analogues, and demonstrate that these compounds exhibit antiviral activity. This work reinforces the understanding that simultaneous variation of both sugar and nucleobase components forms part of standard medicinal chemistry practice in the development of antiviral agents.

67. Taken together, these disclosures establish that the use of modified nucleobases, including deazaadenosine analogues, constitutes a recognised and routine approach in the design of antiviral nucleosides. In this context, the selection or substitution of such nucleobases within a known class of sugar-modified nucleosides falls within the ordinary skill of the art.

68. In view of the foregoing, the prior art offers a consistent and coherent teaching that nucleoside analogues with substitution at the 4′-position of the sugar moiety form a recognised class of antiviral compounds, and that structural variation within this class—including modification of the nucleobase—is routinely undertaken as part of medicinal chemistry practice. The cited documents collectively establish both the framework and the direction for such investigations, as well as a reasonable expectation that these modifications would produce active compounds.

69. The claimed subject matter does not introduce any new technical principle or unexpected technical effect over this established body of knowledge. Instead, it reflects the outcome of systematic and predictable variation within a known chemical space, guided by established structure–activity relationships and standard optimisation strategies. In the absence of any demonstrated technical advance or surprising effect, the claims amount to no more than a modest modification of known compounds.

70. Accordingly, the claimed invention lacks inventive step within the meaning of Section 2(1)(ja) of the Patents Act, 1970, and is liable to be revoked under Section 25(2)(e).

71. In view of the above submissions, impugned patent lacks inventive step and therefore, should be revoked on this ground alone.

GROUND 3: Claims not patentable under Section 25(2)(f)

The claimed subject matter in not patentable under Section 3(d) of the Act

72. It is submitted that the impugned patent application falls within the purview of section 3(d) of the Patents Act, 1970 which states that *"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***."

73. In the above definition, it is clear, that unless a claimed compound (a derivative) is shown to **differ significantly in properties with regard to efficacy, it shall be considered to be the same substance.**

74. From the above, it can be seen that the compound EClдA is close to the claimed compounds (especially MK-8527) so that the claimed compounds might correctly be considered 7-deazaadenosine derivatives of the same

75. The Patentee has not shown in-vivo therapeutic efficacy against such close compound. The Patentee has chosen only to demonstrate activity in comparison to AZT which is not structurally a close compound.

76. Even in the data provided in the specification where AZT is use as a control, many compounds falling within the scope of the claims do not show better activity than AZT For e.g. the following compounds in Table 3, representing IC_{50} (3, 5, 7, 12).

77. In any case, the Patentee has not demonstrated therapeutic efficacy in vivo against any close compound.

78. Therefore, the claimed invention attracts the provisions of section 3(d) of the Act and the patent ought to be revoked on this ground alone.

Invention not Patentable under Section 3(e)

79. Granted claim 25 of the impugned patent claims a composition of the compounds claimed in claim 1-16. It is submitted that the specification does not demonstrate any examples of a composition comprising any of the claimed compounds. Further, unless a composition demonstrates a synergy resulting from its components, or unexpected effects associated with the composition over and above that of the compounds themselves, it is liable to be considered an admixture. In the present case any synergy or unexpected effect has not been demonstrated in the specification. Further, the claimed compounds have no effect over and above the effects shown by compounds already known in the art, the compositions of the claimed compounds also lack any unexpected (synergistic) effect. The Patentee has failed to establish any unexpected effect of the composition comprising claimed compounds

80. Thus, claim 25 of the impugned patent falls squarely within the ambit of section 3(e) of the Act, and ought to be rejected on this ground alone.

GROUND 3: Insufficiency of Disclosure [section 25(2)(g)]

81. It is submitted that claim 1 encompasses a large number of compounds based on the substituents and the number of possible options for those substituents. However, the specification shows the synthesis of only a small number of compounds, which does not enable person skilled in the art to attempt synthesis of each and every claimed compound.

82. Claim 25, although directed towards a composition, is not only inordinately broad (pharmaceutically acceptable carrier with any claimed compound), there is no example of any composition whatsoever in the specification.

83. Thus, the specification does not sufficiently and clearly describe the invention or the method by which it is to be performed and is liable to be rejected on said ground.

Ground 5: Information Relating To Corresponding Applications Under Section 8 [Section 25(2)(h)]

84. The Applicant has failed to disclose to the Patent Office the information required under Section 8. The Applicant is required to provide all the information regarding the prosecution of the equivalent applications till the grant of the Indian application to the Patent Office in writing from time to time and also within the prescribed time.

85. It is observed that Applicant has not provided information about updated the status of corresponding application in the Form-3 which information has not been provided to the learned Controller.

86. Therefore, the applicant has failed to comply with the requirements of the section 8 of the act and the opponent demands rejection on this ground also.

87. It is submitted that the Applicant has failed to disclose the details of corresponding foreign applications and impugned patent application to be refused.

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

CONCLUSION

89. In view of the above, the claims are not inventive and not patentable and insufficient. The post-grant opposition as filed may be allowed and the subject patent may be revoked.

HEARING REQUESTED

90. The Opponent hereby requests a hearing under section 25(2) of the Patents Act, 1970 (hereinafter referred to as “the Patents Act”) and Rule 55 of the Patents Rules (hereinafter referred to as “the Rules”).

P R A Y E R

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

i. that the Controller take the present Opposition on record; that the Indian Patent number 564721 (formerly patent application number 201617031899), be revoked under Section 25(2) of the Patents (Amendment) Act, 2005;

ii. that the Opponent may be allowed to file further documents and evidence if necessary to support their averments;

iii. that the Opponent may be allowed to file rejoinder and affidavit if necessary to support their averments;

iv. that the Opponent may be granted an opportunity of being heard in the matter before any final orders are passed;

v. that the Opponent may be allowed to make further submissions in case the Patentee makes any amendments in the claims;

vi. any other reliefs considering the facts and circumstances may be granted in favour of the Opponent in the interest of justice.

Dated this 02nd day of April, 2026

MARIAM SADAF (IN /PA – 5231)
OF RAJESHWARI AND ASSOCIATES
AGENT FOR THE OPPONENT

TO
THE CONTROLLER OF PATENTS
THE PATENT OFFICE, NEW DELHI

Indian Patent Application No. 201617031899

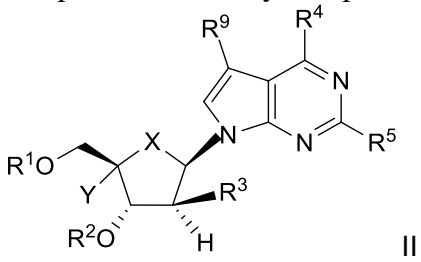
Clean Claims

October 11, 2023

We Claim:

1. A compound

or a pharmaceutically acceptable salt thereof, which has structural formula II:



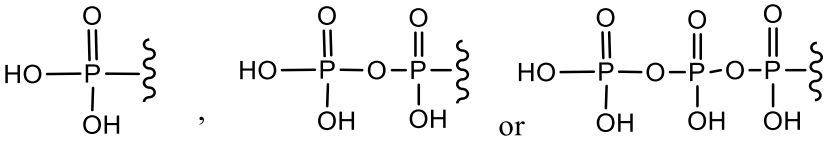
II

;

X is O;

Y is –C≡C-R^8;

R^1 is -H, -C(O)R^6, -C(O)OR^6, -C(O)N(R^6)$_2$,



or a pro-drug modification of the mono-, di- or triphosphate;

R^2 is -H, -C(O)R^{6a}, -C(O)OR^{6a} or -C(O)N(R^{6a})$_2$;

R^3 is –H;

R^4 is -N(R^X)$_2$, -NHC(O)OR^{6b},
-N(C(O)OR^{6b})$_2$, -NHC(O)N(R^{6b})$_2$, or -NHC(O)R^{6b};

R^5 is -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -C_3-C_7 cycloalkyl, halo,
-OR^X, -CN, -N(R^X)$_2$-NHC(O)OR^{6b}, -N(C(O)OR^{6b})$_2$, -NHC(O)N(R^{6b})$_2$, or -NHC(O)R^{6b};

R^6, **R^{6a}** and **R^{6b}** are each independently selected at each occurrence from -H, -C_1-C_6 alkyl,
or -C_1-C_6 haloalkyl;

R^8 is -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -C_3-C_7 cycloalkyl;

R^9 is -H, halo, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -CN, -OR^Y or -N(R^Y)$_2$;

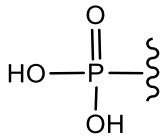
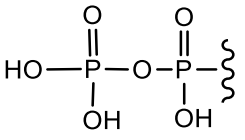
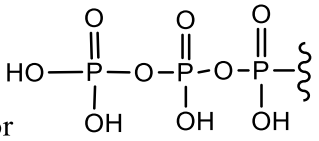
R^X is independently selected at each occurrence from -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl,
aryl, or 5- or 6- membered monocyclic heteroaryl;

or when either or both of **R^4** or **R^5** is -N(R^X)$_2$, each R^X may optionally be joined together with
the nitrogen to which they are both attached to form a 5- or 6-membered monocyclic
heteroaryl or 9- or 10-membered bicyclic heteroaryl; and

R^Y is -H, -C_1-C_6 alkyl or -C_1-C_6 haloalkyl.

2. The compound as claimed in claim 1, or or a pharmaceutically acceptable salt thereof, wherein:

R1 is –H,


, 
or 
; and

R4 is -N(R^X)$_2$.

3. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein **R2** is -H.

4. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof wherein **R4** is -N(R^X)$_2$.

5. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein **R5** is –H, halo, -C_1-C_6 alkyl, -OR^X, or -N(R^X)$_2$.

6. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein **R8** is–H.

7. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein **R9** is –H, halo, -C_1-C_3 alkyl, -C_1-C_3 haloalkyl, -CN, -OR^Y or -N(R^Y)$_2$.

8. The compound as claimed in claim 1 to 7 or a pharmaceutically acceptable salt thereof, wherein

R^X is -H, -C_1-C_6 alkyl or -C_1-C_6 haloalkyl.

9. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein:

R5 is –H, halo, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -CN, -OR^X, -N(R^X)$_2$, -NHC(O)OR^{6b}, -N(C(O)OR^{6b})$_2$, -NHC(O)N(R^{6b})$_2$, or -NHC(O)R^{6b}; and

R9 is –H, halo, -C_1-C_3 alkyl, -C_1-C_3 haloalkyl, -CN, -OR^Y or -N(R^Y)$_2$.

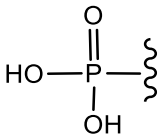
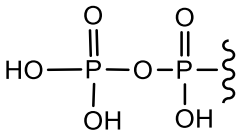
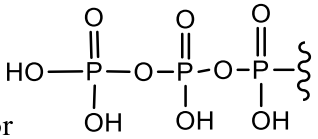
10. The compound as claimed in claim 9 or a pharmaceutically acceptable salt thereof wherein **R4** is -N(R^X)$_2$.

11. The compound as claimed in claim 9 or a pharmaceutically acceptable salt thereof wherein **R1** is –H and **R4** is -N(R^X)$_2$.

12. The compound as claimed in claim 1 or a pharmaceutically acceptable salt thereof, wherein:

Y is –C≡CH;

R1 is -H, -C(O)R^6, -C(O)OR^6, -C(O)N(R^6)$_2$,


, 
or 
;

R2 is H, -C(O)R^{6a}, -C(O)OR^{6a} or -C(O)N(R^{6a})$_2$;

R3 is –H;

R4 is -N(R^X)$_2$, -NHC(O)OR^{6b} or -NHC(O)N(R^{6b})$_2$;

R5 is –H, halo, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -CN, -OR^X or -N(R^X)$_2$;

R6, **R6a**, and **R6b** are each independently selected at each occurrence from -H, -C_1-C_6 alkyl, or -C_1-C_6 haloalkyl;

R9 is –H, halo, -C_1-C_3 alkyl, -C_1-C_3 haloalkyl, -CN, -OR^Y, or -N(R^Y)$_2$;

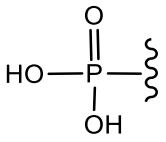
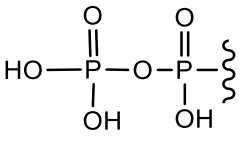
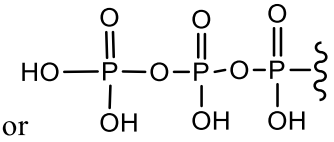
R^X is independently selected at each occurrence from -H, -C_1-C_6 alkyl, or -C_1-C_6 haloalkyl; and

R^Y is independently selected at each occurrence from -H, -C_1-C_6 alkyl or -C_1-C_6 haloalkyl.

13. The compound as claimed in claim 1, or a pharmaceutically acceptable salt thereof, wherein:

Y is -C≡CH;

R1 is -H,


, 
or 
;

R2 is H;

R3 is -H;

R4 is NH_2;

R5 is –H, halo or -NH_2; and

R^9 is –H, halo or -CH_3.

14. The compound as claimed in claims 1 or a pharmaceutically acceptable salt thereof, wherein one or more of R^1, R^2, R^4 or R^5 is selected as follows:

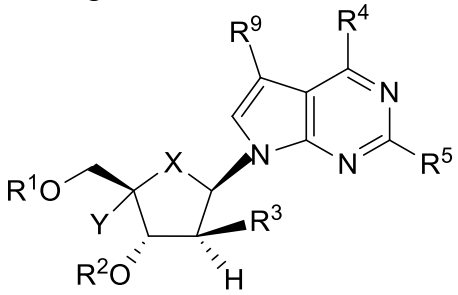
R^1 is -$C(O)R^6$, -$C(O)OR^6$, -$C(O)N(R^6)_2$; and/or

R^2 is -$C(O)R^{6a}$, -$C(O)OR^{6a}$ or -$C(O)N(R^{6a})_2$; and/or

R^4 is -$NHC(O)OR^{6b}$, -$N(C(O)OR^{6b})_2$, -$NHC(O)N(R^{6b})_2$, or -$NHC(O)R^{6b}$; and/or

R^5 is -$NHC(O)OR^{6b}$, -$N(C(O)OR^{6b})_2$, -$NHC(O)N(R^{6b})_2$, or -$NHC(O)R^{6b}$.

15. The compound as claimed in claim **1** or a pharmaceutically acceptable salt thereof, wherein the compound having structural Formula II,



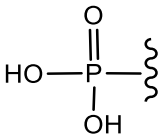
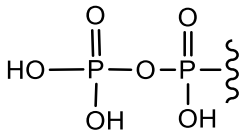
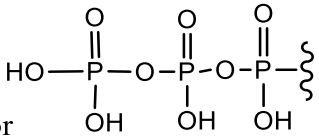
II,

or a pharmaceutically acceptable salt thereof, wherein:

X is O;

Y is –C≡CH;

R^1 is –H,


, 
or 
;

R^2 is –H;

R^3 is –H;

R^4 is –NH_2;

R^5 is –H, halo or -NH_2; and

R^9 is –H, halo or –CH_3.

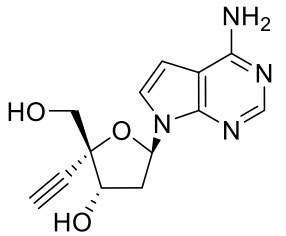
16. The compound as claimed in claim 1 that is:

1)	(2*R*,3*S*,5*R*)-5-(4-amino-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
2)	(2*R*,3*S*,5*R*)-5-(4-amino-5-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;

3)	(2*R*,3*S*,5*R*)-5-(4-amino-5-methyl-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
4)	(2*R*,3*S*,5*R*)-5-(4-amino-2-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
5)	(2*R*,3*S*,5*R*)-5-(4-amino-5-bromo-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
6)	(2*R*,3*S*,5*R*)-5-(4-amino-5-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
7)	(2*R*,3*S*,5*R*)-5-(4-amino-5-iodo-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
8)	(2*R*,3*S*,5*R*)-5-(2,4-diamino-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
9)	(2*R*,3*S*,5*R*)-5-(4-amino-2-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
10)	((2*R*,3*S*,5*R*)-5-(4-amino-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
11)	((2*R*,3*S*,5*R*)-5-(4-amino-5-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
12)	((2*R*,3*S*,5*R*)-5-(4-amino-5-methyl-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
13)	((2*R*,3*S*,5*R*)-5-(4-amino-2-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
14)	((2*R*,3*S*,5*R*)-5-(4-amino-5-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;

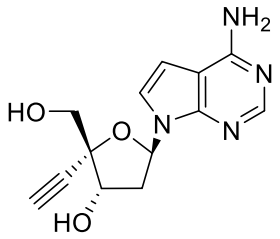
or a pharmaceutically acceptable salt thereof

17. The compound as claimed in claim 1 that is:



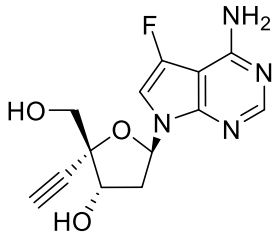
.

18. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



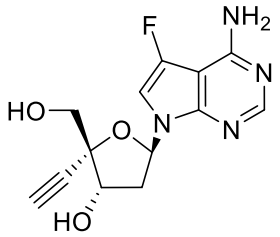
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19. The compound as claimed in claim 1 that is:



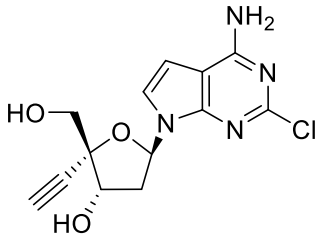
.

20. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



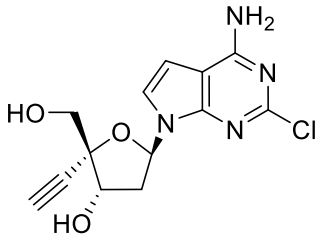
.

21. The compound as claimed in claim 1 that is:



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22. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:

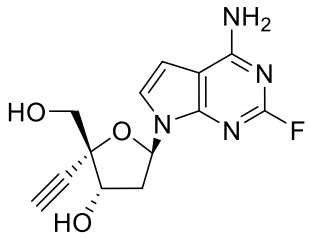


.

23. The compound as claimed in claim 1 that is:

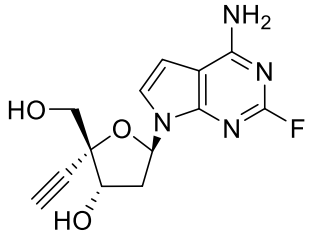
Clean Claims

October 11, 2023



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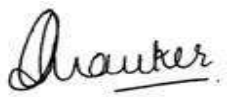
24. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



.

25. A pharmaceutical composition comprising a compound as claimed in any one of claims 1 to 16, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier and optionally comprising at least one anti-HIV agent selected from an HIV antiviral agent, an immunomodulator, and an anti-infective agent such as herein described.

Dated this 19th day of September, 2016



Archana Shanker

IN/PA 149

Of Anand And Anand Advocates

Agents for the applicant



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(54) **SUBSTITUTED NUCLEOSIDE AND NUCLEOTIDE ANALOGS**

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A61P 33/02	(2006.01)

(52) **U.S. Cl.** **514/81**; 544/317; 514/274; 544/243; 514/86; 544/277; 514/263.23; 544/244

(57) **ABSTRACT**

Disclosed herein are nucleotide analogs with protected phosphates, methods of synthesizing nucleotide analogs with protected phosphates and methods of treating diseases and/or conditions such as viral infections, cancer, and/or parasitic diseases with the nucleotide analogs with protected phosphates.

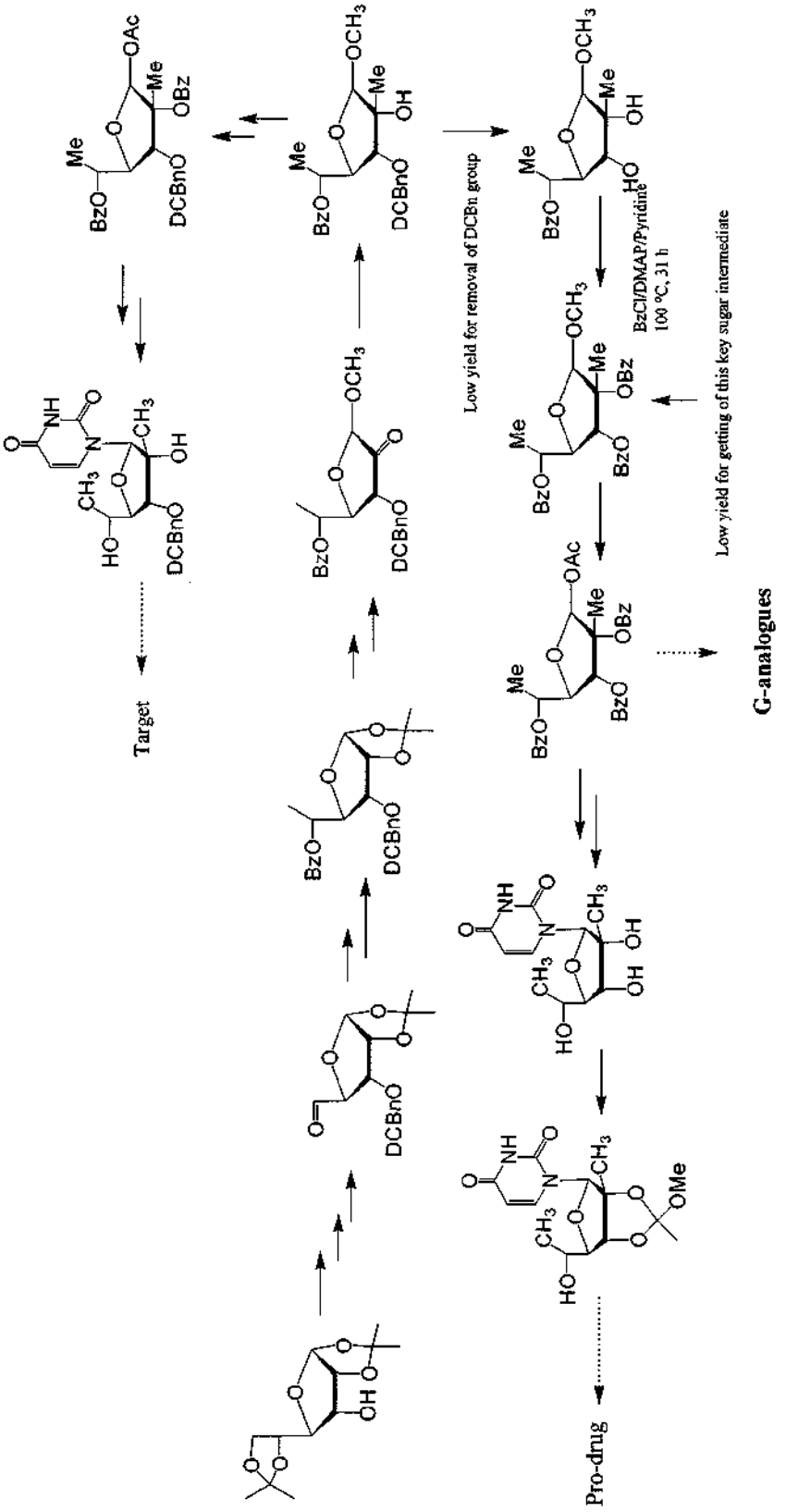

Target
Low yield for removal of DCBn group
BzCl/DMAP/Pyridine
100 °C, 31 h
Low yield for getting of this key sugar intermediate
G-analogues
Pro-drug

Figure 1

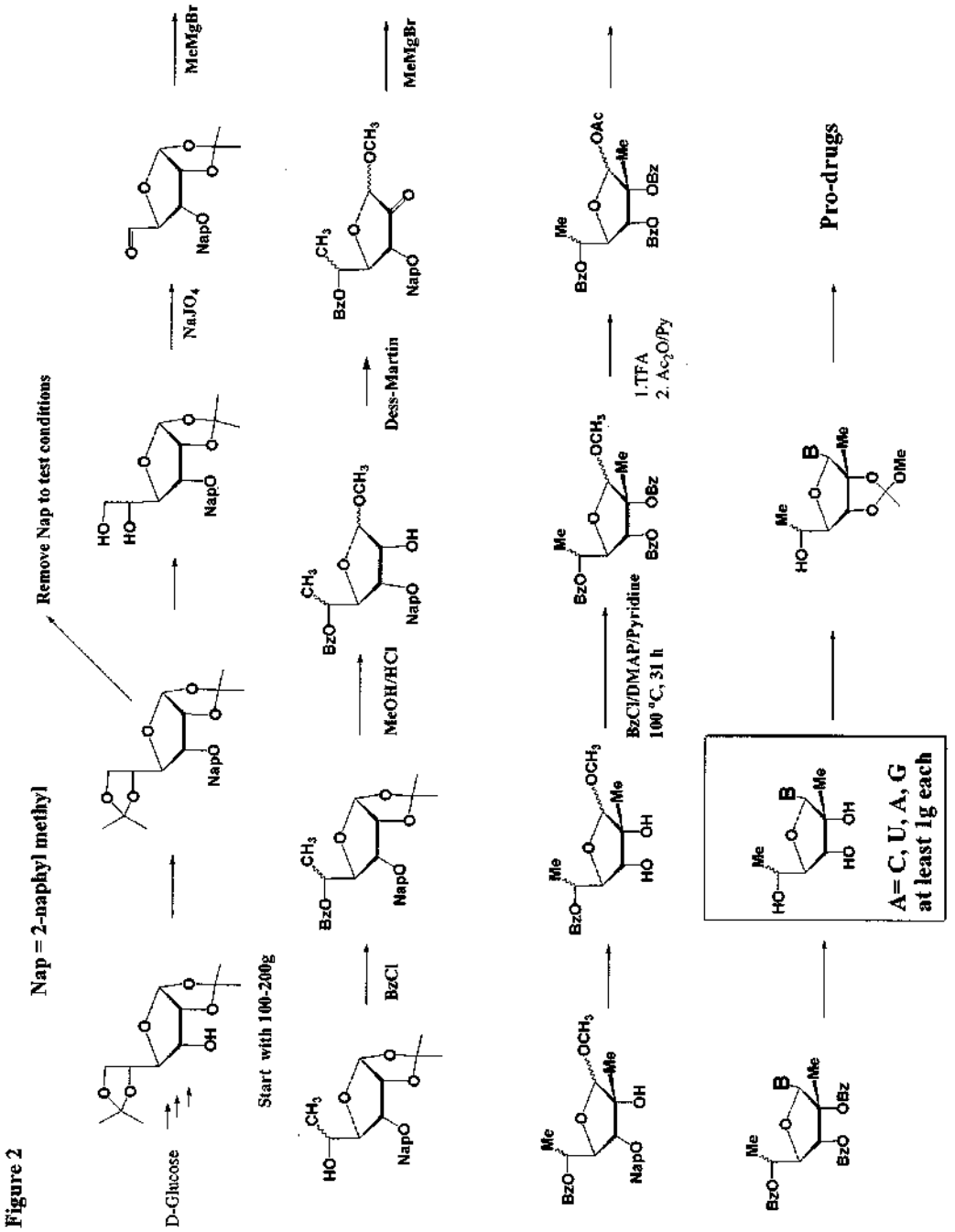
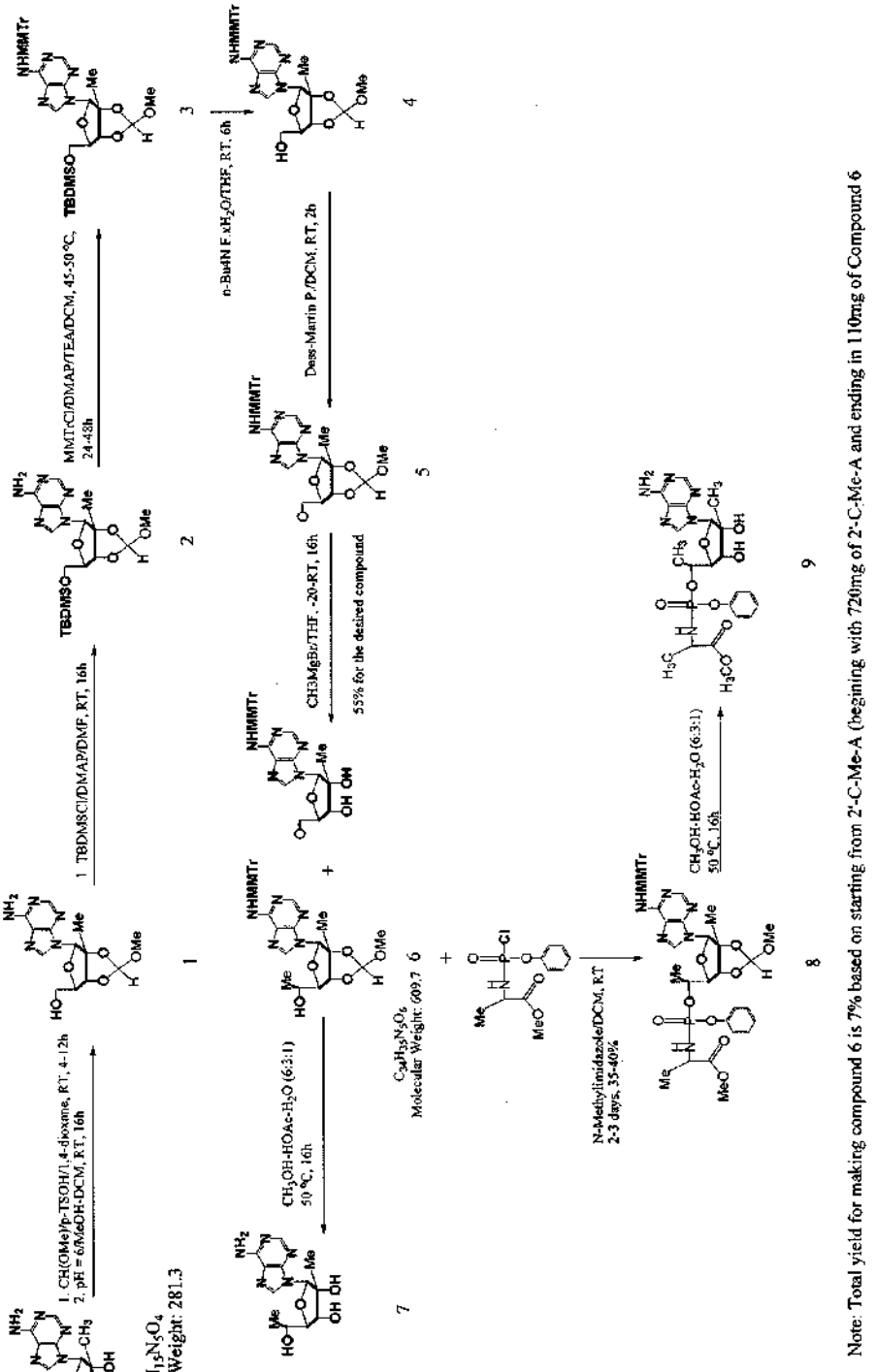

Nap = 2-naphyl methyl
Remove Nap to test conditions
D-Glucose
Start with 100-200g
BzCl
MeOH/HCl
Dess-Martin
MeMgBr
NaJO4
BzCl/DMAP/Pyridine
100 °C, 31 h
1.TFA
2. Ac2O/Py
A= C, U, A, G
at least 1g each
Pro-drugs

Figure 2

Figure 3



Note: Total yield for making compound 6 is 7% based on starting from 2'-C-Me-A (begining with 720mg of 2'-C-Me-A and ending in 110mg of Compound 6

SUBSTITUTED NUCLEOSIDE AND NUCLEOTIDE ANALOGS

BACKGROUND

[0001] 1. Field

[0002] The present application relates to the fields of chemistry, biochemistry and medicine. More particularly, disclosed herein are nucleotide analogs with protected phosphates, pharmaceutical compositions that include one or more nucleotide analogs with protected phosphates and methods of synthesizing the same. Also disclosed herein are methods of treating diseases and/or conditions with the nucleotide analogs with protected phosphates.

[0003] 2. Description of the Related Art

[0004] Nucleoside analogs are a class of compounds that have been shown to exert antiviral and anticancer activity both in vitro and in vivo, and thus, have been the subject of widespread research for the treatment of viral infections and cancer. Nucleoside analogs are therapeutically inactive compounds that are converted by host or viral enzymes to their respective active anti-metabolites, which, in turn, inhibit polymerases involved in viral or cell proliferation. The activation occurs by a variety of mechanisms, such as the addition of one or more phosphate groups and, or in combination with, other metabolic processes.

SUMMARY

[0005] An embodiment disclosed herein relates to a compound of Formula (I), or a pharmaceutically acceptable salt, prodrug or prodrug ester thereof.

[0006] Another embodiment disclosed herein relates to a compound of Formula (II), or a pharmaceutically acceptable salt, prodrug or prodrug ester thereof.

[0007] Some embodiments disclosed herein relate to methods of synthesizing a compound of Formula (I).

[0008] Other embodiments disclosed herein relate to methods of synthesizing a compound of Formula (II).

[0009] An embodiment disclosed herein relates to pharmaceutical compositions that can include one or more compounds of Formulae (I) and (II), or a pharmaceutically acceptable carrier, diluent, excipient or combination thereof. The pharmaceutical compositions of the compounds of Formulae (I) and (II) can be used in the manufacture of a medicament for treating an individual suffering from a neoplastic disease, a viral infection, or a parasitic disease. The pharmaceutical compositions of the compounds of Formulae (I) and (II) can be used for treating a neoplastic disease, a viral infection, or a parasitic disease.

[0010] Some embodiments disclosed herein relate to methods of ameliorating or treating a neoplastic disease that can include administering to a subject suffering from the neoplastic disease a therapeutically effective amount of one or more compounds of Formulae (I) and (II), or a pharmaceutical composition that includes one or more compounds of Formulae (I) and (II). The compounds of Formulae (I) and (II) can be used in the manufacture of a medicament for treating an individual suffering from a neoplastic disease. The compounds of Formulae (I) and (II) can be used for treating a neoplastic disease.

[0011] Other embodiments disclosed herein relate to methods of inhibiting the growth of a tumor that can include administering to a subject having a tumor a therapeutically effective amount of one or more compounds of Formulae (I) and (II), or a pharmaceutical composition that includes one or more compounds of Formulae (I) and (II).

[0012] Still other embodiments disclosed herein relate to methods of ameliorating or treating a viral infection that can include administering to a subject suffering from the viral infection a therapeutically effective amount of one or more compounds of Formulae (I) and (II), or a pharmaceutical composition that includes one or more compounds of Formulae (I) and (II). The compounds of Formulae (I) and (II) can be used in the manufacture of a medicament for treating an individual suffering from a viral infection. The compounds of Formulae (I) and (II) can be used for treating a viral infection.

[0013] Yet still other embodiments disclosed herein relate to methods of ameliorating or treating a parasitic disease that can include administering to a subject suffering from the parasitic disease a therapeutically effective amount of one or more compounds of Formulae (I) and (II), or a pharmaceutical composition that includes one or more compounds of Formulae (I) and (II). The compounds of Formulae (I) and (II) can be used in the manufacture of a medicament for treating an individual suffering from a parasitic disease. The compounds of Formulae (I) and (II) can be used for treating a parasitic disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. **1** shows one method for preparing 2',5'-dimethyl nucleosides and nucleotides in which the base is uracil or guanine.

[0015] FIG. **2** shows one method for preparing 2',5'-dimethyl nucleosides and nucleotides in which the base is cytosine, uracil, adenine or guanine.

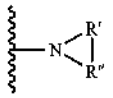
[0016] FIG. **3** shows one method for preparing 2',5'-dimethyl-adenosine phosphoramidate.

DETAILED DESCRIPTION

[0017] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. All patents, applications, published applications and other publications referenced herein are incorporated by reference in their entirety unless stated otherwise. In the event that there are a plurality of definitions for a term herein, those in this section prevail unless stated otherwise.

[0018] As used herein, any "R" group(s) such as, without limitation, R^1, R^{1a} and R^{1b}, represent substituents that can be attached to the indicated atom. A non-limiting list of R groups include, but are not limited to, hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl, heteroalicyclyl, aralkyl, heteroaralkyl, (heteroalicyclyl)alkyl, hydroxy, protected hydroxy, alkoxy, aryloxy, acyl, ester, mercapto, cyano, halogen, thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, S-sulfonamido, N-sulfonamido, C-carboxy, protected C-carboxy, O-carboxy, isocyanato, thiocyanato, isothiocyanato, nitro, silyl, sulfenyl, sulfinyl, sulfonyl, haloalkyl, haloalkoxy, trihalomethanesulfonyl, trihalomethanesulfonamido, and amino, including mono- and di-substituted amino groups, and the protected derivatives thereof. An R group may be substituted or unsubstituted. If two "R" groups are covalently bonded to the same atom or to adjacent atoms, then they may be "taken together" as defined herein to form a cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl or heteroalicyclyl group. For example, without limi-

tation, if R' and R" of an NR'R" group are indicated to be "taken together", it means that they are covalently bonded to one another at their terminal atoms to form a ring that includes the nitrogen:



[0019] Whenever a group is described as being "optionally substituted" that group may be unsubstituted or substituted with one or more of the indicated substituents. Likewise, when a group is described as being "unsubstituted or substituted" if substituted, the substituent may be selected from one or more the indicated substituents. If no substituents are indicated, it is meant that the indicated "optionally substituted" or "substituted" group may be substituted with one or more group(s) individually and independently selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl, heteroalicyclyl, aralkyl, heteroaralkyl, (heteroalicyclyl)alkyl, hydroxy, protected hydroxyl, alkoxy, aryloxy, acyl, ester, mercapto, alkylthio, arylthio, cyano, halogen, thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, S-sulfonamido, N-sulfonamido, C-carboxy, protected C-carboxy, O-carboxy, isocyanato, thiocyanato, isothiocyanato, nitro, silyl, sulfenyl, sulfinyl, sulfonyl, haloalkyl, haloalkoxy, trihalomethanesulfonyl, trihalomethanesulfonamido, and amino, including mono- and di-substituted amino groups, and the protected derivatives thereof. Each of these substituents can be further substituted.

[0020] As used herein, "C_a to C_b" in which "a" and "b" are integers refer to the number of carbon atoms in an alkyl, alkenyl or alkynyl group, or the number of carbon atoms in the ring of a cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl or heteroalicyclyl group. That is, the alkyl, alkenyl, alkynyl, ring of the cycloalkyl, ring of the cycloalkenyl, ring of the cycloalkynyl, ring of the aryl, ring of the heteroaryl or ring of the heteroalicyclyl can contain from "a" to "b", inclusive, carbon atoms. Thus, for example, a "C_1 to C_4 alkyl" group refers to all alkyl groups having from 1 to 4 carbons, that is, CH_3—, CH_3CH_2—, $CH_3CH_2CH_2$, $(CH_3)_2CH$—, $CH_3CH_2CH_2CH_2$—, $CH_3CH_2CH(CH_3)$— and $(CH_3)_3C$—. If no "a" and "b" are designated with regard to an alkyl, alkenyl, alkynyl, cycloalkyl cycloalkenyl, cycloalkynyl, aryl, heteroaryl or heteroalicyclyl group, the broadest range described in these definitions is to be assumed.

[0021] As used herein, "alkyl" refers to a straight or branched hydrocarbon chain that comprises a fully saturated (no double or triple bonds) hydrocarbon group. The alkyl group may have 1 to 20 carbon atoms (whenever it appears herein, a numerical range such as "1 to 20" refers to each integer in the given range; e.g., "1 to 20 carbon atoms" means that the alkyl group may consist of 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms, although the present definition also covers the occurrence of the term "alkyl" where no numerical range is designated). The alkyl group may also be a medium size alkyl having 1 to 10 carbon atoms. The alkyl group could also be a lower alkyl having 1 to 6 carbon atoms. The alkyl group of the compounds may be designated as "C_1-C_6 alkyl" or similar designations. By way of example only, "C_1-C_4 alkyl" indicates that there are one to four carbon atoms in the alkyl chain, i.e., the alkyl chain is selected from methyl, ethyl, propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, and t-butyl. By way of example only, "C_1-C_6 alkyl" indicates that there are one to six carbon atoms in the alkyl chain. Typical alkyl groups include, but are in no way limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tertiary butyl, pentyl, hexyl, and the like. The alkyl group may be substituted or unsubstituted.

[0022] As used herein, "alkenyl" refers to an alkyl group that contains in the straight or branched hydrocarbon chain one or more double bonds. An alkenyl group may be unsubstituted or substituted.

[0023] As used herein, "alkynyl" refers to an alkyl group that contains in the straight or branched hydrocarbon chain one or more triple bonds. An alkynyl group may be unsubstituted or substituted.

[0024] As used herein, "cycloalkyl" refers to a completely saturated (no double or triple bonds) mono- or multi-cyclic hydrocarbon ring system. When composed of two or more rings, the rings may be joined together in a fused fashion. Cycloalkyl groups can contain 3 to 10 atoms in the ring(s) or 3 to 8 atoms in the ring(s). A cycloalkyl group may be unsubstituted or substituted. Typical cycloalkyl groups include, but are in no way limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like.

[0025] As used herein, "cycloalkenyl" refers to a mono- or multi-cyclic hydrocarbon ring system that contains one or more double bonds in at least one ring; although, if there is more than one, the double bonds cannot form a fully delocalized pi-electron system throughout all the rings (otherwise the group would be "aryl," as defined herein). When composed of two or more rings, the rings may be connected together in a fused fashion. A cycloalkenyl group may be unsubstituted or substituted.

[0026] As used herein, "cycloalkynyl" refers to a mono- or multi-cyclic hydrocarbon ring system that contains one or more triple bonds in at least one ring. If there is more than one triple bond, the triple bonds cannot form a fully delocalized pi-electron system throughout all the rings. When composed of two or more rings, the rings may be joined together in a fused fashion. A cycloalkynyl group may be unsubstituted or substituted.

[0027] As used herein, "aryl" refers to a carbocyclic (all carbon) monocyclic or multicyclic aromatic ring system (including fused ring systems where two carbocyclic rings share a chemical bond) that has a fully delocalized pi-electron system throughout all the rings. The number of carbon atoms in an aryl group can vary. For example, the aryl group can be a C_6-C_{14} aryl group, a C_6-C_{10} aryl group, or a C_6 aryl group. Examples of aryl groups include, but are not limited to, benzene, naphthalene and azulene. An aryl group may be substituted or unsubstituted.

[0028] As used herein, "heteroaryl" refers to a monocyclic or multicyclic aromatic ring system (a ring system with fully delocalized pi-electron system) that contain(s) one or more heteroatoms, that is, an element other than carbon, including but not limited to, nitrogen, oxygen and sulfur. The number of atoms in the ring(s) of a heteroaryl group can vary. For example, the heteroaryl group can contain 4 to 14 atoms in the ring(s), 5 to 10 atoms in the ring(s) or 5 to 6 atoms in the ring(s). Furthermore, the term "heteroaryl" includes fused ring systems where two rings, such as at least one aryl ring and at least one heteroaryl ring, or at least two heteroaryl rings, share at least one chemical bond. Examples of het-

eroaryl rings include, but are not limited to, furan, furazan, thiophene, benzothiophene, phthalazine, pyrrole, oxazole, benzoxazole, 1,2,3-oxadiazole, 1,2,4-oxadiazole, thiazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, benzothiazole, imidazole, benzimidazole, indole, indazole, pyrazole, benzopyrazole, isoxazole, benzoisoxazole, isothiazole, triazole, benzotriazole, thiadiazole, tetrazole, pyridine, pyridazine, pyrimidine, pyrazine, purine, pteridine, quinoline, isoquinoline, quinazoline, quinoxaline, cinnoline, and triazine. A heteroaryl group may be substituted or unsubstituted.

[0029] As used herein, "heteroalicyclic" or "heteroalicyclyl" refers to three-, four-, five-, six-, seven-, eight-, nine-, ten-, up to 18-membered monocyclic, bicyclic, and tricyclic ring system wherein carbon atoms together with from 1 to 5 heteroatoms constitute said ring system. A heterocycle may optionally contain one or more unsaturated bonds situated in such a way, however, that a fully delocalized pi-electron system does not occur throughout all the rings. The heteroatoms are independently selected from oxygen, sulfur, and nitrogen. A heterocycle may further contain one or more carbonyl or thiocarbonyl functionalities, so as to make the definition include oxo-systems and thio-systems such as lactams, lactones, cyclic imides, cyclic thioimides, cyclic carbamates, and the like. When composed of two or more rings, the rings may be joined together in a fused fashion. Additionally, any nitrogens in a heteroalicyclic may be quaternized. Heteroalicyclyl or heteroalicyclic groups may be unsubstituted or substituted. Examples of such "heteroalicyclic" or "heteroalicyclyl" groups include but are not limited to, 1,3-dioxin, 1,3-dioxane, 1,4-dioxane, 1,2-dioxolane, 1,3-dioxolane, 1,4-dioxolane, 1,3-oxathiane, 1,4-oxathiin, 1,3-oxathiolane, 1,3-dithiole, 1,3-dithiolane, 1,4-oxathiane, tetrahydro-1,4-thiazine, 2H-1,2-oxazine, maleimide, succinimide, barbituric acid, thiobarbituric acid, dioxopiperazine, hydantoin, dihydrouracil, trioxane, hexahydro-1,3,5-triazine, imidazoline, imidazolidine, isoxazoline, isoxazolidine, oxazoline, oxazolidine, oxazolidinone, thiazoline, thiazolidine, morpholine, oxirane, piperidine N-Oxide, piperidine, piperazine, pyrrolidine, pyrrolidone, pyrrolidione, 4-piperidone, pyrazoline, pyrazolidine, 2-oxopyrrolidine, tetrahydropyran, 4H-pyran, tetrahydrothiopyran, thiamorpholine, thiamorpholine sulfoxide, thiamorpholine sulfone, and their benzo-fused analogs (e.g., benzimidazolidinone, tetrahydroquinoline, 3,4-methylenedioxyphenyl).

[0030] An "aralkyl" is an aryl group connected, as a substituent, via a lower alkylene group. The lower alkylene and aryl group of an aralkyl may be substituted or unsubstituted. Examples include but are not limited to benzyl, substituted benzyl, 2-phenylalkyl, 3-phenylalkyl, and naphtylalkyl.

[0031] A "heteroaralkyl" is heteroaryl group connected, as a substituent, via a lower alkylene group. The lower alkylene and heteroaryl group of heteroaralkyl may be substituted or unsubstituted. Examples include but are not limited to 2-thienylalkyl, 3-thienylalkyl, furylalkyl, thienylalkyl, pyrrolylalkyl, pyridylalkyl, isoxazolylalkyl, and imidazolylalkyl, and their substituted as well as benzo-fused analogs.

[0032] A "(heteroalicyclyl)alkyl" is a heterocyclic or a heteroalicyclylic group connected, as a substituent, via a lower alkylene group. The lower alkylene and heterocyclic or a heterocyclyl of a (heteroalicyclyl)alkyl may be substituted or unsubstituted. Examples include but are not limited tetrahydro-2H-pyran-4-yl)methyl, (piperidin-4-yl)ethyl, (piperidin-4-yl)propyl, (tetrahydro-2H-thiopyran-4-yl)methyl, and (1,3-thiazinan-4-yl)methyl.

[0033] "Lower alkylene groups" are straight-chained tethering groups, forming bonds to connect molecular fragments via their terminal carbon atoms. Examples include but are not limited to methylene (—CH_2—), ethylene (—CH_2CH_2—), propylene (—$CH_2CH_2CH_2$—), and butylene (—$CH_2CH_2CH_2CH_2$—). A lower alkylene group may be substituted or unsubstituted.

[0034] As used herein, "alkoxy" refers to the formula —OR wherein R is an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl, heteroalicyclyl, aralkyl, or (heteroalicyclyl)alkyl is defined as above. Examples of include methoxy, ethoxy, n-propoxy, 1-methylethoxy (isopropoxy), n-butoxy, iso-butoxy, sec-butoxy, tert-butoxy, phenoxy and the like. An alkoxy may be substituted or unsubstituted.

[0035] As used herein, "acyl" refers to a hydrogen, alkyl, alkenyl, alkynyl, or aryl connected, as substituents, via a carbonyl group. Examples include formyl, acetyl, propanoyl, benzoyl, and acryl. An acyl may be substituted or unsubstituted.

[0036] As used herein, "hydroxyalkyl" refers to an alkyl group in which one or more of the hydrogen atoms are replaced by hydroxy group. Examples of hydroxyalkyl groups include but are not limited to, 2-hydroxyethyl, 3-hydroxypropyl, 2-hydroxypropyl, and 2,2-dihydroxyethyl. A hydroxyalkyl may be substituted or unsubstituted.

[0037] As used herein, "haloalkyl" refers to an alkyl group in which one or more of the hydrogen atoms are replaced by halogen (e.g., mono-haloalkyl, di-haloalkyl and tri-haloalkyl). Such groups include but are not limited to, chloromethyl, fluoromethyl, difluoromethyl, trifluoromethyl and 1-chloro-2-fluoromethyl, 2-fluoroisobutyl. A haloalkyl may be substituted or unsubstituted.

[0038] As used herein, "haloalkoxy" refers to an alkoxy group in which one or more of the hydrogen atoms are replaced by halogen (e.g., mono-haloalkoxy, di-haloalkoxy and tri-haloalkoxy). Such groups include but are not limited to, chloromethoxy, fluoromethoxy, difluoromethoxy, trifluoromethoxy and 1-chloro-2-fluoromethoxy, 2-fluoroisobutoxy. A haloalkoxy may be substituted or unsubstituted.

[0039] A "sulfenyl" group refers to an "—SR" group in which R can be hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl, heteroalicyclyl, aralkyl, or (heteroalicyclyl)alkyl. A sulfenyl may be substituted or unsubstituted.

[0040] A "sulfinyl" group refers to an "—S(=O)—R" group in which R can be the same as defined with respect to sulfenyl. A sulfinyl may be substituted or unsubstituted.

[0041] A "sulfonyl" group refers to an "SO_2R" group in which R can be the same as defined with respect to sulfenyl. A sulfonyl may be substituted or unsubstituted.

[0042] An "O-carboxy" group refers to a "RC(=O)O—" group in which R can be hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl, heteroalicyclyl, aralkyl, or (heteroalicyclyl)alkyl, as defined herein. An O-carboxy may be substituted or unsubstituted.

[0043] The terms "ester" and "C-carboxy" refer to a "—C(=O)OR" group in which R can be the same as defined with respect to O-carboxy. An ester and C-carboxy may be substituted or unsubstituted.

[0044] A "thiocarbonyl" group refers to a "—C(=S)R" group in which R can be the same as defined with respect to O-carboxy. A thiocarbonyl may be substituted or unsubstituted.

[0045] A "trihalomethanesulfonyl" group refers to an "X_3CSO_2—" group wherein X is a halogen.

[0046] A "trihalomethanesulfonamido" group refers to an "$X_3CS(O)_2RN$—" group wherein X is a halogen and R defined with respect to O-carboxy.

[0047] The term "amino" as used herein refers to a —NH_2 group.

[0048] As used herein, the term "hydroxy" refers to a —OH group.

[0049] A "cyano" group refers to a "—CN" group.

[0050] The term "azido" as used herein refers to a —N_3 group.

[0051] An "isocyanato" group refers to a "—NCO" group.

[0052] A "thiocyanato" group refers to a "—CNS" group.

[0053] An "isothiocyanato" group refers to an "—NCS" group.

[0054] A "mercapto" group refers to an "—SH" group.

[0055] A "carbonyl" group refers to a C═O group.

[0056] An "S-sulfonamido" group refers to a "—$SO_2NR_AR_B$" group in which R_A and R_B can be the same as R defined with respect to O-carboxy. An S-sulfonamido may be substituted or unsubstituted.

[0057] An "N-sulfonamido" group refers to a "$R_BSO_2N(R_A)$—" group in which R_A and R_B can be the same as R defined with respect to O-carboxy. A N-sulfonamido may be substituted or unsubstituted.

[0058] An "O-carbamyl" group refers to a "—$OC(=O)NR_AR_B$" group in which R_A and R_B can be the same as R defined with respect to O-carboxy. An O-carbamyl may be substituted or unsubstituted.

[0059] An "N-carbamyl" group refers to an "$R_BOC(=O)NR_A$—" group in which R_A and R_B can be the same as R defined with respect to O-carboxy. An N-carbamyl may be substituted or unsubstituted.

[0060] An "O-thiocarbamyl" group refers to a "—$OC(=S)$—NR_AR_B" group in which R_A and R_B can be the same as R defined with respect to O-carboxy. An O-thiocarbamyl may be substituted or unsubstituted.

[0061] An "N-thiocarbamyl" group refers to an "$R_BOC(=S)NR_A$—" group in which R_A and R_B can be the same as R defined with respect to O-carboxy. An N-thiocarbamyl may be substituted or unsubstituted.

[0062] A "C-amido" group refers to a "—$C(=O)NR_AR_B$" group in which R_A and R_B can be the same as R defined with respect to O-carboxy. A C-amido may be substituted or unsubstituted.

[0063] An "N-amido" group refers to a "$R_BC(=O)NR_A$—" group in which R_A and R_B can be the same as R defined with respect to O-carboxy. An N-amido may be substituted or unsubstituted.

[0064] As used herein, "organylcarbonyl" refers to a group of the formula —C(═O)R' wherein R' can be alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl, heteroalicyclyl, aralkyl, or (heteroalicyclyl)alkyl. An organylcarbonyl can be substituted or unsubstituted.

[0065] The term "alkoxycarbonyl" as used herein refers to a group of the formula —C(═O)OR' wherein R' can be the same as defined with respect to organylcarbonyl. An alkoxycarbonyl can be substituted or unsubstituted.

[0066] As used herein, "organylaminocarbonyl" refers to a group of the formula C(═O)NR'R" wherein R' and R" can each be independently selected from the same substituents as defined with respect to organylcarbonyl. An organylaminocarbonyl can be substituted or unsubstituted.

[0067] As used herein, the term "levulinoyl" refers to a $C(=O)CH_2CH_2C(=O)CH_3$ group.

[0068] The term "halogen atom," as used herein, means any one of the radio-stable atoms of column 7 of the Periodic Table of the Elements, i.e., fluorine, chlorine, bromine, or iodine, with fluorine and chlorine being preferred.

[0069] Where the numbers of substituents is not specified (e.g. haloalkyl), there may be one or more substituents present. For example "haloalkyl" may include one or more of the same or different halogens. As another example, "C_1-C_3 alkoxyphenyl" may include one or more of the same or different alkoxy groups containing one, two or three atoms.

[0070] As used herein, the term "nucleoside" refers to a compound composed of any pentose or modified pentose moiety attached to a specific portion of a heterocyclic base, tautomer, or derivative thereof such as the 9-position of a purine, 1-position of a pyrimidine, or an equivalent position of a heterocyclic base derivative. Examples include, but are not limited to, a ribonucleoside comprising a ribose moiety and a deoxyribonucleoside comprising a deoxyribose moiety. In some instances, the nucleoside can be a nucleoside drug analog.

[0071] As used herein, the term "nucleoside drug analog" refers to a compound composed of a nucleoside that has therapeutic activity, such as antiviral, anti-neoplastic, antiparasitic and/or antibacterial activity.

[0072] As used herein, the term "nucleotide" refers to a nucleoside having a phosphate ester substituted on the 5'-position or an equivalent position of a nucleoside derivative.

[0073] As used herein, the term "heterocyclic base" refers to a purine, a pyrimidine and derivatives thereof. The term "purine" refers to a substituted purine, its tautomers and analogs thereof. Similarly, the term "pyrimidine" refers to a substituted pyrimidine, its tautomers and analogs thereof. Examples of purines include, but are not limited to, purine, adenine, guanine, hypoxanthine, xanthine, theobromine, caffeine, uric acid and isoguanine. Examples of pyrimidines include, but are not limited to, cytosine, thymine, uracil, and derivatives thereof. An example of an analog of a purine is 1,2,4-triazole-3-carboxamide.

[0074] Other non-limiting examples of heterocyclic bases include diaminopurine, 8-oxo-N^6-methyladenine, 7-deazaxanthine, 7-deazaguanine, N^4,N^4-ethanocytosin, N^6,N^6-ethano-2,6-diaminopurine, 5-methylcytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, isocytosine, isoguanine, and other heterocyclic bases described in U.S. Pat. Nos. 5,432,272 and 7,125,855, which are incorporated herein by reference for the limited purpose of disclosing additional heterocyclic bases.

[0075] The term "—O-linked amino acid" refers to an amino acid that is attached to the indicated moiety via its main-chain carboxyl function group. When the amino acid is attached, the hydrogen that is part of the —OH portion of the carboxyl function group is not present and the amino acid is attached via the remaining oxygen. An —O-linked amino acid can be protected at any nitrogen group that is present on the amino acid. For example, an —O-linked amino acid can contain an amide or a carbamate group. Suitable amino acid protecting groups include, but are not limited to, carbobenzyloxy (Cbz), p-methoxybenzyl carbonyl (Moz or MeOZ), tert-butyloxycarbonyl (BOC), 9-fluorenylmethyloxycarbonyl (FMOC), benzyl (Bn), p-methoxybenzyl (PMB), 3,4-dimethoxybenzyl (DMPM), and tosyl (Ts) groups. The term "—N-linked amino acid" refers to an amino acid that is

attached to the indicated moiety via its main-chain amino or mono-substituted amino group. When the amino acid is attached in an —N-linked amino acid, one of the hydrogens that is part of the main-chain amino or mono-substituted amino group is not present and the amino acid is attached via the nitrogen. An —N-linked amino acid can be protected at any hydroxyl or carboxyl group that is present on the amino acid. For example, an —N-linked amino acid can contain an ester or an ether group. Suitable amino acid protecting groups include, but are not limited to, methyl esters, ethyl esters, propyl esters, benzyl esters, tert-butyl esters, silyl esters, orthoesters, and oxazoline. As used herein, the term "amino acid" refers to any amino acid (both standard and non-standard amino acids), including, but limited to, α-amino acids β-amino acids, γ-amino acids and δ-amino acids. Examples of suitable amino acids, include, but are not limited to, alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, tyrosine, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

[0076] The terms "derivative," "variant," or other similar terms refer to a compound that is an analog of the other compound.

[0077] The terms "protecting group" and "protecting groups" as used herein refer to any atom or group of atoms that is added to a molecule in order to prevent existing groups in the molecule from undergoing unwanted chemical reactions. Examples of protecting group moieties are described in T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis,* 3. Ed. John Wiley & Sons (1999), and in J. F. W. McOmie, *Protective Groups in Organic Chemistry* Plenum Press (1973), both of which are hereby incorporated by reference for the limited purpose of disclosing suitable protecting groups. The protecting group moiety may be chosen in such a way, that they are stable to certain reaction conditions and readily removed at a convenient stage using methodology known from the art. A non-limiting list of protecting groups include benzyl; substituted benzyl; alkylcarbonyls (e.g., t-butoxycarbonyl (BOC)); arylalkylcarbonyls (e.g., benzyloxycarbonyl, benzoyl); substituted methyl ether (e.g. methoxymethyl ether); substituted ethyl ether; a substituted benzyl ether; tetrahydropyranyl ether; silyl ethers (e.g., trimethylsilyl, triethylsilyl, triisopropylsilyl, t-butyldimethylsilyl, or t-butyldiphenylsilyl); esters (e.g. benzoate ester); carbonates (e.g. methoxymethylcarbonate); sulfonates (e.g. tosylate, mesylate); acyclic ketal (e.g. dimethyl acetal); cyclic ketals (e.g., 1,3-dioxane or 1,3-dioxolanes); acyclic acetal; cyclic acetal; acyclic hemiacetal; cyclic hemiacetal; and cyclic dithioketals (e.g., 1,3-dithiane or 1,3-dithiolane).

[0078] "Leaving group" as used herein refers to any atom or moiety that is capable of being displaced by another atom or moiety in a chemical reaction. More specifically, in some embodiments, "leaving group" refers to the atom or moiety that is displaced in a nucleophilic substitution reaction. In some embodiments, "leaving groups" are any atoms or moieties that are conjugate bases of strong acids. Examples of suitable leaving groups include, but are not limited to, tosylates and halogens. Non-limiting characteristics and examples of leaving groups can be found, for example in *Organic Chemistry,* 2d ed., Francis Carey (1992), pages 328-331; *Introduction to Organic Chemistry,* 2d ed., Andrew Streitwieser and Clayton Heathcock (1981), pages 169-171; and *Organic Chemistry,* 5th ed., John McMurry (2000), pages 398 and 408; all of which are incorporated herein by reference for the limited purpose of disclosing characteristics and examples of leaving groups.

[0079] As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (See, *Biochem.* 1972 11:942-944).

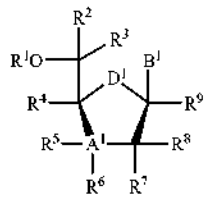
[0080] A "prodrug" refers to an agent that is converted into the parent drug in vivo. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. Examples of prodrugs include compounds that have one or more biologically labile groups attached to the parent drug (e.g., a compound of Formula I and/or a compound of Formula II). For example, one or more biologically labile groups can be attached to a functional group of the parent drug (for example, by attaching one or more biologically labile groups to a phosphate). When more than one biologically labile groups is attached, the biologically labile groups can be the same or different. The biologically labile group(s) can be linked (for example, through a covalent bond), to an oxygen or a heteroatom, such as a phosphorus of a monophosphate, diphosphate, triphosphate, and/or a stabilized phosphate analog containing carbon, nitrogen or sulfur (referred to hereinafter in the present paragraph as "phosphate"). In instances where the prodrug is form by attaching one or more biologically labile groups to the phosphate, removal of the biologically labile group in the host produces a phosphate. The removal of the biologically labile group(s) that forms the prodrug can be accomplished by a variety of methods, including, but not limited to, oxidation, reduction, amination, deamination, hydroxylation, dehydroxylation, hydrolysis, dehydrolysis, alkylation, dealkylation, acylation, deacylation, phosphorylation, dephosphorylation, hydration and/or dehydration. An example, without limitation, of a prodrug would be a compound which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but which then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water-solubility is beneficial. A further example of a prodrug might comprise a short peptide (polyaminoacid) bonded to an acid group where the peptide is metabolized or cleaved to reveal the active moiety. Additional examples of prodrug moieties include the following: R*, R*C(=O)OCH_2—, R*C(=O)SCH_2CH_2—, R*C(=O)SCHR'NH—, phenyl-O—, N-linked amino acids, O-linked amino acids, peptides, carbohydrates, and lipids, wherein each R* can be independently selected from an alkyl, an alkenyl, an alkynyl, an aryl, an aralkyl, acyl, sulfonate ester, a lipid, an —N-linked amino acid, an —O-linked amino acid, a peptide and a cholesterol. The prodrug can be a carbonate. The carbonate can be a cyclic carbonate. The cyclic carbonate can contain a carbonyl group between two hydroxyl groups that results in the formation of a five or six memebered ring. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in *Design of Prodrugs,* (ed. H. Bundgaard, Elsevier, 1985), which is hereby incorporated herein by reference for the limited purpose of describing procedures and preparation of suitable prodrug derivatives.

[0081] The term "pro-drug ester" refers to derivatives of the compounds disclosed herein formed by the addition of any of several ester-forming groups that are hydrolyzed under physiological conditions. Examples of pro-drug ester groups include pivaloyloxymethyl, acetoxymethyl, phthalidyl, indanyl and methoxymethyl, as well as other such groups known in the art, including a (5-R-2-oxo-1,3-dioxolen-4-yl)methyl group. Other examples of pro-drug ester groups can be found in, for example, T. Higuchi and V. Stella, in "Pro-drugs as Novel Delivery Systems", Vol. 14, A.C.S. Symposium Series, American Chemical Society (1975); and "Bioreversible Carriers in Drug Design: Theory and Application", edited by E. B. Roche, Pergamon Press: New York, 14-21 (1987) (providing examples of esters useful as prodrugs for compounds containing carboxyl groups). Each of the above-mentioned references is herein incorporated by reference for the limited purpose of disclosing ester-forming groups that can form prodrug esters.

[0082] The term "pharmaceutically acceptable salt" refers to a salt of a compound that does not cause significant irritation to an organism to which it is administered and does not abrogate the biological activity and properties of the compound. In some embodiments, the salt is an acid addition salt of the compound. Pharmaceutical salts can be obtained by reacting a compound with inorganic acids such as hydrohalic acid (e.g., hydrochloric acid or hydrobromic acid), sulfuric acid, nitric acid, phosphoric acid and the like. Pharmaceutical salts can also be obtained by reacting a compound with an organic acid such as aliphatic or aromatic carboxylic or sulfonic acids, for example acetic, succinic, lactic, malic, tartaric, citric, ascorbic, nicotinic, methanesulfonic, ethanesulfonic, p-toluensulfonic, salicylic or naphthalenesulfonic acid. Pharmaceutical salts can also be obtained by reacting a compound with a base to form a salt such as an ammonium salt, an alkali metal salt, such as a sodium or a potassium salt, an alkaline earth metal salt, such as a calcium or a magnesium salt, a salt of organic bases such as dicyclohexylamine, N-methyl-D-glucamine, tris(hydroxymethyl)methylamine, C_1-C_7 alkylamine, cyclohexylamine, triethanolamine, ethylenediamine, and salts with amino acids such as arginine, lysine, and the like.

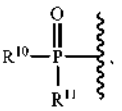
[0083] It is understood that, in any compound described herein having one or more chiral centers, if an absolute stereochemistry is not expressly indicated, then each center may independently be of R-configuration or S-configuration or a mixture thereof. Thus, the compounds provided herein may be enatiomerically pure or be stereoisomeric mixtures. In addition it is understood that, in any compound described herein having one or more double bond(s) generating geometrical isomers that can be defined as E or Z, each double bond may independently be E or Z a mixture thereof. Likewise, all tautomeric forms are also intended to be included.

[0084] An embodiment disclosed herein relates to a compound of Formula (I), or a pharmaceutically acceptable salt or a prodrug thereof:

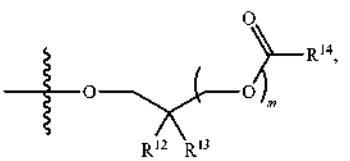


(I)

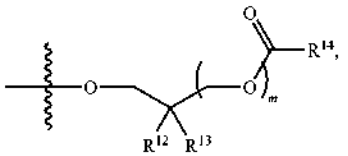
[0085] wherein: A^1 can be selected from C (carbon), O (oxygen) and S (sulfur); B^1 can be an optionally substituted heterocyclic base or a derivative thereof; D^1 can be selected from C═CH_2, CH_2, O (oxygen), S (sulfur), CHF, and CF_2; R^1 can be hydrogen, an optionally substituted alkyl, an optionally substituted cycloalkyl, an optionally substituted aralkyl, dialkylaminoalkylene, alkyl-C(═O)—, aryl-C(═O)—, alkoxyalkyl-C(═O)—, aryloxyalkyl-C(═O)—, alkylsulfonyl, arylsulfonyl, aralkylsulfonyl,



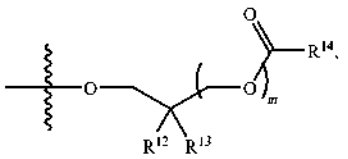
an —O-linked amino acid, diphosphate, triphosphate or derivatives thereof; R^2 and R^3 can be each independently selected from hydrogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl and an optionally substituted C_{1-6} haloalkyl, provided that at least one of R^2 and R^3 is not hydrogen; or R^2 and R^3 are taken together to form a group selected from among C_{3-6} cycloalkyl, C_{3-6} cycloalkenyl, C_{3-6} aryl, and a C_{3-6} heteroaryl; R^4, R^7 and R^9 can be independently selected from hydrogen, halogen, —NH_2, —NHR^{a1}, $NR^{a1}R^{b1}$, —OR^{a1}, —SR^{a1}, —CN, —NC, —N_3, —NO_2, —$N(R^{c1})$—$NR^{a1}R^{b1}$, —$N(R^{c1})$—OR^{a1}, —S—SR^{a1}, —C(═O)R^{a1}, —C(═O)OR^{a1}, —C(═O)$NR^{a1}R^{b1}$, —O—(C═O)R^{a1}, —O—(C═O)R^{a1}, —O—C(═O)OR^{a1}, —O—C(═O)$NR^{a1}R^{b1}$, —$N(R^{c1})$—C(═O)$NR^{a1}R^{b1}$, —S(═O)R^{a1}, S(═O)$_2R^{a1}$, —O—S(═O)$_2NR^{a1}R^{b1}$, —$N(R^{c1})$—S(═O)$_2NR^{a1}R^{b1}$, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl, an optionally substituted aralkyl and an —O-linked amino acid; R^5 and R^6 can be independently absent or selected from hydrogen, halogen, —NH_2, —NHR^{a1}, $NR^{a1}R^{b1}$, —OR^{a1}, —SR^{a1}, —CN, —NC, —N_3, —NO_2, —$N(R^{c1})$—$NR^{a1}R^{b1}$, —$N(R^{c1})$—OR^{a1}, —S—SR^{a1}, —C(═O)R^{a1}, —C(═O)OR^{a1}, —C(═O)$NR^{a1}R^{b1}$, —O—C(═O)OR^{a1}, —O—C(═O)$NR^{a1}R^{b1}$, —$N(R^{c1})$—C(═O)$NR^{a1}R^{b1}$, —S(═O)R^{a1}, S(═O)$_2R^{a1}$, —O—S(═O)$_2NR^{a1}R^{b1}$, —$N(R^{c1})$—S(═O)$_2NR^{a1}R^{b1}$, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl and an —O-linked amino acid; or R^6 and R^7 taken together form —O—C(═O)—O—; R^8 can be halogen, —OR^{a1}, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl and an optionally substituted C_{1-6} haloalkyl; R^{a1}, R^{b1} and R^{c1} can be each independently selected from hydrogen, an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted alkynyl, an optionally substituted aryl, an optionally substituted heteroaryl, an optionally substituted aralkyl and an optionally substituted heteroaryl(C_{1-6} alkyl); R^{10} can be selected from O^-, —OH, an optionally substituted aryloxy or aryl-O—,



alkyl-C(=O)—O—CH_2—O—, alkyl-C(=O)—S—CH_2CH_2—O— and an —N-linked amino acid; R^{11} can be selected from O^-, —OH, an optionally substituted aryloxy or aryl-O—,



alkyl-C(=O)—O—CH_2—O—, alkyl-C(=O)—S—CH_2CH_2—O— and an —N-linked amino acid; each R^{12} and each R^{13} can be independently —C≡N or an optionally substituted substituent selected from C_{1-8} organylcarbonyl, C_{1-8} alkoxycarbonyl and C_{1-8} organylaminocarbonyl; each R^{14} can be hydrogen or an optionally substituted C_{1-6}-alkyl; each m can be independently 1 or 2, and if both R^{10} and R^{11} are

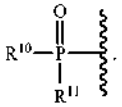


each R^{12}, each R^{13}, each R^{14} and each m can be the same or different.

[0086] In an embodiment, m can be 1. In another embodiment, m can be 2. In some embodiments, A^1 can be carbon. In some embodiments, D^1 can be oxygen. In an embodiment, A^1 can be carbon and D^1 can be oxygen. In other embodiments, A^1 can be carbon, D^1 can be oxygen and m can be 1. In an embodiment, A^1 can be carbon, D^1 can be oxygen and m can be 2.

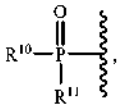
[0087] In some embodiments, the optionally substituted C_{1-6} alkyl can be selected from methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, tert-butyl, pentyl, and hexyl. In an embodiment, the optionally substituted C_{1-6} alkyl can be methyl. In an embodiment, R^2 can be methyl and R^3 can be hydrogen. In some embodiments, R^2 and R^8 can both be methyl. In some embodiments, the optionally substituted C_{1-6} alkoxy can be selected from methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, iso-butoxy and tert-butoxy. In an embodiment, the optionally substituted C_{1-6} haloalkyl can be trifluoromethyl. In some embodiments, R^2 can be trifluoromethyl and R^3 can be hydrogen. In some embodiments, R^2 can be trifluoromethyl and R^8 can be methyl.

[0088] In some embodiments, a compound of Formula (I) can be a nucleoside or nucleoside derivative. In an embodiment, R^1 can be hydrogen. In some embodiments, a compound of Formula (I) can be a nucleotide or nucleotide derivative. In an embodiment, R^1 can be monophosphate. In another embodiment, R^1 can be a diphosphate. In still another embodiment, R^1 can be a triphosphate. In yet still another embodiment, R^1 can be

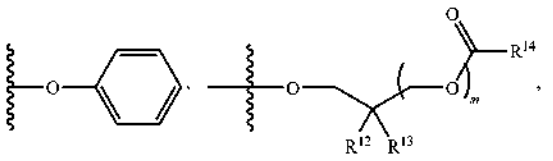


When R^1 is

[0089]

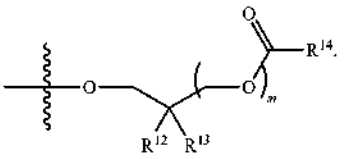


R^{10} and R^{11} can both be O^-. In some embodiments, to facilitate entry into a cell, the charge on the phosphate of the nucleotide or nucleotide derivative can be neutralized with an appropriate moiety. In some embodiments, the moiety can be

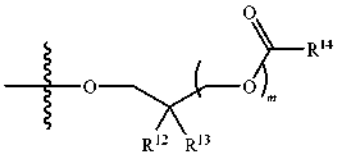


—O-naphthol and/or an —N-linked amino acid, such as those described herein.

[0090] In some embodiments, at least one of R^{10} and R^{11} can be



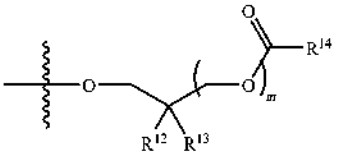
The substitutents on



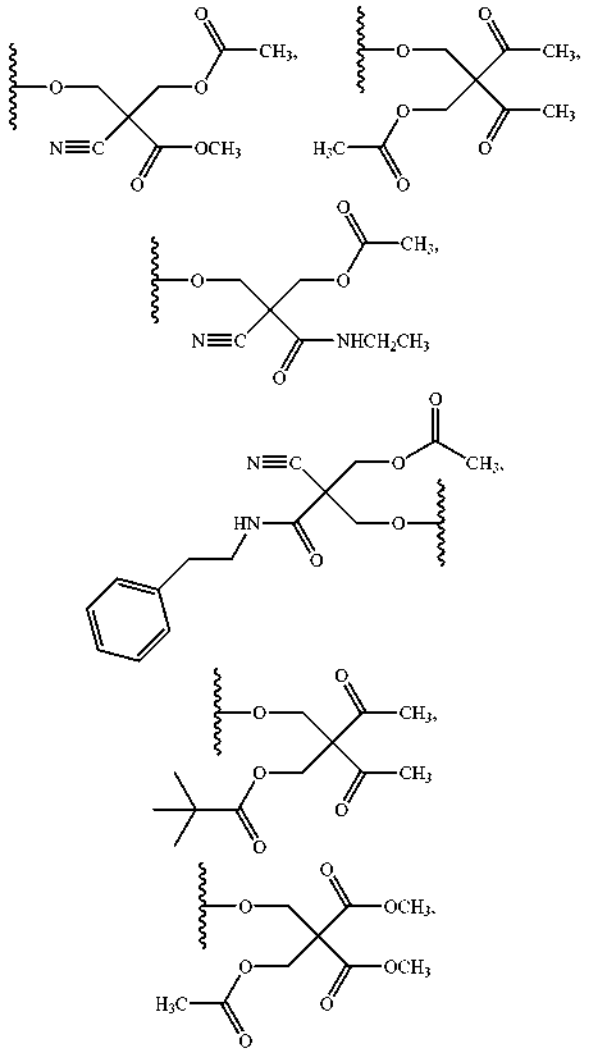
can vary. In some embodiments, R^{12} can be —C≡N and R^{13} can be an optionally substituted C_{1-8} alkoxycarbonyl such as —C(=O)OCH_3. In other embodiments, R^{12} can be —C≡N and R^{13} can be an optionally substituted C_{1-8} organylaminocarbonyl, for example, —C(=O)$NHCH_2CH_3$ and —C(=O)$NHCH_2CH_2$phenyl. In still other embodiments, both R^{12} and R^{13} can be an optionally substituted C_{1-8} organylcarbonyl. In an embodiment, both R^{12} and R^{13} can be —C(=O)CH_3. In yet still other embodiments, both R^{12} and

R^{13} can be an optionally substituted C_{1-8} alkoxycarbonyl. In an embodiment, both R^{12} and R^{13} can be —C(=O)OCH_3 or —C(=O)OCH_2CH_3. In an embodiment, both R^{12} and R^{13} can be an optionally substituted C_{1-8} alkoxycarbonyl, for example —C(=O)OCH_2CH_3, and m can be 2. In some embodiments, including those in this paragraph, R^{14} can be an optionally substituted C_{1-6}-alkyl. In an embodiment, including those in this paragraph, R^{14} can be methyl or tert-butyl.

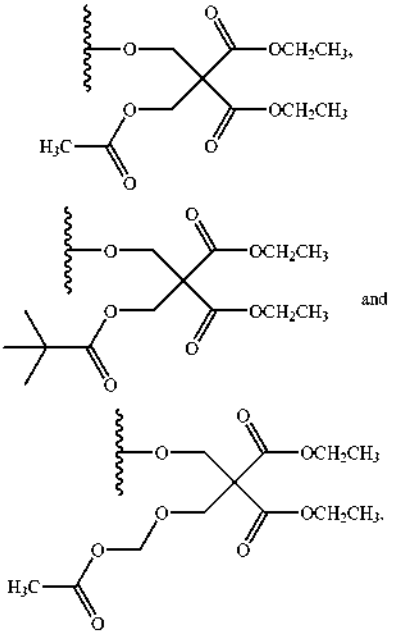
[0091] Examples of suitable



groups, include but are not limited to, the following:

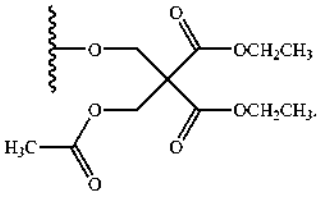


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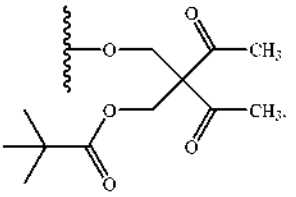


and

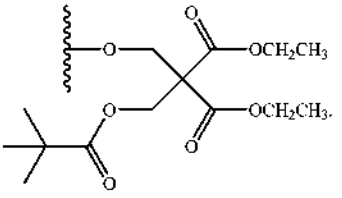
[0092] In an embodiment, R^{10} and/or R^{11} can be



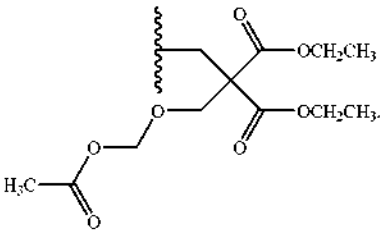
In another embodiment, R^{10} and/or R^{11} can be



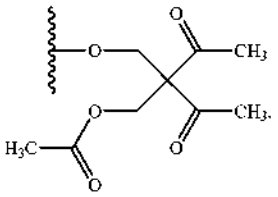
In still another embodiment, R^{10} and/or R^{11} can be



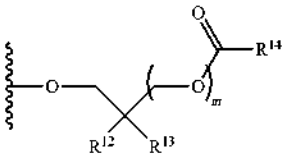
In yet still another embodiment, R^{10} and/or R^{11} can be



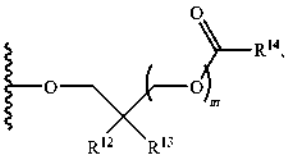
In an embodiment, R^{10} and/or R^{11} can be



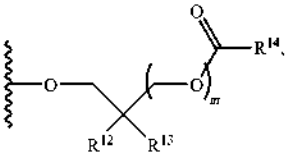
[0093] In some embodiments, both R^{10} and R^{11} can be



wherein each R^{12}, each R^{13}, each R^{14} and each m can be the same or different. In some embodiments, when both R^{10} and R^{11} are

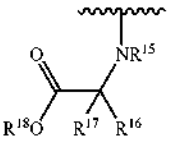


R^{10} and R^{11} can be the same. In other embodiments, when both R^{10} and R^{11} are

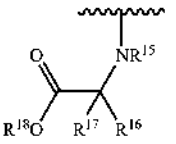


R^{10} and R^{11} can be different.

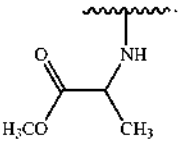
[0094] In an embodiment, at least one of R^{10} and R^{11} can be an —N-linked amino acid. Various amino acids can be utilized as a substituent for R^{10} or R^{11} . In some embodiments, R^{10} or R^{11} can have the structure



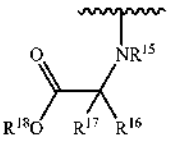
wherein: R^{15} can be hydrogen or an optionally substituted C_{1-4}-alkyl; R^{16} can be selected from hydrogen, an optionally substituted C_{1-6}-alkyl, an optionally substituted aryl, an optionally substituted aryl(C_{1-6} alkyl) and haloalkyl; R^{17} can be hydrogen or an optionally substituted C_{1-6}-alkyl; and R^{18} can be selected from an optionally substituted C_{1-6} alkyl, an optionally substituted C_6 aryl, an optionally substituted C_{10} aryl, and an optionally substituted C_{3-6} cycloalkyl. In an embodiment, R^{15} can be hydrogen. In some embodiments, R^{16} can be an optionally substituted C_{1-6}-alkyl, for example, methyl. In an embodiment, R^{17} can be hydrogen or an optionally substituted C_{1-6}-alkyl such as methyl. In some embodiment, R^{18} can be an optionally substituted C_{1-6}-alkyl. In an embodiment, R^{18} can be methyl. One example of a suitable



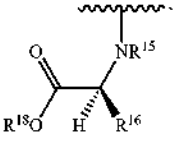
group includes, but are not limited to,



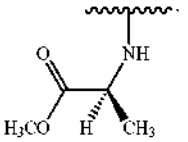
In some embodiments, the amino acid can be in the L-configuration. In other embodiments, the amino acid can be in the D-configuration. For example,



can be

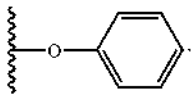


such as

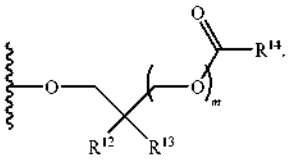


Additional suitable amino acids that can be used in embodiments disclosed herein are described in Cahard et al., *Mini-Reviews in Medicinal Chemistry*, 2004, 4:371-381 and McGuigan et al., *J. Med. Chem.*, 2008, 51(18):5807-5812, which hereby incorporated by reference for the limited purpose of describing additional suitable amino acids.

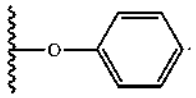
[0095] In some embodiments, at least one of R^{10} and R^{11} can be an —N-linked amino acid, such as those described herein, and the other of at least one of R^{10} and R^{11} can be



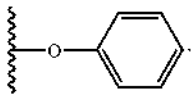
In other embodiments, at least one of R^{10} and R^{11} can be an —N-linked amino acid, such as those described herein, and the other of at least one of R^{10} and R^{11} can be



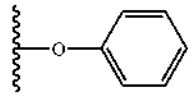
In some embodiments, at least one of R^{10} and R^{11} can be



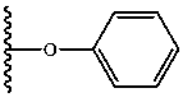
In an embodiment, R^{10} can be



In some embodiments, at least one of R^{10} and R^{11} can be an —N-linked amino acid. In an embodiment, R^{10} can be

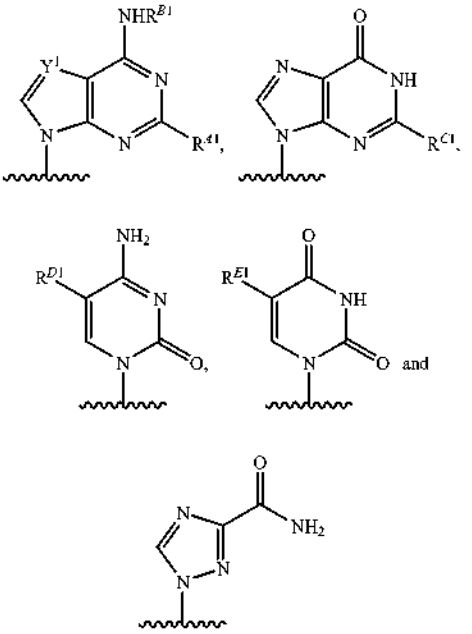


and R^{11} can be an —N-linked amino acid. In another embodiment, R^{10} cannot be

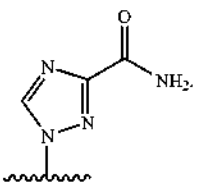


when R^{11} is an —N-linked amino acid.

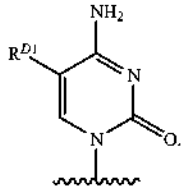
[0096] The substituent B^1 can also vary. In some embodiments, B^1 can be selected from:



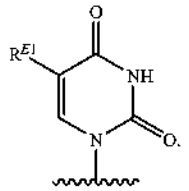
wherein: R^{A1} can be hydrogen or halogen; R^{B1} can be hydrogen, an optionally substituted C_{1-6}alkyl, or an optionally substituted C_{3-8} cycloalkyl; R^{C1} can be hydrogen or amino; R^{D1} can be hydrogen, halogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl and an optionally substituted C_{2-6} alkynyl; R^{E1} can be hydrogen, halogen, an optionally substituted C_{1-6}alkyl, an optionally substituted C_{2-6} alkenyl and an optionally substituted C_{2-6} alkynyl; and Y^1 can be N (nitrogen) or CR^{F1}, wherein R^{F1} can be selected from hydrogen, halogen, an optionally substituted C_{1-6}-alkyl, an optionally substituted C_{2-6}-alkenyl and an optionally substituted C_{2-6}-alkynyl. In some embodiments, B^1 can be



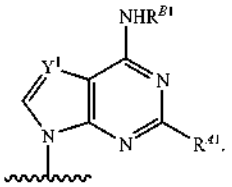
In other embodiments, B^1 can be



In yet other embodiments, B^1 can be



In an embodiment, R^E can be hydrogen. In yet still other embodiments, B^1 can be



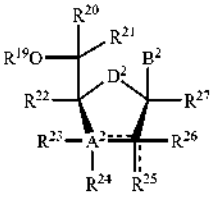
In an embodiment Y^1 can be nitrogen; R^{A1} can be hydrogen and R^{B1} can be hydrogen. In another embodiment, Y^1 can be CR^{F1}, wherein R^{F1} can be selected from hydrogen, halogen, an optionally substituted C_{1-6}-alkyl, an optionally substituted C_{2-6}-alkenyl and an optionally substituted C_{2-6}-alkynyl; R^{A1} can be hydrogen and R^{B1} can be hydrogen. When B^1 is any of the aforementioned moieties shown above, in some embodiments, A^1 can be carbon. In an embodiment, B^1 can be any of the aforementioned moieties shown above, A^1 can be carbon and D^1 can be oxygen.

[0097] In some embodiments, R^4 can be selected from hydrogen, halogen, $—OR^{a1}$, —CN, $—N_3$ and an optionally substituted C_{1-6} alkyl. In some embodiments, R^5 can be absent or selected from hydrogen, halogen, $—OR^{a1}$ and an optionally substituted C_{1-6} alkyl. In some embodiments, R^6 can be absent or selected from hydrogen, halogen, $—NH_2$, $—OR^{a1}$, $—N_3$, an optionally substituted C_{1-6} alkyl and an —O-linked amino acid. In some embodiments, R^7 can be absent or selected from hydrogen, halogen, $—OR^{a1}$, —CN, —NC, an optionally substituted C_{1-6} alkyl and an —O-linked amino acid. In an embodiment, R^6 can be $—OR^{a1}$, wherein R^{a1} is hydrogen. In another embodiment, R^6 can be an —O-linked amino acid. In some embodiments, R^7 can be $—OR^{a1}$, wherein R^{a1} is hydrogen. In other embodiments, R^7 can be a C_{1-6} alkoxy such as methoxy. In still other embodiments, R^7 can be an —O-linked amino acid. In some embodiments, both R^6 and R^7 can be hydroxy groups. In other embodiments, R^7 can be a hydroxyl group and R^6 can be —O-linked amino acid. A non-limiting list of suitable —O-linked amino acid include, but are not limited to the following: alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, tyrosine, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. In an embodiment, the —O-linked amino acid can be valine. In some embodiments, the —O-linked amino acid can be selected from-O-linked α-amino acid, —O-linked β-amino acid, —O-linked γ-amino acid and —O-linked δ-amino acid. In an embodiment, the —O-linked amino acid can be in the L-configuration. In some embodiments, R^9 can be selected from hydrogen, halogen and an optionally substituted C_{1-6} alkyl.

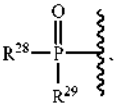
[0098] In some embodiments, the compound of Formula (I) can be an anti-neoplastic agent. In other embodiments, the compound of Formula (I) can be an anti-viral agent. In still other embodiments, the compound of Formula (I) can be an anti-parasitic agent.

[0099] An embodiment disclosed herein relates to a compound of Formula (II), or a pharmaceutically acceptable salt or a prodrug thereof:

(II)



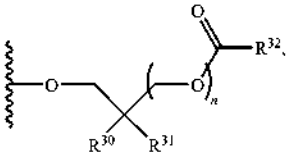
wherein: ------ each can be independently a double or single bond; A^2 can be selected from C (carbon), O (oxygen) and S (sulfur); B^2 can be an optionally substituted heterocyclic base or a derivative thereof; D^2 can be selected $C{=}CH_2$, CH_2, O (oxygen), S (sulfur), CHF, and CF_2; R^{19} can be hydrogen, an optionally substituted alkyl, an optionally substituted cycloalkyl, an optionally substituted aralkyl, dialkylaminoalkylene, alkyl-C(=O)—, aryl-C(=O)—, alkoxyalkyl-C(=O)—, aryloxyalkyl-C(=O)—, alkylsulfonyl, arylsulfonyl, aralkylsulfonyl,



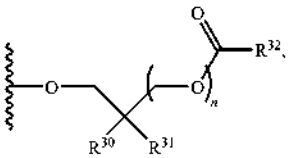
an —O-linked amino acid, diphosphate, triphosphate or derivatives thereof; R^{20} and R^{21} can be each independently selected from hydrogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl and an optionally substituted C_{1-6} haloalkyl, provided that at least one of R^{20} and R^{21} is not hydrogen; or R^{20} and R^{21} are taken together to form a group selected from among C_{3-6} cycloalkyl, C_{3-6} cycloalkenyl, C_{3-6} aryl, and a C_{3-6} heteroaryl; R^{22} and R^{27} can be independently selected from hydrogen, halogen, $—NH_2$, $—NHR^{a2}$, $NR^{a2}R^{b2}$, $—OR^{a2}$, $—SR^{a2}$, —CN, —NC, $—N_3$, $—NO_2$, $—N(R^{c2})—NR^{a2}R^{b2}$, $—N(R^{c2})—OR^{a2}$, $—S—SR^{a2}$, $—C(=O)R^{a2}$, $—C(=O)OR^{a2}$, $—C(=O)NR^{a2}R^{b2}$, $—O—C(=O)OR^{a2}$, $—O—C(=O)NR^{a2}R^{b2}$, $—N(R^{c2})—C(=O)NR^{a2}R^{b2}$, $—S(=O)R^{a2}$, $S(=O)_2R^{a2}$, $—O—S(=O)_2NR^{a2}R^{b2}$, $—N(R^{c2})—S(=O)_2NR^{a2}R^{b2}$, an optionally

substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl and an —O-linked amino acid; R^{23}, R^{24} and R^{25} can be independently absent or selected from the group consisting of hydrogen, halogen, $—NH_2$, $—NHR^{a2}$, $NR^{a2}R^{b2}$, $—OR^{a2}$, $—SR^{a2}$, —CN, —NC, $—N_3$, $—NO_2$, $—N(R^{c2})—NR^{a2}R^{b2}$, $—N(R^{c2})—OR^{a2}$, $—S—SR^{a2}$, $—C(=O)R^{a2}$, $—C(=O)OR^{a2}$, $—C(=O)NR^{a2}R^{b2}$, $—O—C(=O)R^{a2}$, $—O—C(=O)OR^{a2}$, $—O—C(=O)NR^{a2}R^{b2}$, $—N(R^{c2})—C(=O)NR^{a2}R^{b2}$, $—S(=O)R^{a2}$, $S(=O)_2R^{a2}$, $—O—S(=O)_2NR^{a2}R^{b2}$, $—N(R^{c2})—S(=O)_2NR^{a2}R^{b2}$, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl, an optionally substituted aralkyl and an —O-linked amino acid; or R^{24} and R^{25} taken together form —O—C(=O)—O—; R^{26} can be absent or selected from hydrogen, halogen, $—NH_2$, $—NHR^{a2}$, $NR^{a2}R^{b2}$, $—OR^{a2}$, $—SR^{a2}$, —CN, —NC, $—N_3$, $—NO_2$, $—N(R^{c2})—NR^{a2}$, R^{b2}, $—N(R^{c2})—OR^{a2}$, $—S—SR^{a2}$, $C(=O)R^{a2}$, $—C(=O)OR^{a2}$, $—C(=O)NR^{a2}R^{b2}$, $—O—C(=O)OR^{a2}$, $—O—C(=O)NR^{a2}R^{b2}$, $—N(R^{c2})—C(=O)NR^{a2}R^{b2}$, $—S(=O)R^{a2}$, $S(=O)_2R^{a2}$, $—O—S(=O)_2NR^{a2}R^{b2}$, $—N(R^{c2})—S(=O)_2NR^{a2}R^{b2}$, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl, an optionally substituted haloalkyl, an optionally substituted hydroxyalkyl and an —O-linked amino acid, or when the bond to R^{25} indicated by ====== is a double bond, then R^{25} is a C_{2-6} alkylidene and R^{26} is absent; R^{a2}, R^{b2} and R^{c2} can be each independently selected from hydrogen, an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted alkynyl, an optionally substituted aryl, an optionally substituted heteroaryl, an optionally substituted aralkyl and an optionally substituted heteroaryl(C_{1-6} alkyl);

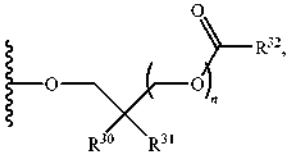
[0100] R^{28} can be selected from O^-, —OH, an optionally substituted aryloxy or aryl-O—,



alkyl-C(=O)—O—CH_2—O—, alkyl-C(=O)—S—CH_2CH_2—O— and an —N-linked amino acid; R^{29} can be selected from O^-, —OH, an optionally substituted aryloxy or aryl-O—,



alkyl-C(=O)—O—CH_2—O—, alkyl-C(=O)—S—CH_2CH_2—O—and an —N-linked amino acid; each R^{30} and each R^{31} can be independently —C≡N or an optionally substituted substituent selected from C_{1-8} organylcarbonyl, C_{1-8} alkoxycarbonyl and C_{1-8} organylaminocarbonyl; each R^{32} can be hydrogen or an optionally substituted C_{1-6}-alkyl; and each n can be independently 1 or 2, and if both R^{28} and R^{29} are

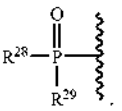


each R^{30}, each R^{31}, each R^{32} and each n can be the same or different.

[0101] In an embodiment, n can be 1. In another embodiment, n can be 2. In some embodiments, A^2 can be carbon. In some embodiments, D^2 can be oxygen. In an embodiment, each ====== can be a single bond. In an embodiment, A^2 can be carbon, D^2 can be oxygen and each ====== can be a single bond. In other embodiments, A^2 can be carbon, D^2 can be oxygen, each ====== can be a single bond and n can be 1. In an embodiment, A^2 can be carbon, D^2 can be oxygen, each ====== can be a single bond and n can be 2.

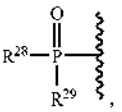
[0102] In some embodiments, the optionally substituted C_{1-6} alkyl can be selected from methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, tert-butyl, pentyl and hexyl. In an embodiment, the optionally substituted C_{1-6} alkyl can be methyl. For example, in an embodiment, R^{20} can be methyl and R^{21} can be hydrogen. In some embodiments, the optionally substituted C_{1-6} alkoxy can be selected from methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, iso-butoxy and tert-butoxy. In some embodiments, the optionally substituted C_{1-6} haloalkyl can be trifluoromethyl. In an embodiment, R^{20} can be trifluoromethyl and R^{21} can be hydrogen.

[0103] In some embodiments, a compound of Formula (II) can be a nucleoside or nucleoside derivative. In an embodiment, R^{19} can be hydrogen. In some embodiments, a compound of Formula (II) can be a nucleotide or nucleotide derivative. In an embodiment, R^{19} can be a monophosphate. In another embodiment, R^{19} can be a diphosphate. In yet another embodiment, R^{19} can be a triphosphate. In still yet another embodiment, R^{19}can be

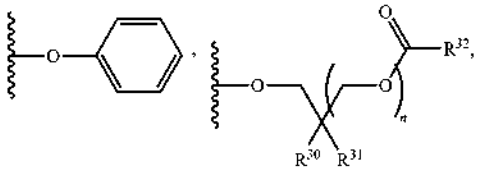


When R^{19} is

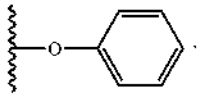
[0104]



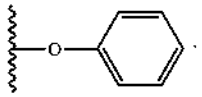
R^{28} and R^{29} can both be O^-. In some embodiments, neutralizing the charge on the phosphate of the nucleotide or nucleotide derivative may facilitate the entry of the nucleotides and nucleotides analogs in a cell. In some embodiments, R^{28} and R^{29} can each be independently



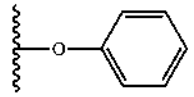
—O-naphthol and/or an —N-linked amino acid. In some embodiments, at least one of R^{28} and R^{29} can be



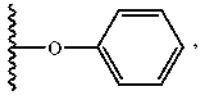
In an embodiment, R^{28} can be



In some embodiments, at least one of R^{28} and R^{29} can be an —N-linked linked amino acid. In an embodiment, R^{28} can be

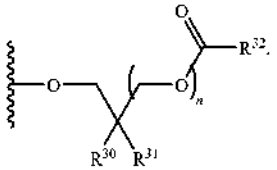


and R^{29} can be an —N-linked amino acid, such as those described herein. In another embodiment, when R^{28} is

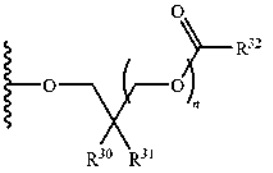


R^{29} cannot be an —N-linked amino acid.

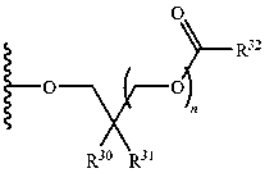
[0105] In an embodiment, at least one of R^{28} and R^{29} can be



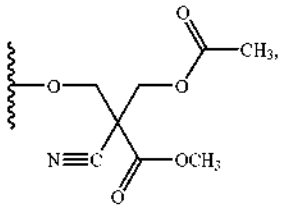
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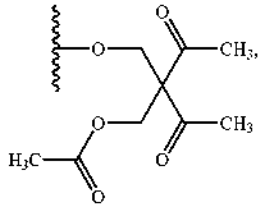


can vary. In some embodiments, R^{30} can be —C≡N and R^{31} can be an optionally substituted C_{1-8} alkoxycarbonyl such as —C(═O)OCH_3. In other embodiments, R^{30} can be —C≡N and R^{31} can be an optionally substituted C_{1-8} organylaminocarbonyl, for example, —C(═O)$NHCH_2CH_3$ and —C(═O)$NHCH_2CH_2$phenyl. In still other embodiments, both R^{30} and R^{31} can be an optionally substituted C_{1-8} organylcarbonyl. In an embodiment, both R^{30} and R^{31} can be —C(═O)CH_3. In yet still other embodiments, both R^{30} and R^{31} can be an optionally substituted C_{1-8} alkoxycarbonyl. In an embodiment, both R^{30} and R^{31} can be —C(═O)OCH_3 or —C(═O)OCH_2CH_3. In an embodiment, both R^{30} and R^{31} can be an optionally substituted C_{1-8} alkoxycarbonyl, for example —C(═O)OCH_2CH_3, and n can be 2. In some embodiments, including those in this paragraph, R^{32} can be an optionally substituted C_{1-6}-alkyl. In an embodiment, including those in this paragraph, R^{32} can be methyl or tert-butyl. Examples of

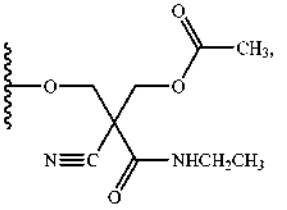


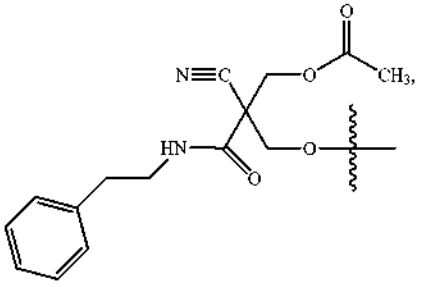
groups, include but are not limited to the following:

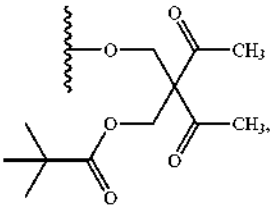


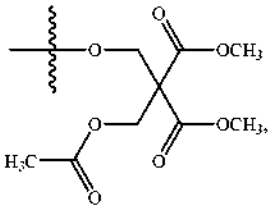


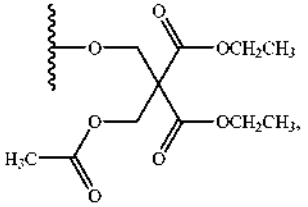
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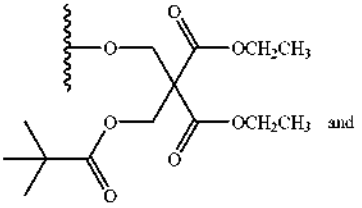




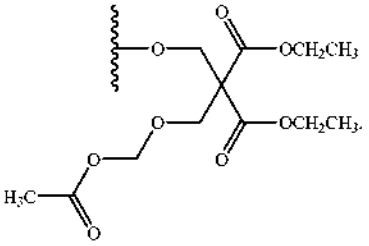




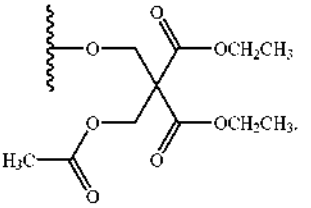




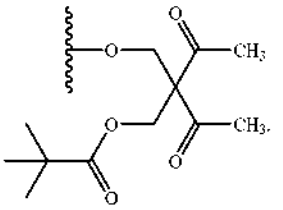
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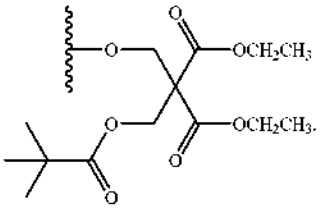
[0106] In an embodiment, at least one of R^{28} and R^{29} can be



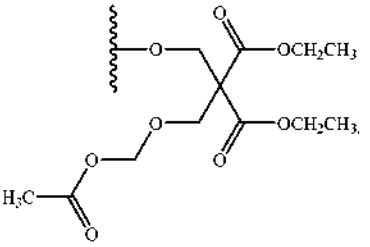
In another embodiment, at least one of R^{28} and R^{29} can be



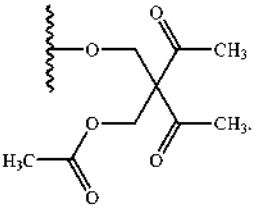
In still another embodiment, at least one of R^{28} and R^{29} can be



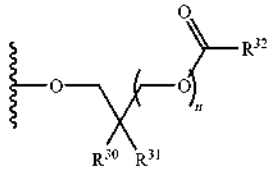
In yet still another embodiment, at least one of R^{28} and R^{29} can be



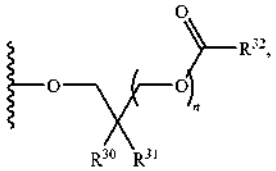
In some embodiments, at least one of R^{28} and R^{29} can be



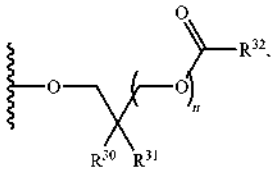
In some embodiments, both R^{28} and R^{29} can be



wherein each R^{30}, each R^{31}, each R^{32} and each n can be the same or different. In an embodiment, when R^{28} and R^{29} are

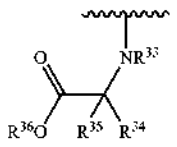


R^{28} and R^{29} can be the same. In another embodiment, when R^{28} and R^{29} are

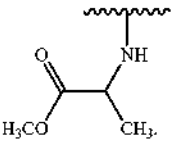


R^{28} and R^{29} can be different.

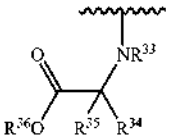
[0107] In some embodiments, at least one of R^{28} and R^{29} can be an —N-linked amino acid. Suitable amino acids include those described herein. In some embodiments, an —N-linked amino acid can have the structure



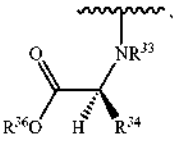
wherein: R^{33} can be hydrogen or an optionally substituted C_{1-4}-alkyl; R^{34} can be selected from hydrogen, an optionally substituted C_{1-6}-alkyl, an optionally substituted aryl, an optionally substituted aryl(C_{1-6} alkyl) and an optionally substituted haloalkyl; R^{35} can be hydrogen or an optionally substituted C_{1-6}-alkyl; and R^{36} can be selected from an optionally substituted C_{1-6} alkyl, an optionally substituted C_6 aryl, an optionally substituted C_{10} aryl, and an optionally substituted C_{3-6} cycloalkyl. In an embodiment, R^{33} can be hydrogen. In some embodiments, R^{34} can be an optionally substituted C_{1-6}-alkyl, for example, methyl. In an embodiment, R^{35} can be hydrogen or an optionally substituted C_{1-6}-alkyl. In an embodiment, R^{35} can be methyl. In some embodiment, R^{36} can be an optionally substituted C_{1-6}-alkyl. One example of a suitable an —N-linked amino acid is



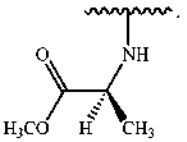
In some embodiments, the amino acid can be in the L-configuration. In other embodiments, the amino acid can be in the D-configuration. For example,



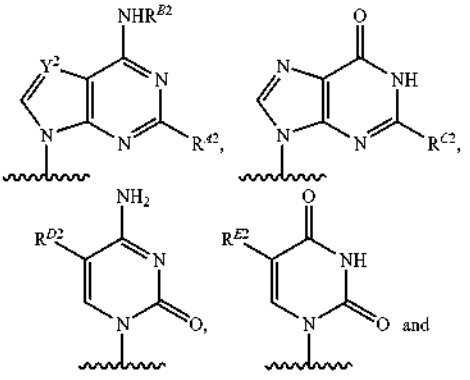
can be



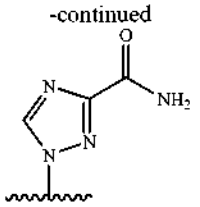
such as



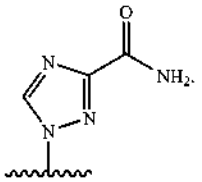
[0108] Various optionally substituted heterocyclic bases and optionally substituted heterocyclic base derivatives can be present in a compound of Formula (II). Examples of suitable optionally substituted heterocyclic bases and optionally substituted heterocyclic base derivatives are shown below.



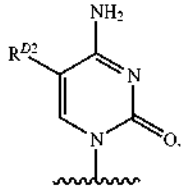
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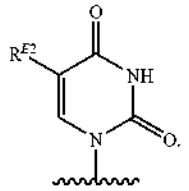
wherein: R^{A2} can be hydrogen or halogen; R^{B2} can be hydrogen, an optionally substituted C_{1-6}alkyl, or an optionally substituted C_{3-8} cycloalkyl; R^{C2} can be hydrogen or amino; R^{D2} can be hydrogen, halogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl and an optionally substituted C_{2-6} alkynyl; R^{E2} can be hydrogen, halogen, an optionally substituted C_{1-6}alkyl, an optionally substituted C_{2-6} alkenyl and an optionally substituted C_{2-6} alkynyl; and Y^2 can be N (nitrogen) or CR^{F2}, wherein R^{F2} can be selected from hydrogen, halogen, an optionally substituted C_{1-6}-alkyl, an optionally substituted C_{2-6}-alkenyl and an optionally substituted C_{2-6}-alkynyl. In some embodiments, B^2 can be



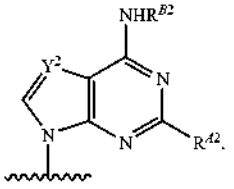
In other embodiments, B^2 can be



In yet other embodiments, B^2 can be



In yet still other embodiments, B^2 can be

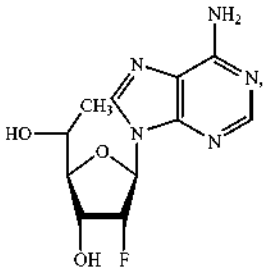


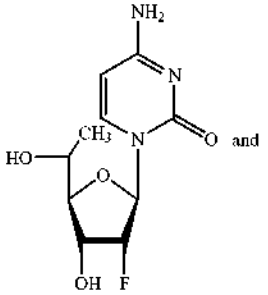
In an embodiment Y^2 can be nitrogen; R^{A2} can be hydrogen and R^{B2} can be hydrogen. In another embodiment, Y^2 can be CR^{F2}, wherein R^{F2} can be selected from hydrogen, halogen, an optionally substituted C_{1-6}-alkyl, an optionally substituted C_{2-6}-alkenyl and an optionally substituted C_{2-6}-alkynyl; R^{A2} can be hydrogen and R^{B2} can be hydrogen. When B^2 is any of the aforementioned moieties shown above, in some embodiments, A^2 can be carbon. In an embodiment, B^2 can be any of the aforementioned moieties shown above, A^2 can be carbon and D^2 can be oxygen. In some embodiments, B^2 can be any of the aforementioned moieties shown above, A^2 can be carbon, D^2 can be oxygen and each ------ can be a single bond.

[0109] In some embodiments, R^{22} can be selected from hydrogen, halogen, $—OR^{a2}$, —CN, $—N_3$ and an optionally substituted C_{1-6} alkyl. In some embodiments, R^{23} can be absent or selected from hydrogen, halogen, $—OR^{a2}$ and an optionally substituted C_{1-6} alkyl. In some embodiments, R^{24} can be absent or selected from hydrogen, halogen, $—NH_2$, $—OR^{a2}$, $—N_3$, an optionally substituted C_{1-6} alkyl and an —O-linked amino acid. In some embodiments, R^{24} can be $—OR^{a2}$, wherein R^{a2} is hydrogen. In other embodiments, R^{24} can be an —O-linked amino acid. In some embodiments, R^{25} can be selected from hydrogen, halogen, $—OR^{a2}$, —CN, —NC, an optionally substituted C_{1-6} alkyl and an —O-linked amino acid. In some embodiments, R^{25} can be $—OR^{a2}$, wherein R^{a2} is hydrogen. In other embodiments, R^{25} can be a C_{1-6} alkoxy such as methoxy. In still other embodiments, R^{25} can be an —O-linked amino acid. In some embodiments, both R^{24} and R^{25} can be hydroxy groups. In other embodiments, R^{25} can be a hydroxyl group and R^{24} can be an —O-linked amino acid. Suitable —O-linked amino acids are described herein. In some embodiments, R^{26} can be selected from hydrogen, halogen, an optionally substituted C_{1-6} alkyl, an optionally substituted haloalkyl, an optionally substituted hydroxyalkyl, and the bond to R^{25} indicated by ------ is a double bond, R^{25} is a C_{2-6} alkenyl and R^{26} is absent. In some embodiments, R^{27} can be selected from hydrogen, halogen and an optionally substituted C_{1-6} alkyl.

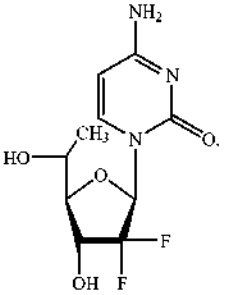
[0110] In some embodiments, at least one of R^{25} and R^{26} can be a halogen. In other embodiments, both R^{25} and R^{26} can be a halogen.

[0111] Examples of compounds of Formula (II) are shown below.



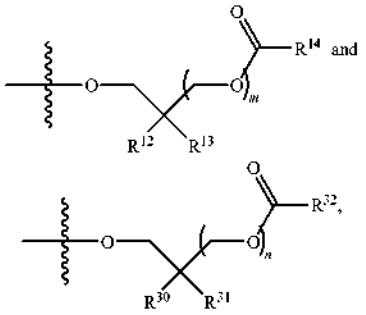


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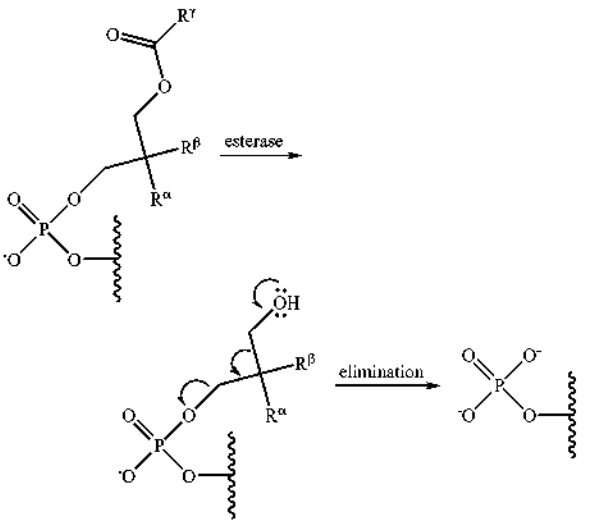
[0112] In some embodiments, B^1 and B^2 cannot be an optionally substituted pyridinyl group, an optionally substituted tricyclic heterocyclic group, an optionally substituted piperizinyl, an optionally substituted pyrrolo-pyrimidinone, a triazole substituted with an amidine, an optionally substituted pyrido-pyrimidine. In some embodiments, B^1 and B^2 cannot be any of moieties attached to the 1'-position disclosed in U.S. Application Nos. 2006-0229265 (filed Mar. 30, 2006), 2005-0203044 (filed Jan. 26, 2005) and 2007-0258921 (filed Apr. 30, 2007); U.S. Pat. No. 7,268,119 (filed Feb. 14, 2007), U.S. Pat. No. 6,815,542 (filed Dec. 13, 2002), U.S. Pat. No. 6,495,677 (filed Jun. 16, 2000), U.S. Pat. No. 7,081,449 (filed Jul. 3, 2001), U.S. Pat. No. 6,130,326 (filed Apr. 14, 1999), U.S. Pat. No. 6,552,183 (filed Aug. 7, 2000) U.S. Pat. No. 6,573,248 (Dec. 31, 2001) U.S. Pat. No. 6,642,206 (Apr. 9, 2002), U.S. Pat. No. 5,767,097 (filed Jan. 23 1996); International Publication Nos. WO 2004/106356 (filed May 27, 2004), WO 2004/080466 (filed Mar. 7, 2003), WO 03/039523 (filed Nov. 5, 2002); and Canadian Patent No. 02252144 (filed Oct. 26, 1998).

[0113] As stated previously, in some embodiments, neutralizing the charge on the phosphate group may facilitate the penetration of the cell membrane by compounds of Formulae (I) and (II) by making the compound more lipophilic. Furthermore, it is believed that the 2,2-disubstituted-acyl(oxyalkyl) groups, such as



attached to the phosphate impart increased plasma stability to compounds of Formulae (I) and (II) by inhibiting the degradation of the compound. Once inside the cell, the 2,2-disubstituted-acyl(oxyalkyl) group attached to the phosphate can be easily removed by esterases via enzymatic hydrolysis of the acyl group. The remaining portions of the group on the phosphate can then be removed by elimination. The general reaction scheme is shown below in Scheme 1a.

Scheme 1a

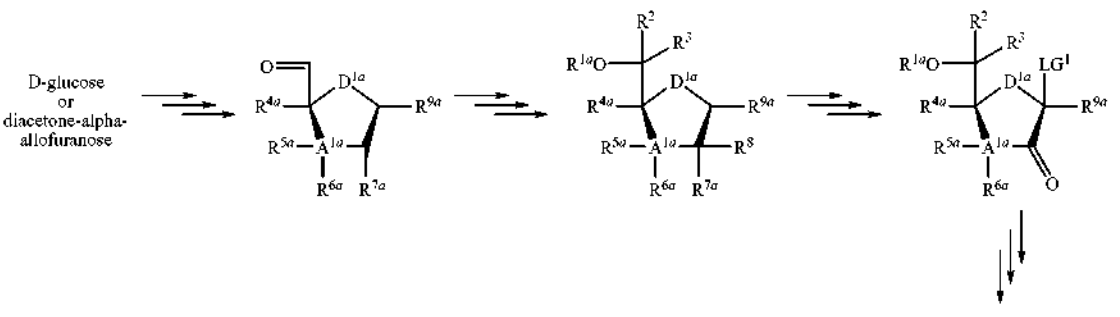


[0114] A further advantage of the 2,2-disubstituted-acyl (oxyalkyl) groups described herein is the rate of elimination of the remaining portion of the 2,2-disubstituted-acyl(oxyalkyl) group is modifiable. Depending upon the identity of the substituents on the 2-carbon, shown in Scheme 1a as R^{α} and R^{β}, the rate of elimination may be adjusted from several seconds to several hours. As a result, the removal of the remaining portion of the 2,2-disubstituted-acyl(oxyalkyl) group can be retarded, if necessary, to enhance cellular uptake but, readily eliminated upon entry into the cell. Upon removal of the groups on the oxygen atoms of the phosphate, the resulting nucleotide analog possesses a monophosphate. Thus, the necessity of an initial intracellular phosphorylation is no longer a prerequisite to obtaining the biologically active phosphorylated form.

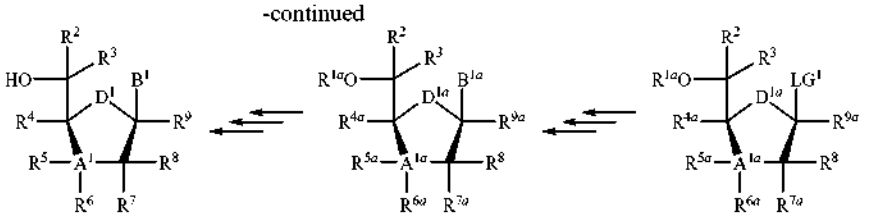
Synthesis

[0115] Compounds of Formulae (I) and (II), and those described herein may be prepared in various ways. General synthetic routes to the compounds of Formulae (I) and (II), and the starting materials used to synthesize the compounds of Formulae (I) and (II) are shown in Schemes 1-3 and FIGS. **1-3**. The routes shown are illustrative only and are not intended, nor are they to be construed, to limit the scope of the claims in any manner whatsoever. Those skilled in the art will be able to recognize modifications of the disclosed synthesis and to devise alternate routes based on the disclosures herein; all such modifications and alternate routes are within the scope of the claims.

Scheme 2



-continued



[0116] One method for forming a compound of Formula (I) is shown in Scheme 2 in which R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, A^1, B^1 and D^1 can be the same as disclosed herein, and R^{1a} can be hydrogen or a protecting group. Examples of suitable protecting groups include, but are not limited to, an optionally substituted benzoyl and silyl ethers such as trimethylsilyl (TMS), tert-butyldimethylsilyl (TBDMS), triisopropylsilyl (TIPS) and tert-butyldiphenylsilyl (TBDPS). Also, in Scheme 2, R^{4a}, R^{5a}, R^{6a}, R^{7a}, R^{9a}, A^{1a}, B^{1a} and D^{1a} can be the same as R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively, or can be each a protected version of R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively. By "protected versions, the substituents listed herein for R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1 may be altered to include one or more protecting groups. For example, the hydrogen of a hydroxy group may be exchanged for a protecting group, two hydroxy groups may be cyclized to form an acetal or an ortho-ester, the hydrogen on a NH group may be exchanged for a protecting group and/or one or both hydrogens on a —NH_2 group may be replaced for one or more protecting groups. Additionally, in Scheme 2, LG^1 can be a suitable leaving group, such as those described herein.

[0117] A five membered heterocyclic ring can be formed via an addition/cyclization reaction from D-glucose. In some embodiments, the five-membered heterocyclic ring can be an optionally substituted ribose sugar. In other embodiments, the five membered can be an optionally substituted deoxyribose sugar. Alternatively, diacetone-alpha-allofuranose, a commercially available reagent can be used.

[0118] The 5'-OH group can be oxidized to an aldehyde using methods known to those skilled in the art. Suitable oxidizing agents include, but are not limited to, Dess-Martin periodinane, TPAP/NMO (tetrapropylammonium perruthenate/N-methylmorpholine N-oxide), Swern oxidation reagent, PCC (pyridinium chlorochromate), and/or PDC (pyridinium dichromate), sodium periodate, Collin's reagent, ceric ammonium nitrate CAN, $Na_2Cr_2O_7$ in water, Ag_2CO_3 on celite, hot HNO_3 in aqueous glyme, O_2-pyridine CuCl, $Pb(OAc)_4$-pyridine and benzoyl peroxide-$NiBr_2$.

[0119] An optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl or an optionally substituted C_{1-6} haloalkyl can be added to the 5'-carbon using methods known to those skilled in the art. For example, an optionally substituted C_{1-6} alkyl or an optionally substituted C_{1-6} haloalkyl can be added to the 5'-carbon using alkylation methods are known to those skilled in the art, such as through the use of an organometallic moiety. A non-limiting list of suitable organometallic moieties include organomagnesium compounds, organolithium compounds, organotin compounds, organocuprates compounds, organozinc, and organopalladium compounds, metal carbonyls, metallocenes, carbine complexes, and organometalloids (e.g., organoboranes and organo silanes). In some embodiments, the organometallic moiety can be an organomagnesium compound. In an embodiment, the organomagnesium compound can be an optionally substituted C_{1-6} alkyl or an optionally substituted C_{1-6} haloalkyl-Mg-halo, for example, MeMgBr.

[0120] If not already present, addition of an optionally substituted C_{1-6} alkyl to the 2'-position can also be accomplished using methods known to a person of ordinary skill in the art. When a hydroxy group is present on the 2'-position, in some embodiment, the hydroxy group can be oxidized to a ketone using one or more suitable methods. For example, the hydroxy group can be oxidized to a ketone using one or more oxidizing agents. Suitable oxidizing agent include, but are not limited to, acid dichromates, $KMnO_4$, Br_2, MnO_2, ruthenium tetraoxide, Jones reagent, Collin' s reagent, Corey's reagent, pyridnium dichromate, Swern oxidation reagent, DMSO and trifluoroacetic anhydride (TFAA), and those previously described herein. In an embodiment, the oxidizing agent can be Dess-Martin periodinane or DMSO and TFAA.

[0121] An optionally substituted C_{1-6} alkyl can be added to the 2'-carbon using methods known to those skilled in the art. In some embodiments, the 2'-carbon can be alkylated using a suitable organometallic moiety such as those described herein. In an embodiment, the organometallic moiety can be MeMgBr.

[0122] The substitutent at the 1'-position can be converted to an appropriate leaving group, for example a nucleofuge, using methods known to those skilled in the art. For example, the 1'-position can be converted to an appropriate leaving group via an hydrolysis reaction followed by acetylation using a suitable reagent such as acetic anhydride. As another example, the 1'-position can be converted to an appropriate leaving group by transforming the acetal to a hemiacetal under acid conditions followed by acetylation with an appropriate reagent (e.g., acetic anhydride).

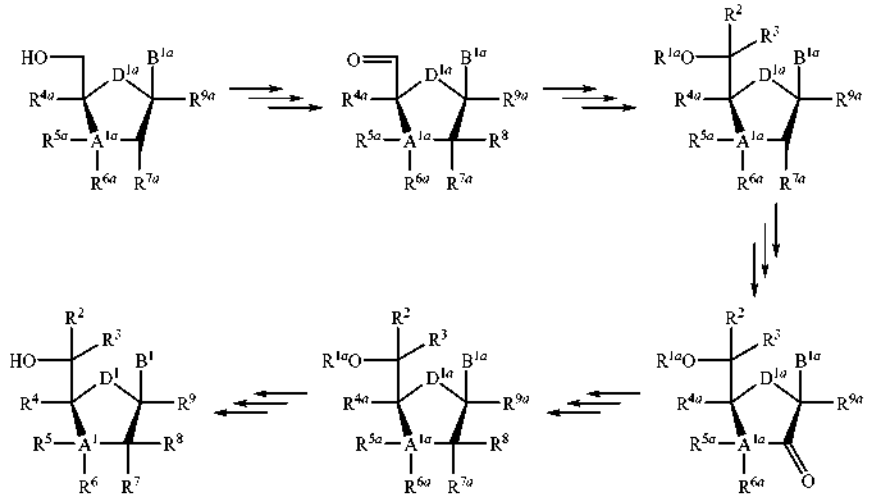
[0123] An optionally substituted heterocyclic base or an optionally substituted heterocyclic base derivative can be added to the 1'-position using a catalyst. Suitable catalysts are known in the art. In an embodiment, the catalysts can be trimethylsilyl trifluoromethanesulfonate. To facilitate the reaction, in some embodiments, the addition of the optionally substituted heterocyclic base or the optionally substituted heterocyclic base derivative can take place in the presence of a base. Examples of suitable bases include amine-based bases such as triethylamine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN). After addition of the optionally substituted heterocyclic base or the optionally substituted heterocyclic base derivative, a compound of Formula (I) in which R^1 is H can be obtained after removal of any protecting groups that may be present.

[0124] If needed and/or desired, any hydroxy groups present on the 2', 3' and 4'-positions can be protected with one or more suitable protecting groups. The hydroxy groups can be protected with an individual protecting group. Alternatively, two adjacent hydroxy groups can be cyclized to form

an acetal or an ortho ester. In some embodiments, some of the hydroxy groups can be protected with individual protecting groups and other hydroxy groups can be protected through the formation of an acetal or an ortho ester.

[0125] Alternatively, if an optionally substituted heterocyclic base or an optionally substituted heterocyclic base derivative is already present on the 5-membered heterocyclic ring, an optionally substituted C_{1-6} alkyl or an optionally substituted C_{1-6} haloalkyl (e.g., CF_3) can be added to the 5'-position as shown below in Scheme 3. The substituents R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, A^1, B^1 and D^1 can be the same as disclosed herein, and R^{4a}, R^{5a}, R^{6a}, R^{7a}, R^{9a}, A^{1a}, B^{1a} and D^{1a} can be the same as R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively, or can be each a protected version of R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively. R^{1a} can be hydrogen or a protecting group, including those described herein.

Scheme 3



[0126] As described herein, the hydroxy group at the 5'-position can be oxidized to aldehyde using a suitable oxidizing reagent such as those described herein. An optionally substituted C_{1-6} alkyl or an optionally substituted C_{1-6} haloalkyl can be added the 5'-position using an appropriate alkylation method. Appropriate alkylation methods are described herein. In an embodiment, the 5'-position can be alkylated using an organometallic reagent, for example, an organomagnesium compound.

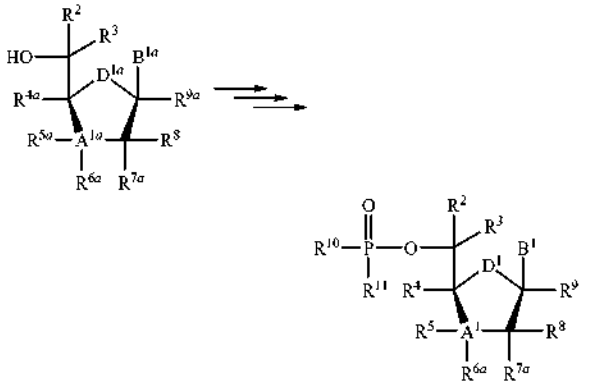
[0127] If an optionally substituted C_{1-6} alkyl is not already present on the 2'-position, the optionally substituted C_{1-6} alkyl can be added using known to those skilled in the art. For example, when a hydroxy group is present on the 2'-position, in some embodiment, the hydroxy group can be oxidized to a ketone using one or more suitable methods. In an embodiment, the hydroxy group can be oxidized to a ketone using one or more oxidizing agents disclosed herein. An optionally substituted C_{1-6} alkyl can then be added to the 2'-carbon using methods known to those skilled in the art. In some embodiments, the 2'-carbon can be alkylated using a suitable organometallic moiety such as those described herein. In an embodiment, the organometallic moiety can be MeMgBr.

[0128] If needed and/or desired, the optionally substituted heterocyclic base or the optionally substituted heterocyclic base derivative can be protected with one or more suitable protecting groups during the formation of a compound of Formula (I). For example, one or more amino groups attached to a ring and/or any —NH groups present in a ring of the optionally substituted heterocyclic base and/or optionally substituted heterocyclic base derivative can be protected with one or more suitable protecting groups. In an embodiment, the optionally substituted heterocyclic base and/or optionally substituted heterocyclic base derivative can be protected with one or more triarylmethyl protecting groups. A non-limiting list of triarylmethyl protecting groups are trityl, monomethoxytrityl (MMTr), 4,4'-dimethoxytrityl (DMTr), 4,4',4"-trimethoxytrityl (TMTr), 4,4',4"-tris-(benzoyloxy)trityl (TBTr), 4,4',4"-tris (4,5-dichlorophthalimido)trityl (CPTr), 4,4',4"-tris(levulinyloxy)trityl (TLTr), p-anisyl-1-naphthylphenylmethyl, di-o-anisyl-1-naphthylmethyl, p-tolyldipheylmethyl, 3-(imidazolylmethyl)-4,4'-dimethoxytrityl, 9-phenylxanthen-9-yl (Pixyl), 9-(p-methoxyphenyl) xanthen-9-yl (Mox), 4-decyloxytrityl, 4-hexadecyloxytrityl, 4,4'-dioctadecyltrityl, 9-(4- octadecyloxyphenyl)xanthen-9-yl, 1,1'-bis-(4-methoxyphenyl)-1'-pyrenylmethyl, 4,4',4"-tris-(tert-butylphenyl)methyl (TTTr) and 4,4'-di-3,5-hexadienoxytrityl. Any protecting groups on the 5-membered heterocyclic ring can also be protected with one or more suitable protecting groups, including those described herein.

[0129] The protecting groups can be removed and other protecting groups can be added at different times during the general reaction schemes shown in Schemes 2 and 3, for example, before the formation of the aldehyde at the 5'-position, after the alkylation of the 5'-position, before the oxidation of the 2'-position, after alkylation of the 2'-position, before the addition of the optionally substituted heterocyclic base or optionally substituted heterocyclic base derivative and/or after the addition of the optionally substituted heterocyclic base or optionally substituted heterocyclic base derivative. Removal and replacement of a protecting group may be useful because of the reactions conditions. The protecting groups may assistant in preventing unwanted side reaction and/or make the separation of the desired product simpler

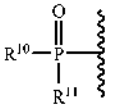
[0130] A phosphate group can be added to 5'-position as shown in Scheme 4. The substituents R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, A^1, B^1 and D^1 can be the same as disclosed herein, and R^{4a}, R^{5a}, R^{6a}, R^{7a}, R^{9a}, A^{1a}, B^{1a} and D^{1a} can be the same as R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively, or can be each a protected version of R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively.

Scheme 4

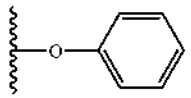


[0131] A variety of methods can be used to add a phosphate group to the 5'-position. Suitable methods are described in Current Protocals in Nucleic Acid Chemistry. Donald E. Bergstrom *Nucleoside Phosphorylation and Related Modifications* in Current Protocals in Nucleic Acid Chemistry, Chapter 1, (2008) John Wiley & Sons, Inc. For example, a phosphate at the 5'-position can be formed via a phosphoamidite and oxidation methods.

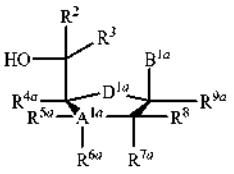
[0132] To add a



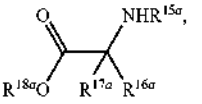
group wherein one of R^{10} and R^{11} is



and the other of R^{10} and R^{11} is an —N-linked amino acid, a (O-phenyl-N-linked amino acid))phosphoramidohalide can be reacted with the 5'-position of a nucleoside or a nucleoside derivative, such as

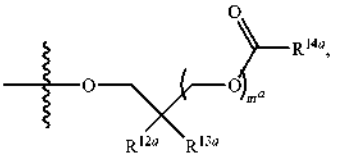


where R^2, R^3 and R^8 can be the same as previously defined herein, and R^{4a}, R^{51}, R^{6a}, R^{7a}, R^{9a}, A^{1a}, B^{1a} and D^{1a} can be the same as R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively, or can be each a protected version of R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively. A variety of amino acids can be used to form the —N-linked amino acid. In some embodiments, the amino acid can have the following structure



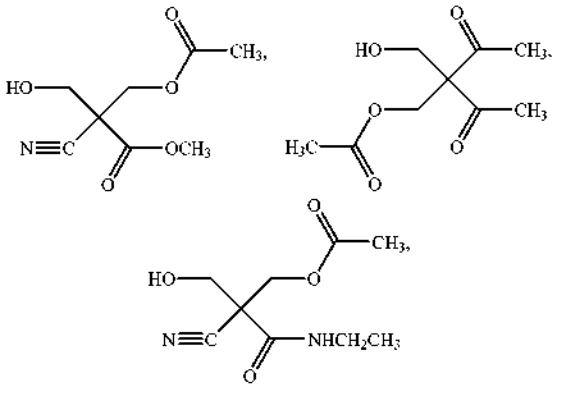
wherein R^{15a}, R^{16a}, R^{17a}, and R^{18a} can be the same as R^{15}, R^{16}, R^{17} and R^{18}, as described herein with respect to Formula (I). If needed and/or desired, any hydroxy groups present on the 5-membered heterocyclic ring can be protected with one or more protecting groups such as those described herein. In some embodiments, any hydroxy groups on the 2'- and 3'-positions can be protected with one or more protecting groups. For example, when the 5' -membered heterocyclic ring has hydroxy groups at the 2'- and 3'-positions, the oxygens can be protected by forming an acetal or an ortho ester.

[0133] The hydroxy precursor,

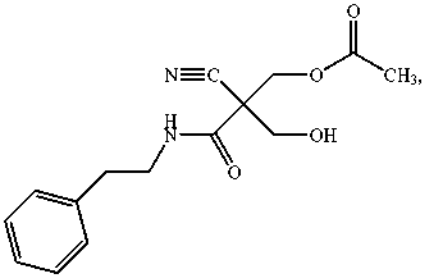


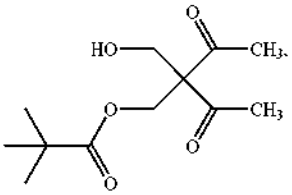
in which R^{12a}, R^{13a}, R^{14a} and m^a are the same as R^{12}, R^{13}, R^{14} and m, respectively, as described herein, of the 2,2-disubstituted-acyl(oxyalkyl) groups can be synthesized according in a manner similar to those described in the following articles. Ora, et al., *J. Chem. Soc. Perkin Trans.* 2, 2001 6: 881-5; Poijärvi, P. et al., *Helv. Chim. Acta.* 2002 85:1859-76; Poijarvi, P. et al., *Lett. Org. Chem.*, 2004, 1:183-88; and Poijärvi, P. et al., *Bioconjugate Chem.*, 2005 16(6):1564-71, all of which are hereby incorporated by reference in their entireties.

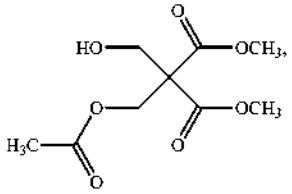
[0134] Examples of hydroxy precursors can include the following:

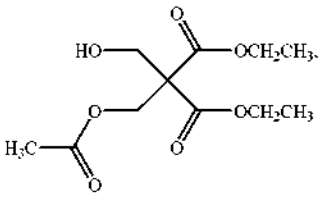


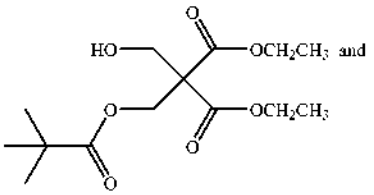
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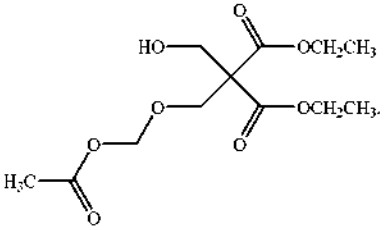




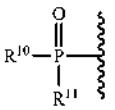




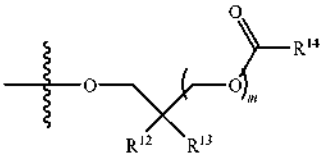




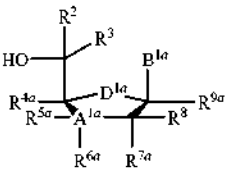
[0135] To add a



group wherein one of R^{10} and R^{11} is

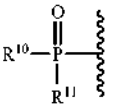


and the other R^{10} and R^{11} is an —N-linked amino acid, diphenylphosphite can be reacted with one or more of the hydroxy precursors described herein, a nucleoside or nucleoside derivative (for example,

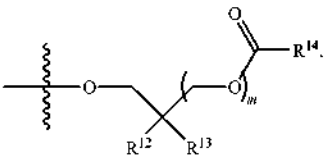


where R^2, R^3 and R^8 can be the same as previously defined herein, and R^{4a}, R^{5a}, R^{6a}, R^{7a}, R^{9a}, A^{1a}, B^{1a} and D^{1a} can be the same as R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively, or can be each a protected version of R^4, R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively), an amino acid, and a suitable oxidizing agent to form a compound of Formula (I). As previously discussed, various amino acids can be used, including those described herein Likewise, any suitable oxidizing agent can be used. In an embodiment, the oxidizing agent can be carbon tetrachloride (CCl_4). In some embodiments, the oxidizing agent, such as CCl_4, oxidizes the phosphorus from (III) to (V).

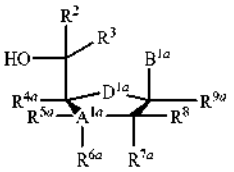
[0136] Various methods can also be used to add a



group wherein R^{10} and R^{11} are

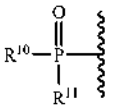


In some embodiments, diphenylphosphite can be reacted with one or more of the hydroxy precursors described herein, a nucleoside or nucleoside derivative (such as

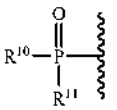


where R^2, R^3 and R^8 can be the same as previously defined herein, and R^{4a}, R^{5a}, R^{6a}, R^{7a}, R^{9a}, A^{1a}, B^{1a} and D^{1a} can be the same as R^4 R^5, R^6, R^7, R^9, A^1, B^1 and D^1, respectively, or can be each a protected version of R^4, R^5, R^6R^7, R^9, A^1, B^1 and D^1, respectively) and a suitable oxidizing agent.

[0137] If desired and/or needed, one or more suitable protecting groups, including those described herein, can be used to protect the optionally substituted heterocyclic base, the optionally substituted heterocyclic base derivative, and/or any hydroxy groups presented on the 5-membered heterocyclic ring. For example, any hydroxy groups can be protected with individual protecting groups, as acetals and/or as ortho esters. Similarly, one or more amino groups attached to a ring and/or any —NH groups present in a ring of the optionally substituted heterocyclic base and/or optionally substituted heterocyclic base derivative can be protected with one or more suitable protecting groups, for example, one or more triarylmethyl protecting groups. As discussed herein, the protecting groups can be removed, replaced and exchanged at different times during the formation of a compound of Formula (I). For example, a variety of protecting groups can be used to protect the optionally substituted heterocyclic base and/or optionally substituted heterocyclic base derivative when the



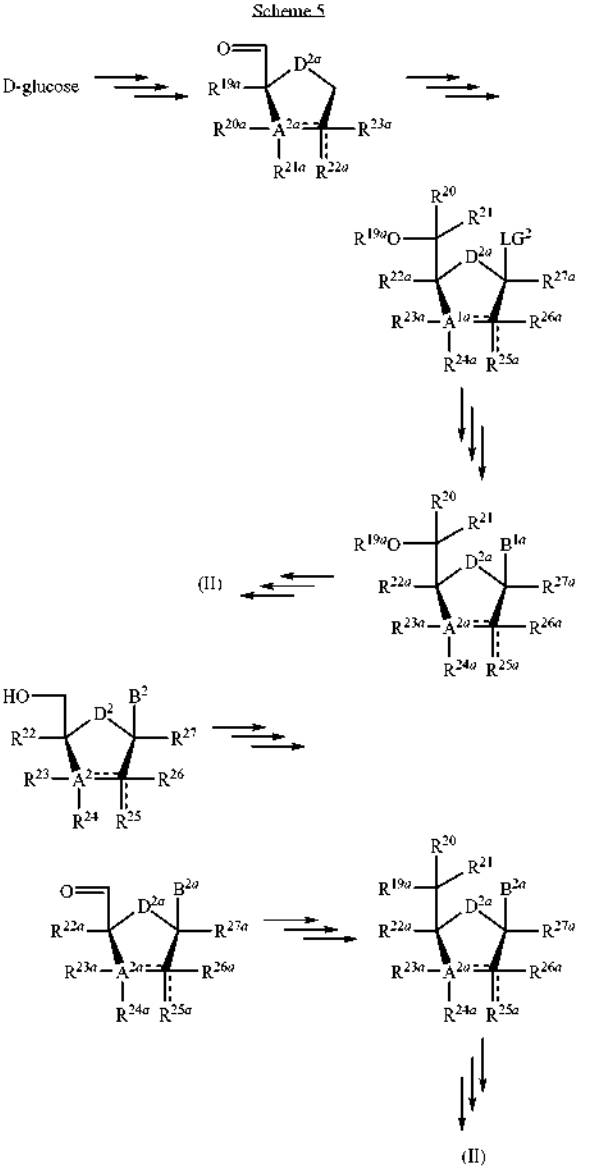
moiety is added to the 5'-position. Suitable protecting groups are known to those skilled in the art, including those described herein. The protecting groups present on the optionally substituted heterocyclic base and/or optionally substituted heterocyclic base derivative can be removed and other protecting groups can be added at different times during the addition of the phosphate groups. Likewise, any protecting groups present on the optionally substituted 5-membered heterocyclic ring can be removed and/or changed at different times during the addition of the



moiety. In some instances, removal and replacement of a protecting group may be useful because of the reactions conditions. The protecting groups can also assistant in preventing unwanted side reaction and/or make the separate of the desired product more facile.

[0138] In situations where the optionally substituted heterocyclic ring already has an optionally substituted C_{1-6} alkyl at the 2'-position, the steps needed to add an optionally substituted C_{1-6} alkyl at the 2'-position may be omitted.

Scheme 5

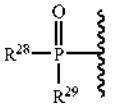


[0139] Compounds of Formula (II) can be formed using methods similar to those as described herein with respect to the preparation of compounds of Formula (I). As shown above in Scheme 5, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl or an optionally substituted C_{1-6} haloalkyl can be added to the 5'-position after the 5'-position has been oxidized to aldehyde using one or more suitable reagents. The substituents R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, A^2, B^2 and D^2 can be the same as disclosed herein, and R^{22a}, R^{23a}, R^{24a}, R^{25a}, R^{26a}, R^{27a}, A^{2a}, B^{2a} and D^{2a} can be the same as R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, A^2, B^2 and D^2, respectively, or can be each a protected version of R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, A^2, B^2 and D^2,

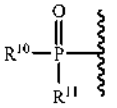
respectively. The substituent R^{19a} can be hydrogen or a protecting group, and LG^2 can be a suitable leaving group. Examples of suitable protecting groups include, but are not limited to, an optionally substituted benzoyl and silyl ethers such as trimethylsilyl (TMS), tert-butyldimethylsilyl (TBDMS), triisopropylsilyl (TIPS) and tert-butyldiphenylsilyl (TBDPS).

[0140] If an optionally substituted heterocyclic base or an optionally substituted heterocyclic base derivative is not already present on the 5-membered heterocyclic ring, the optionally substituted heterocyclic base or an optionally substituted heterocyclic base can added using methods known to those skilled in the art. For example, the substitutent at the 1'-position can be converted to an appropriate leaving group, for example a nucleofuge, using methods known to those skilled in the art. As an example, the 1'-position can be converted to an appropriate leaving group via an hydrolysis reaction followed by acetylation using a suitable reagent such as acetic anhydride. As another example, the 1'-position can be converted to an appropriate leaving group by transforming the acetal to a hemiacetal under acid conditions followed by acetylation with an appropriate reagent (e.g., acetic anhydride).

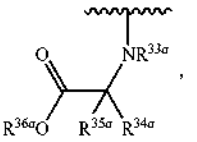
[0141] An optionally substituted heterocyclic base or an optionally substituted heterocyclic base derivative can be added to the 1'-position using a catalyst. Suitable catalysts are known in the art. In an embodiment, the catalysts can be trimethylsilyl trifluoromethanesulfonate. To facilitate the reaction, in some embodiments, the addition of the optionally substituted heterocyclic base or the optionally substituted heterocyclic base derivative can take place in the presence of a base. Examples of suitable bases include amine-based bases such as triethylamine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN). After addition of the optionally substituted heterocyclic base or the optionally substituted heterocyclic base derivative, a compound of Formula (II) in which R^{19} is H can be obtained after removal of any protecting groups that may be present. A



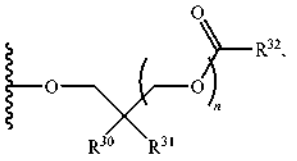
moiety can be added to the 5'-position using the same or similar methods for adding



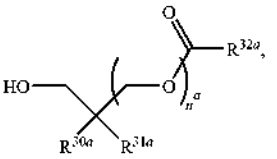
described herein. When one of R^{28} and R^{29} is an —N-linked amino acid, in some embodiments, the amino acid can have the structure,



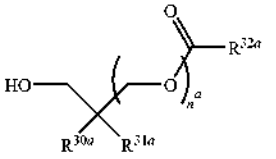
wherein R^{33a}, R^{34a}, R^{35a}, and R^{36a} can be the same as R^{33}, R^{34}, R^{35} and R^{36}, as described herein with respect to Formula (II). In an embodiment, when one of R^{28} and R^{29} is



the hydroxy precursor can have the structure,

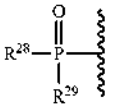


wherein R^{30a}, R^{31a}, R^{32a}, and n^a are the same as R^{30}, R^{31}, R^{32} and n, respectively, as described herein. Examples of suitable hydroxy precursors having the structure



and method of obtaining the same are previously described herein.

[0142] If desired and/or needed, one or more suitable protecting groups, including those described herein, can be used to protect the optionally substituted heterocyclic base, the optionally substituted heterocyclic base derivative, and/or any hydroxy groups presented on the 5-membered heterocyclic ring during the synthesis of a compound of Formula (II). For example, any hydroxy groups can be protected with individual protecting groups, as acetals and/or as ortho esters. Similarly, one or more amino groups attached to a ring and/or any —NH groups present in a ring of the optionally substituted heterocyclic base and/or optionally substituted heterocyclic base derivative can be protected with one or more suitable protecting groups, for example, one or more triarylmethyl protecting groups. As discussed herein, the protecting groups can be removed, replaced and exchanged at different times during the formation of a compound of Formula (II), for example, during the addition of a



group.

Pharmaceutical Compositions

[0143] An embodiment described herein relates to a pharmaceutical composition, that can include a therapeutically effective amount of one or more compounds described herein (e.g., a compound of Formula (I) and/or a compound of Formula (II)) and a pharmaceutically acceptable carrier, diluent, excipient or combination thereof.

[0144] The term "pharmaceutical composition" refers to a mixture of a compound disclosed herein with other chemical components, such as diluents or carriers. The pharmaceutical composition facilitates administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to, oral, intramuscular, intraocular, intranasal, intravenous, injection, aerosol, parenteral, and topical administration. Pharmaceutical compositions can also be obtained by reacting compounds with inorganic or organic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. Pharmaceutical compositions will generally be tailored to the specific intended route of administration.

[0145] The term "physiologically acceptable" defines a carrier, diluent or excipient that does not abrogate the biological activity and properties of the compound.

[0146] As used herein, a "carrier" refers to a compound that facilitates the incorporation of a compound into cells or tissues. For example, without limitation, dimethyl sulfoxide (DMSO) is a commonly utilized carrier that facilitates the uptake of many organic compounds into cells or tissues of a subject.

[0147] As used herein, a "diluent" refers to an ingredient in a pharmaceutical composition that lacks pharmacological activity but may be pharmaceutically necessary or desirable. For example, a diluent may be used to increase the bulk of a potent drug whose mass is too small for manufacture or administration. It may also be a liquid for the dissolution of a drug to be administered by injection, ingestion or inhalation. A common form of diluent in the art is a buffered aqueous solution such as, without limitation, phosphate buffered saline that mimics the composition of human blood.

[0148] As used herein, an "excipient" refers to an inert substance that is added to a pharmaceutical composition to provide, without limitation, bulk, consistency, stability, binding ability, lubrication, disintegrating ability etc., to the composition. A "diluent" is a type of excipient.

[0149] The pharmaceutical compositions described herein can be administered to a human patient per se, or in pharmaceutical compositions where they are mixed with other active ingredients, as in combination therapy, or carriers, diluents, excipients or combinations thereof. Proper formulation is dependent upon the route of administration chosen. Techniques for formulation and administration of the compounds described herein are known to those skilled in the art.

[0150] The pharmaceutical compositions disclosed herein may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or tableting processes. Additionally, the active ingredients are contained in an amount effective to achieve its intended purpose. Many of the compounds used in the pharmaceutical combinations disclosed herein may be provided as salts with pharmaceutically compatible counterions.

[0151] Suitable routes of administration may, for example, include oral, rectal, topical transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intravenous, intramedullary injections, as well as intrathecal, direct intraventricular, intraperitoneal, intranasal, intraocular injections or as an aerosol inhalant.

[0152] One may also administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into the infected area, often in a depot or sustained release formulation. Furthermore, one may administer the compound in a targeted drug delivery system, for example, in a liposome coated with a tissue-specific antibody. The liposomes will be targeted to and taken up selectively by the organ.

[0153] The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied with a notice associated with the container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, may be the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert. Compositions that include a compound disclosed herein formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Methods of Use

[0154] One embodiment disclosed herein relates to a method of treating and/or ameliorating a disease or condition that can include administering to a subject a therapeutically effective amount of one or more compounds described herein, such as a compound of Formula (I) and/or a compound of Formula (II), or a pharmaceutical composition that includes a compound described herein.

[0155] Some embodiments disclosed herein relate to a method of ameliorating or treating a neoplastic disease that can include administering to a subject suffering from the neoplastic disease a therapeutically effective amount of one or more compounds described herein (e.g., a compound of Formula (I) and/or a compound of Formula (II)) or a pharmaceutical composition that includes one or more compounds described herein. In an embodiment, the neoplastic disease can be cancer. In some embodiments, the neoplastic disease can be a tumor such as a solid tumor. In an embodiment, the neoplastic disease can be leukemia. Examples of leukemias include, but are not limited to, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and juvenile myelomonocytic leukemia (JMML).

[0156] An embodiment disclosed herein relates to a method of inhibiting the growth of a tumor that can include administering to a subject having the tumor a therapeutically effective amount of one or more compounds described herein or a pharmaceutical composition that includes one or more compounds described herein.

[0157] Other embodiments disclosed herein relates to a method of ameliorating or treating a viral infection that can include administering to a subject suffering from the viral infection a therapeutically effective amount of one or more compounds described herein or a pharmaceutical composition that includes one or more compounds described herein. In an embodiment, the viral infection can be caused by a virus selected from an adenovirus, an Alphaviridae, an Arbovirus, an Astrovirus, a Bunyaviridae, a Coronaviridae, a Filoviridae, a Flaviviridae, a Hepadnaviridae, a Herpesviridae, an Alphaherpesvirinae, a Betaherpesvirinae, a Gammaherpesvirinae, a Norwalk Virus, an Astroviridae, a Caliciviridae, an Orthomyxoviridae, a Paramyxoviridae, a Paramyxoviruses, a Rubulavirus, a Morbillivirus, a Papovaviridae, a Parvoviridae, a Picornaviridae, an Aphthoviridae, a Cardioviridae, an Enteroviridae, a Coxsackie virus, a Polio Virus, a Rhinoviridae, a Phycodnaviridae, a Poxviridae, a Reoviridae, a Rotavirus, a Retroviridae, an A-Type Retrovirus, an Immunodeficiency Virus, a Leukemia Viruses, an Avian Sarcoma Viruses, a Rhabdoviruses, a Rubiviridae and/or a Togaviridae. In an embodiment, the viral infection is a hepatitis C viral infection. In another embodiment, the viral infection is a HIV infection.

[0158] One embodiment disclosed herein relates to a method of ameliorating or treating a parasitic disease that can include administering to a subject suffering from the parasitic disease a therapeutically effective amount of one or more compounds described herein or a pharmaceutical composition that includes one or more compounds described herein. In an embodiment, the parasite disease can be Chagas' disease.

[0159] As used herein, a "subject" refers to an animal that is the object of treatment, observation or experiment. "Animal" includes cold- and warm-blooded vertebrates and invertebrates such as fish, shellfish, reptiles and, in particular, mammals. "Mammal" includes, without limitation, mice, rats, rabbits, guinea pigs, dogs, cats, sheep, goats, cows, horses, primates, such as monkeys, chimpanzees, and apes, and, in particular, humans.

[0160] As used herein, the terms "treating," "treatment," "therapeutic," or "therapy" do not necessarily mean total cure or abolition of the disease or condition. Any alleviation of any undesired signs or symptoms of a disease or condition, to any extent can be considered treatment and/or therapy. Furthermore, treatment may include acts that may worsen the patient's overall feeling of well-being or appearance.

[0161] The term "therapeutically effective amount" is used to indicate an amount of an active compound, or pharmaceutical agent, that elicits the biological or medicinal response indicated. For example, a therapeutically effective amount of compound can be the amount need to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated This response may occur in a tissue, system, animal or human and includes alleviation of the symptoms of the disease being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. The therapeutically effective amount of the compounds disclosed herein required as a dose will depend on the route of administration, the type of animal, including human, being treated, and the physical characteristics of the specific animal under consideration. The dose can be tailored to achieve a desired effect, but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

[0162] As will be readily apparent to one skilled in the art, the useful in vivo dosage to be administered and the particular mode of administration will vary depending upon the age, weight, the severity of the affliction, and mammalian species treated, the particular compounds employed, and the specific use for which these compounds are employed. (See e.g., Fingl et al. 1975, in "The Pharmacological Basis of Therapeutics", which is hereby incorporated herein by reference in its entirety, with particular reference to Ch. 1, p. 1). The determination of effective dosage levels, that is the dosage levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine pharmacological methods. Typically, human clinical applications of products are commenced at lower dosage levels, with dosage level being increased until the desired effect is achieved. Alternatively, acceptable in vitro studies can be used to establish useful doses and routes of administration of the compositions identified by the present methods using established pharmacological methods.

[0163] Although the exact dosage will be determined on a drug-by-drug basis, in most cases, some generalizations regarding the dosage can be made. The daily dosage regimen for an adult human patient may be, for example, an oral dose of between 0.01 mg and 3000 mg of each active ingredient, preferably between 1 mg and 700 mg, e.g. 5 to 200 mg. The dosage may be a single one or a series of two or more given in the course of one or more days, as is needed by the patient. In some embodiments, the compounds will be administered for a period of continuous therapy, for example for a week or more, or for months or years.

[0164] In instances where human dosages for compounds have been established for at least some condition, those same dosages, or dosages that are between about 0.1% and 500%, more preferably between about 25% and 250% of the established human dosage will be used. Where no human dosage is established, as will be the case for newly-discovered pharmaceutical compositions, a suitable human dosage can be inferred from ED_{50} or ID_{50} values, or other appropriate values derived from in vitro or in vivo studies, as qualified by toxicity studies and efficacy studies in animals.

[0165] In cases of administration of a pharmaceutically acceptable salt, dosages may be calculated as the free base. As will be understood by those of skill in the art, in certain situations it may be necessary to administer the compounds disclosed herein in amounts that exceed, or even far exceed, the above-stated, preferred dosage range in order to effectively and aggressively treat particularly aggressive diseases or infections.

[0166] Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the modulating effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

[0167] Dosage intervals can also be determined using MEC value. Compositions should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

[0168] It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity or organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

[0169] In non-human animal studies, applications of potential products are commenced at higher dosage levels, with dosage being decreased until the desired effect is no longer achieved or adverse side effects disappear. The dosage may range broadly, depending upon the desired effects and the therapeutic indication. Alternatively dosages may be based and calculated upon the surface area of the patient, as understood by those of skill in the art.

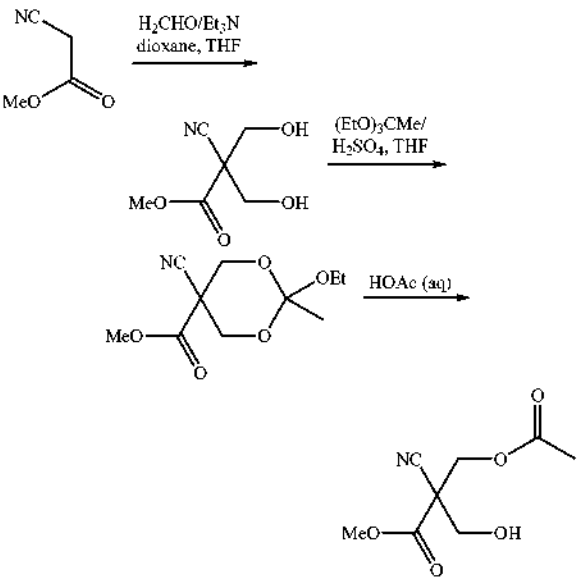
[0170] Compounds disclosed herein can be evaluated for efficacy and toxicity using known methods. For example, the toxicology of a particular compound, or of a subset of the compounds, sharing certain chemical moieties, may be established by determining in vitro toxicity towards a cell line, such as a mammalian, and preferably human, cell line. The results of such studies are often predictive of toxicity in animals, such as mammals, or more specifically, humans. Alternatively, the toxicity of particular compounds in an animal model, such as mice, rats, rabbits, or monkeys, may be determined using known methods. The efficacy of a particular compound may be established using several recognized methods, such as in vitro methods, animal models, or human clinical trials. Recognized in vitro models exist for nearly every class of condition, including but not limited to cancer, cardiovascular disease, and various immune dysfunction. Similarly, acceptable animal models may be used to establish efficacy of chemicals to treat such conditions. When selecting a model to determine efficacy, the skilled artisan can be guided by the state of the art to choose an appropriate model, dose, and route of administration, and regime. Of course, human clinical trials can also be used to determine the efficacy of a compound in humans.

Examples

[0171] Additional embodiments are disclosed in further detail in the following examples, which are not in any way intended to limit the scope of the claims.

Example 1
1-METHYL 3-ACETOXY-2-CYANO-2-(HYDROXYMETHYL)PROPANOATE (1)

[0172]



[0173] Methyl 2-cyano-3-hydroxy-2-hydroxymethylpropanoate. Formaldehyde (66.7 mmol, 2.0 g) was added as 20% aq solution (10 g) to 1,4-dioxane (30 mL) on an ice-bath. Methyl cyanoacetate (30.3 mmol, 2.12 mL) and Et_3N (0.61 mmol, 0.61 mL of 1 mol L^{-1} solution in THF) were added and the mixture was stirred for 20 min. Another portion of Et_3N (0.61 mmol) was added and the ice-bath was removed. The mixture was stirred for 1.5 h at room temperature. The mixture was then diluted with water (200 mL) and extracted with benzene (3×50 mL) to remove side products. The aqueous phase was evaporated under reduced pressure at 30° C. to one fourth of the original volume and extracted 5 times with ethyl acetate. The combined extracts were dried over Na_2SO_4 and evaporated to a clear oil. The yield was 72% (4.82 g). The compound was used without characterization to the next step.

[0174] Methyl 5-cyano-2-ethoxy-2-methyl-1,3-dioxane-5-carboxylate. Methyl 2-cyano-3-hydroxy-2-hydroxymethylpropanoate (23.3 mmol, 3.7 g) was dissolved in dry THF (8 mL) and triethyl orthoacetate (34.9 mmol, 6.55 mL) was added. A catalytic amount of concentrated sulfuric acid (0.70 mmol, 37 μL) was added and the mixture was stirred over night at room temperature. The mixture was poured into a stirred ice-cold aq. $NaHCO_3$ (5%, 50 mL). The product was extracted into Et_2O (2×50 mL) and the extracts were washed with saturated aq. NaCl and dried over Na_2SO_4. The solvent was evaporated and purified by Silica gel chromatography applying a stepwise gradient from 5% ethyl acetate in dichloromethane to pure ethyl acetate. The product was obtained in 42% yield (5.33 g) as a clear oil that started to crystallize ^1H NMR for the major diastereomer ($CDCl_3$) 4.34 (d, J=7.0 Hz, 2H, —CH_2O—), 4.03 (d, J=8.5 Hz, 2H, —CH_2O—), 3.84 (s, 3H, OMe), 3.54 (q, J=7.2 Hz, 2H, —CH_2CH_3), 1.55 (s, 3H, —CH_3), 1.25 (t, J=7.2, 3H, —CH_2CH_3). ^{13}C NMR for the major diastereomer ($CDCl_3$) 164.8 (C=O), 117.0 (CN), 111.4 (C2), 62.3 (C4 and C6), 59.1 (—CH_2CH_3), 53.9 (—OCH_3), 42.4 (C5), 22.3 (2-CH_3), 15.0 (CH_2CH_3).

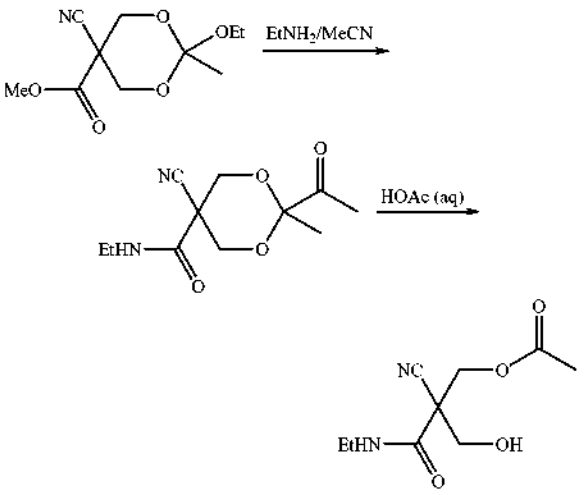
[0175] Methyl 3-acetyloxy-2-cyano-2-(hydroxymethyl) propanoate. Methyl 5-cyano-2-ethoxy-2-methyl-1,3-dioxane-5-carboxylate (2.18 mmol, 0.50 g) was dissolved in a

mixture of acetic acid and water (4:1, v/v, 20 mL) and the mixture was stirred for 2 h at room temperature, after which the mixture was evaporated to dryness and the residue was coevaporated 3 times with water. The product was purified by Silica gel chromatography, eluting with dichloromethane containing 5% MeOH. The yield was 52% (0.23 g). ^{1}H NMR ($CDCl_3$) 4.53 (d, J=11.0 Hz, 1H, —CH_2OAc), 4.50 (d, J=11.0 Hz, 1H, —CH_2OAc), 4.04 (d, J=6.5 Hz, 2H, —CH_2OH), 3.91 (s, 3H, —OMe), 2.90 (t, J=6.5 Hz, —OH), 2.16 (s, 3H, —$C(O)CH_3$). ^{13}C NMR ($CDCl_3$) 170.4 (C═O), 166.0 (C═O), 116.0 (CN), 63.1 (—CH_2OH), 62.3 (—CH_2OAc), 54.1 (—OMe), 51.0 (C2), 20.6 (—$C(O)CH_3$).

Example 2

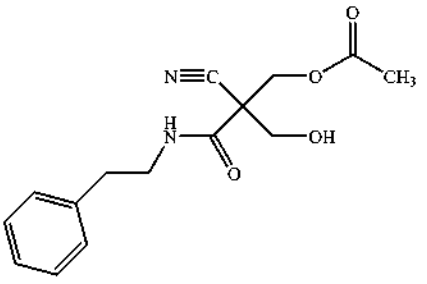
2-CYANO-3-(ETHYLAMINO)-2-(HYDROXYMETHYL)-3-OXOPROPYL ACETATE (2)

[0176]



2-CYANO-3-(2-PHENYLETHYLAMINO)-2-(HYDROXYMETHYL)-3-OXOPROPYL ACETATE(2b)

[0177]

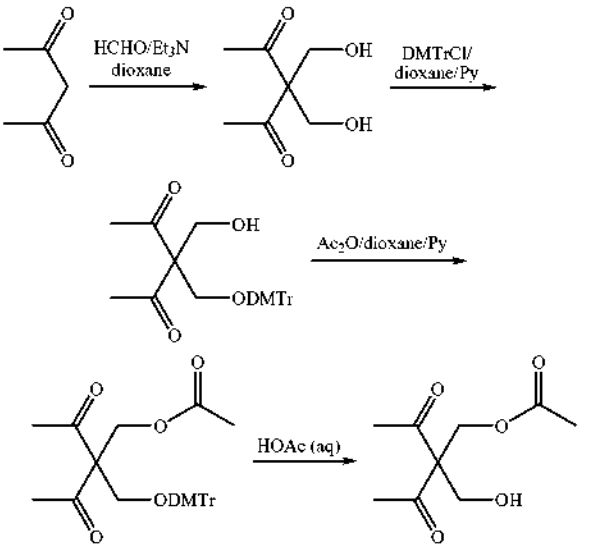


[0178] 2-cyano-3-(2-phenylethylamino)-2-(hydroxymethyl)-3-oxopropyl acetate was prepared according to the procedure described in Poijärvi, P.; Mäki, E.; Tomperi, J.; Ora, M.; Oivanen, M.; Lönnberg, H., *Helve. Chim. Acta.* 2002 85:1869-1876, which is hereby incorporated by reference for the limited purpose of describing the method of synthesizing and purifying 2-cyano-3-(2-phenylethylamino)-2-(hydroxymethyl)-3-oxopropyl acetate.

Example 3

2-ACETYL-2-(HYDROXYMETHYL)-3-OXOBUTYL ACETATE (3)

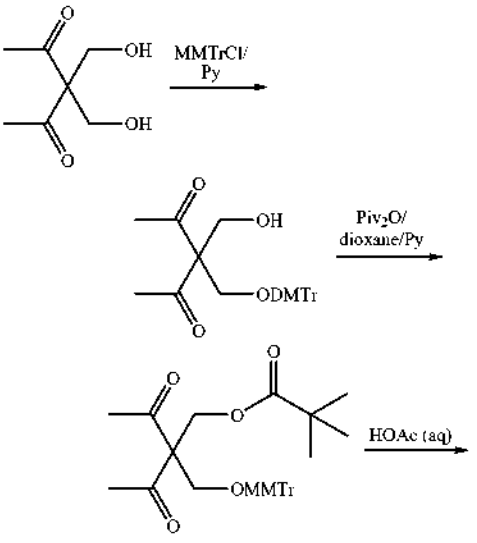
[0179]



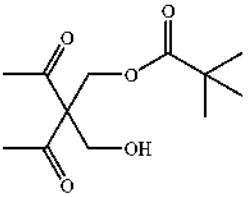
Example 4

2-ACETYL-2-(HYDROXYMETHYL)-3-OXOBUTYL PIVALATE (4)

[0180]



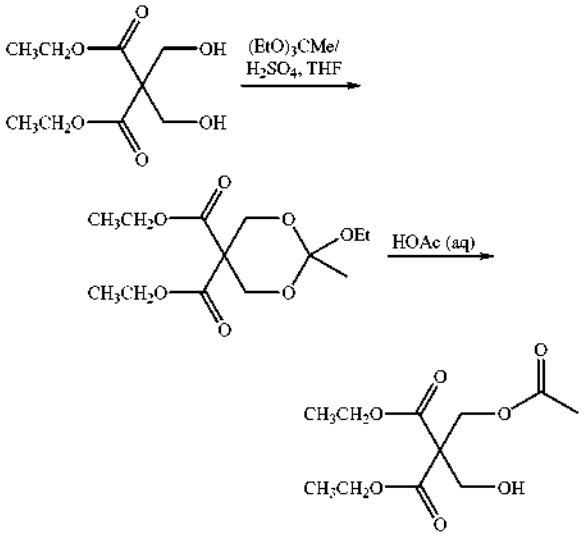
-continued



Example 5

2-ACETYL-2-HYDROXYMETHYL-3-OXOBUTYL ACETATE (5)

[0181]



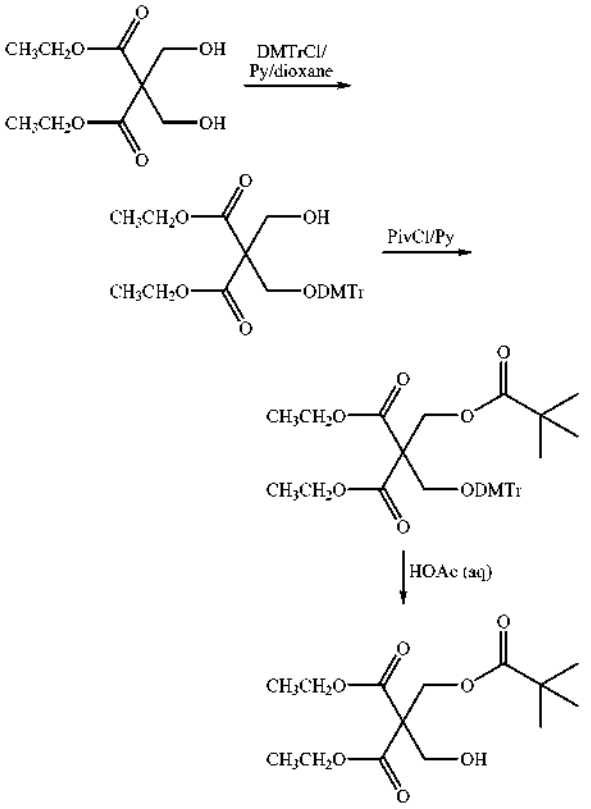
[0182] Diethyl 2-ethoxy-2-methyl-1,3-dioxane-5,5-dicarboxylate. Concentrated H_2SO_4 (1.3 mmol; 71 μL) was added to a mixture of diethyl 2,2-bis(hydroxymethyl)malonate (43.5 mmol, 9.6 g) and triethyl orthoacetate (65.2 mmol; 11.9 mL) in dry THF (15 mL). The reaction was allowed to proceed overnight and the mixture was the poured into an ice-cold solution of 5% $NaHCO_3$ (50 mL). The product was extracted with diethyl ether (2×50 mL), washed with saturated aqueous NaCl (2×50 mL) and dried over Na_2SO_4. The solvent was evaporated and the crude product was purified on a silica gel column eluting with a mixture of dichloromethane and methanol (95:5, v/v). The product was obtained as clear oil in 89% yield (11.3 g). ^1H NMR δ_H (500 MHz, $CDCl_3$): 4.30-4.36 (m, 6H, 4-CH_2, 6-CH_2 and 5-$COOCH_2Me$), 4.18 (q, J=7.1 Hz, 5-$COOCH_2Me$), 3.54 (q, J=7.10 Hz, 2H, 2-OCH_2Me), 1.46 (s, 3H, 2-CH_3), 1.32 (t, J=7.10 Hz, 3H, 2-OCH_2Me), 1.27 (t, J=7.1 Hz 3H, 5-$COOCH_2Me$), 1.26 (t, J=7.1 Hz 3H, 5-$COOCH_2Me$). ^{13}C NMR (500 MHz, $CDCl_3$): δ=168.0 and 167.0 (5-COOEt), 111.1 (C2), 62.0 and 61.9 (5-$COOCH_2Me$), 61.6 (C4 and C6), 58.7 (2-OCH_2Me), 52.3 (C5), 22.5 (2-Me), 15.1 (2-OCH_2CH_3), 14.0 and 13.9 (5-$COOCH_2CH_3$).

[0183] Diethyl 2-(acetyloxymethyl)-2-(hydroxymethyl) malonate. Diethyl 2-ethoxy-2-methyl-1,3-dioxane-5,5-dicarboxylate (17.9 mmol; 5.2 g) was dissolved in 80% aqueous acetic acid (30 mL) and left for 2 h at room temperature. The solution was evaporated to dryness and the residue was coevaporated three times with water. The product was purified by silica gel column chromatography eluting with ethyl acetate in dichloromethane (8:92, v/v). The product was obtained as yellowish oil in 75% yield (3.6 g). ^1H NMR δ_{11} (500 MHz, $CDCl_3$): 4.76 (s, 2H, CH_2OAc), 4.26 (q, J=7.10 Hz, 4H, OCH_2Me), 4.05 (d, J=7.10 Hz, 2H, CH_2OH), 2.72 (t, J=7.1 Hz, 1H, CH_2OH), 2.08 (s, 3H, Ac), 1.27 (t, J=7.10 Hz, 6H, OCH_2CH_3). ^{13}C NMR (500 MHz, $CDCl_3$): δ=170.9 (C=O Ac), 168.1 (2×C=O malonate), 62.3 and 62.2 (CH_2OH and CH_2OAc), 61.9 (2×OCH_2CH_3) 59.6 (spiro C), 20.7 (CH_3 Ac 14.0 (2×OCH_2CH_3).

Example 6

2,2-BIS(ETHOXYCARBONYL)-3-HYDROXYPROPYL PIVALATE (6)

[0184]



[0185] 2,2-Bis(ethoxycarbonyl)-3-(4,4'-dimethoxytrityloxy)propyl pivalate. Diethyl 2,2-bis(hydroxymethyl)malonate was reacted with 1 equiv. of 4,4'-dimethoxytrityl chloride in 1,4-dioxane containing 1 equivalent of pyridine. Diethyl 2-(4,4'-dimethoxytrityloxymethyl)-2-(hydroxymethyl)malonate (2.35 g, 4.50 mmol) was acylated with pivaloyl chloride (0.83 mL, 6.75 mmol) in dry MeCN (10 mL) containing 3 equivalent pyridine (1.09 mL, 13.5 mmol). After 3 days at room temperature, the reaction was quenched with MeOH (20 mL) and a conventional CH_2Cl_2/aq HCO_3^-

workup was carried out. Silica gel chromatography (EtOAc/hexane 1:1, v/v) gave 2.47 g (90%) of the desired product as yellowish syrup. ^{1}H NMR ($CDCl_3$, 200 MHz): 7.13-7.39 [m, 9H, $(MeO)_2$ Tr]; 6.81 (d, 4H, $[MeO]_2$ Tr); 4.71 (s, 2H, CH_2OPiv); 4.15 (q, J=7.1, 4H, OCH_2CH_3); 3.78 [s, 6H, $(CH_3O)_2Tr$]; 3.67 (s, 2H, CH_2ODMTr); 1.27 (t, J=7.1, 6H, OCH_2CH_3); 1.02 [s, 9H, $COC(CH_3)_3$].

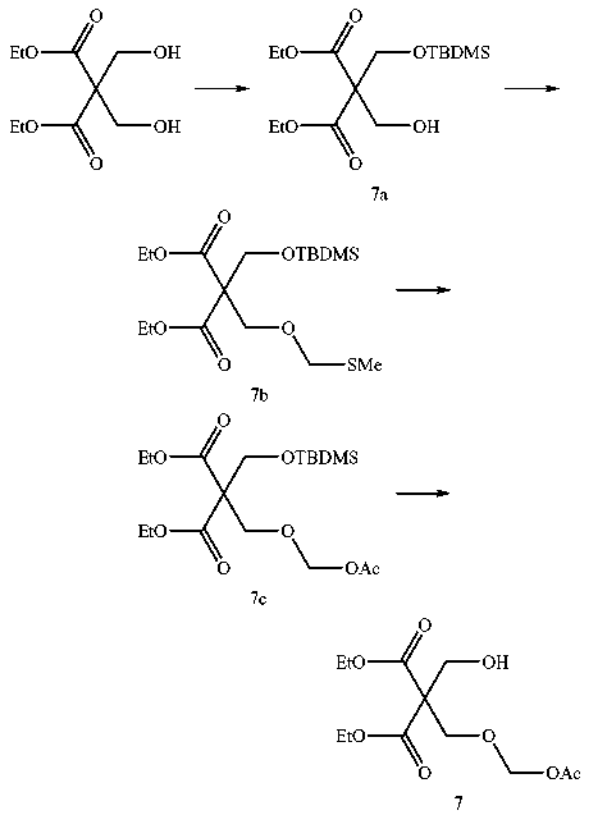
[0186] 2,2-Bis(ethoxycarbonyl)-3-hydroxypropyl pivalate. 2,2-Bis(ethoxycarbonyl)-3-(4,4'-dimethoxytrityloxy) propyl pivalate (2.47 g, 4.07 mmol) in a 4:1 mixture of CH_2Cl_2 and MeOH (20 mL) was treated for 4 h at room temperature with TFA (2.00 mL, 26.0 mmol) to remove the dimethoxytrityl group. The mixture was neutralized with pyridine (2.30 mL, 28.6 mmol), subjected to CH_2Cl_2/aq workup and purified by silica gel chromatography (EtOAc/hexane 3:7, v/v) to obtain 1.15 g (93%) of the desired product.

[0187] ^{1}H NMR ($CDCl_3$, 200 MHz): 4.59 (s, 2H, CH_2OPiv); 4.25 (q, J=7.1, 4H, OCH_2CH_3); 4.01 (s, 2H, CH_2OH); 1.28 (t, J=7.1, 6H, OCH_2CH_3); 1.18 [s, 9H, $COC(CH_3)_3$], ESI-MS$^+$; m/z 305.4 ($[MH]^+$), 322.6 ($[MNH_4]^+$), 327.6 ($[MNa]^+$), 343.5 ($[MK]^+$).

Example 7

DIETHYL 2-ACETYLOXYMETHYL-2-HYDROXYMETHYLMALONATE (7)

[0188]



[0189] Diethyl 2-(tert-butyldimethylsilyloxymethyl)-2-hydroxymethylmalonate (7a). Diethyl 2,2-bis(hydroxymethyl)malonate (28.3 mmol; 6.23 g) was coevaporated twice from dry pyridine and dissolved in the same solvent (20 mL). tert-Butyldimethylsilyl chloride (25.5 mmol; 3.85 g) in dry pyridine (10 mL) was added portionwise. The reaction was allowed to proceed for 4 days. The mixture was evaporated to a solid foam, which was then equilibrated between water (200 mL) and DCM (4×100 mL). The organic phase was dried on Na_2SO_4. The product was purified by silica gel chromatography eluting with 10% ethyl acetate in DCM. The yield was 78%. ^{1}H NMR ($CDCl_3$) δ 4.18-4.25 (m, 4H, OCH_2Me), 4.10 (s, 2H, CH_2OSi), 4.06 (s, 2H, CH_2OH), 2.63 (br s, 1H, OH), 1.26 (t, J=7.0 Hz, 6H, OCH_2CH_3), 0.85 (s, 9H, Si—SMe_3), 0.05 (s, 6H, Me—Si). ^{13}C NMR ($CDCl_3$) δ 169.2 (C═O), 63.3 (CH_2OH), 62.8 (CH_2OSi), 61.6 (spiro C), 61.4 (OCH_2Me), 25.6 [$C(CH_3)_3$], 18.0 (Si—CMe_3), 14.0 (OCH_2CH_3), −3.6 (Si—CH_3). MS $[M+H]^+$ obsd. 335.7, calcd. 335.2; [M+Na] obsd. 357.6, calcd. 357.2.

[0190] Diethyl 2-(tert-butyldimethylsilyloxymethyl)-2-methylthiomethylmalonate (7b). Compound 7a (19.7 mmol; 6.59 g) was dissolved into a mixture of acetic anhydride (40 mL), acetic acid (12.5 mL) and DMSO (61 mL) and the mixture was stirred overnight. The reaction was stopped by dilution with cold aq. Na_2CO_3 (290 ml 10% aq. solution) and the product was extracted in diethyl ether (4×120 mL). The combined organic phase was dried on Na_2SO_4. The product was purified by silica gel chromatography using DCM as an eluent. The yield was 91%. ^{1}H NMR ($CDCl_3$) δ 4.61 (s, 2H, OCH_2S), 4.14-4.19 (m, 4H, OCH_2Me), 4.06 (s, 2H, CH_2OSi), 4.00 (s, 2H, CH_2OCH_2SMe), 2.06 (SCH_3), 1.22 (t, J=7.0 Hz, 6H, OCH_2CH_3), 0.83 (s, 9H, Si—SMe_3), 0.02 (s, 6H, Me—Si). ^{13}C NMR ($CDCl_3$) δ 168.3 (C═O), 75.6 (CH_2S), 65.7 (CH_2OCH_2SMe), 61.4 (CH_2OSi), 61.2 (spiro C), 60.9 (OCH_2Me), 25.6 [$C(CH_3)_3$], 18.0 (Si—CMe_3), 14.0 (OCH_2CH_3), 13.7 (SCH_3), −3.6 (Si—CH_3). MS $[M+H]^+$ obsd. 395.4, calcd. 395.2; [M+Na] obsd. 417.6, calcd. 417.2.

[0191] Diethyl 2-acetyloxymethyl-2-(tert-butyldimethylsilyloxymethyl)malonate (7c). Compound 7b (17.9 mmol; 7.08 g) was dissolved in dry DCM (96 mL) under nitrogen. Sulfurylchloride (21.5 mmol; 1.74 mL of 1.0 mol L^{-1} solution in DCM) was added in three portions and the mixture was stirred for 70 min under nitrogen. The solvent was removed under reduced pressure and the residue was dissolved into dry DCM (53 mL). Potassium acetate (30.9 mmol; 3.03 g) and dibenzo-18-crown-6 (13.5 mmol; 4.85 g) in DCM (50 mL) were added and the mixture was stirred for one hour and a half. Ethyl acetate (140 mL) was added, the organic phase was washed with water (2×190 mL) and dried on Na_2SO_4. The product was purified by silica gel chromatography using DCM as an eluent. The yield was 71%. ^{1}H NMR ($CDCl_3$) δ 5.24 (s, 2H, OCH_2O), 4.15-4.22 (m, 4H, OCH_2Me), 4.13 (s, 2H), CH_2OSi), 4.08 (s, 2H, CH_2OAc), 2.08 (Ac), 1.26 (t, J=8.0 Hz, 6H, OCH_2CH_3), 0.85 (s, 9H), Si—SMe_3), 0.04 (s, 6H, Me—Si). ^{13}C NMR ($CDCl_3$) δ 170.2 (Ac), 168.0 (C═O), 89.3 (OCH_2O), 67.5 (CH_2OAc), 61.4 (OCH_2Me), 61.1 (CH_2OSi), 60.2 (spiro C), 25.6[$C(CH_3)_3$], 21.0 (Ac), 18.1 (Si—CMe_3), 14.0 (OCH_2CH_3), −5.7 (Si—CH_3). MS $[M+Na]^+$ obsd. 429.6, calcd. 429.2.

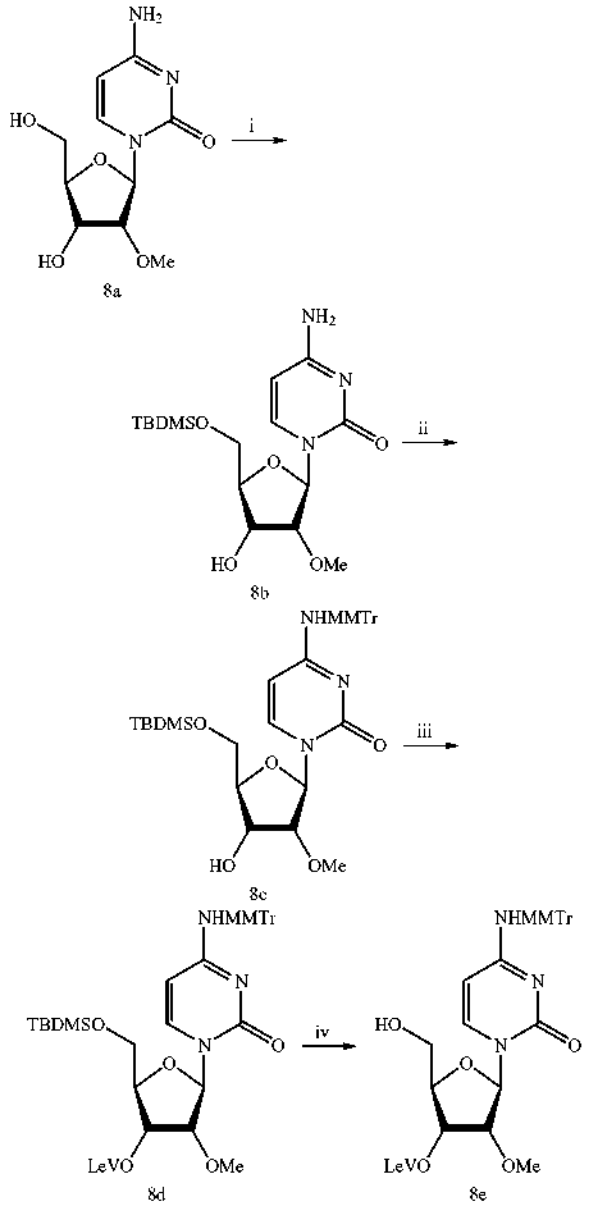
[0192] Diethyl 2-acetyloxymethyl-2-hydroxymethylmalonate (7). Compound 7c (7.2 mmol; 2.93 g) was dissolved in dry THF (23 mL) and triethylamine trihydrogenfluoride (8.64 mmol; 1.42 mL) was added. The mixture was stirred for one week. Aq. triethylammonium acetate (13 mL of 2.0 mol L^{-1} solution) was added. The mixture was evaporated to dryness and the residue was purified by silica gel chromatography using DCM containing 2-5% MeOH as an eluent. The yield

was 74%. ^{1}H NMR ($CDCl_3$) δ 5.25 (s, 2H, OCH_2O), 4.16-4.29 (m, 6H, OCH_2Me and CH_2OAc), 4.13 (s, 2H, CH_2OH), 2.10 (Ac), 1.81 (br s, 1H, OH), 1.26 (t, J=9.0 Hz, 6H, OCH_2CH_3). MS $[M+Na]^+$ obsd. 315.3, calcd. 315.1.

Example 8

3'-O-LEVULINOYL-N^4-(4-METHOXYTRITYL)-2'-O-METHYLCYTIDINE (8e)

[0193]



[0194] 5'-O-(tert-Butyldimethylsilyl)-2'-O-methylcytidine (8b). 2'-O-methylcytidine (8a; 18.4 mmol; 4.74 g) was coevaporated twice from dry pyridine, dried over P_2O_5 (24 h) and dissolved in dry pyridine (20 mL). tert-Butyldimethylsilyl chloride (TBDMSCl; 20.2 mmol; 3.05 g) was added and the mixture was agitated at room temperature overnight. The unreacted TBDMSCl was quenched with MeOH, the mixture was evaporated to dryness and the residue was subjected to chloroform/aq. $NaHCO_3$ work-up. The yield of the crude product dried on Na_2SO_4 was nearly quantitative. It was used for 4-methoxytritylation of the amino group without further purification. ^{1}H NMR ($CDCl_3$): δ 8.14 (d, J=7.5 Hz, 1H, H6), 6.00 (d, J=1.1 Hz; 1H, H1'), 6.82 (d, J=7.5 Hz, 1H, H5), 4.22 (dd, J=8.0 and 5.1 Hz, 1H, H3'), 4.09 (dd, J=11.8 and 1.8 Hz, 1H, H5'), 3.97 (m, 1H, H4'), 3.87 (dd, J=11.8 and 1.6, 1H, H5"), 3.73 (dd, J=5.1 and 1.0 Hz, 1H, H2'), 3.67 (s, 3H, 2'-OMe), 0.94 (s, 9H, Me_3C—Si), 0.13 (s, 3H, Me-Si), 0.13 (s, 3H, Me-Si).

[0195] 5-O-(tert-Butyldimethylsilyl)-N^4-(4-methoxytrityl)-2'-O-methylcytidine (8c). Compound 8b (18.4 mmol; 6.84 g) was coevaporated twice from dry pyridine and dissolved in the same solvent (20 mL). 4-Methoxytrityl chloride (18.4 mmol; 5.69 g) was added and the mixture was agitated at 45 °o for 24 h. MeOH (20 mL) was added, the mixture was evaporated to dryness and the residue was subjected to chloroform/aq. $NaHCO_3$ work-up. Silica gel chromatography with DCM containing 2-5% MeOH gave compound 8c as a solid foam in 46% overall yield starting from 2'-O-methylcytidine. ^{1}H NMR ($CDCl_3$) δ 7.91 (d, J=7.7 Hz, 1H, H6), 7.26-7.33 (m, 6H, MMTr), 7.21-7.23 (m, 4H, MMTr), 7.13-7.15 (m, 2H, MMTr), 6.82-6.85 (m, 2H, MMTr), 6.77 (br. s, 1H, NH), 5.99 (s, 1H, H1'), 5.00 (d, J=7.7 Hz, 1H, H5), 4.12 (m, 1H, H3'), 4.02 (dd, J=11.9 and 1.2 Hz, 1H, H5'), 3.86-3.88 (m, 1H, H4'), 3.81 (dd, J=11.9 and 1.2 Hz, 1H, H5"), 3.81 (s, 3H, MeO-MMTr), 3.72-3.74 (m, 4H, H2' and 2'-OMe), 2.63 (br s, 1H, 3'-OH), 0.75 (s, 9H, Me_3C—Si), −0.03 (s, 3H, MeSi), −0.05 (s, 3H, Me-Si). ^{13}C NMR ($CDCl_3$) δ 165.6 (C4), 158.7 (MMTr), 155.1 (C2), 144.4 (MMTr), 144.3 (MMTr), 140.9 (C6), 136.0 (MMTr), 130.0 (MMTr), 128.6 (MMTr), 128.3 (MMTr), 127.5 (MMTr), 113.6 (MMTr), 94.2 (C5), 87.6 (C1'), 83.9 (C2'), 83.7 (C4'), 70.5 (MMTr), 66.8 (C3'), 60.5 (C5'), 58.8 (2'-OMe), 55.2 (MMTr), 25.8 (TBDMS), 18.3 (TBDMS), −5.6 (TBDMS), −5.7 (TBDMS).

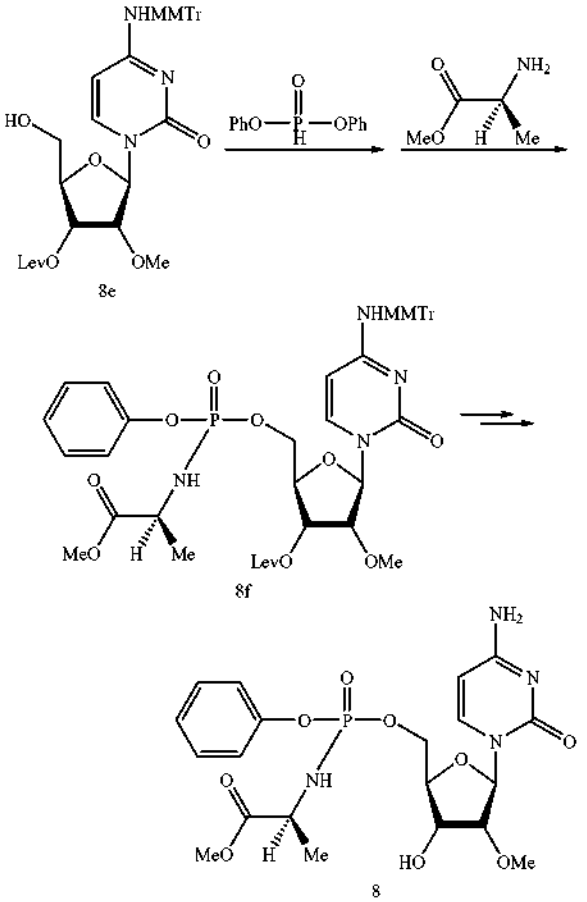
[0196] 5'-O-(tert-Butyldimethylsilyl)-3'-O-levulinoyl-N^4-(4-methoxytrityl)-2'-O-methylcytidine (8d). Levulinic acid (21.6 mmol; 2.51 g) was dissolved in dry dioxane and dicyclohexylcarbodiimide (11.1 mmol; 2.28 g) was added portionwise during 1 h at 0° C. The mixture was allowed to warm up to reduce its viscosity and it was then filtrated to a solution of compound 8c (8.46 mmol; 5.45 g) in pyridine (18 mL). The mixture was agitated overnight, evaporated to dryness and the residue was subjected to DCM/$NaHCO_3$ work-up. The organic phase was dried on Na_2SO_4, evaporated to dryness and the residue was purified by Silica gel chromatography using DCM containing 1% MeOH as an eluent. Yield 86%. ^{1}H NMR ($CDCl_3$) δ 7.81 (d, J=7.7 Hz, 1H, H6), 7.27-7.34 (m, 6H, MMTr), 7.22-7.23 (m, 4, MMTr), 7.14-7.15 (m, 2H, MMTr), 6.84-6.86 (m, 2H, MMTr), 6.80 (br. s, 1H, NH), 6.07 (d, J=1.5 Hz, 1H, H1'), 4.99 (d, J=7.7 Hz, 1H, H5), 4.97 (dd, J=7.9 and 5.0 Hz, 1H, H3'), 4.21 (m, 1H, H2'), 3.99-4.01 (m, 2H, H4' and H5'), 3.81 (s, 3H, MeO—MMTr), 3.70 (dd, J=12.0 and 1.3 Hz, 1H, H5"), 3.57 (s, 3H, 2'-OMe), 2.63-2.83 (m, 4H, Lev), 2.21 (s, 3H, Lev), 0.74 (s, 9H, Me_3C—Si), −0.05 (s, 3H, Me-Si), −0.07 (s, 3H, Me-Si). ^{13}C NMR ($CDCl_3$) δ 206.1 (Lev), 172.0 (Lev), 165.5 (C4), 158.7 (MMTr), 155.1 (C2), 144.4 (MMTr), 144.3 (MMTr), 140.7 (C6), 136.0 (MMTr), 130.0 (MMTr), 128.6 (MMTr), 128.3 (MMTr), 127.5 (MMTr), 113.6 (MMTr), 94.4 (C5), 88.4 (C1'), 82.5 (C2'), 81.3 (C4'), 70.6 (MMTr), 69.1 (C3'), 60.8

(C5'), 58.9 (2'-OMe), 55.2 (MMTr), 37.8 (Lev), 29.8 (Lev), 27.8 (Lev), 25.7 (TBDMS), 18.2 (TBDMS), −5.7 (TBDMS), −5.8 (TBDMS).

[0197] 3'-O-Levulinoyl-N^4-(4-methoxytrityl)-2'-O-methylcytidine (8e). Compound 8d (3.40 mmol; 2.52 g) was dissolved into a mixture THF (48 mL) and AcOH (9 mL) containing tetrabutylammonium fluoride (6.85 mmol; 1.79 g). The mixture was agitated for 2 days and then evaporated to dryness. The residue was dissolved into EtOAc (50 mL), washed with water, aq. $NaHCO_3$ and brine, and dried on Na_2SO_4. The compound 8e was obtained as a white foam in virtually quantitative yield. ^{1}H NMR ($CDCl_3$) δ 7.22-7.34 (m, 11H, H6 and MMTr), 7.12-7.15 (m, 2H, MMTr), 6.89 (br. s, 1H, NH), 6.83-6.85 (m, 2H, MMTr), 5.41 (d, J=5.0 Hz, 1H, H1'), 5.31 (dd, J=4.6 and 4.7, 1H, H4'), 5.07 (d, J=7.6 Hz, 1H, H5), 4.58 (dd, J=5.0 and 5.0 Hz, 1H, H3'), 4.18 (m, 1H, H2'), 3.90 (d, J=12.7 Hz, 1H, H5'), 3.81 (s, 3H, MeO—MMTr), 3.71 (dd, J=12.7 and 4.7 Hz, 1H, H5"), 3.45 (s, 3H, 2'-OMe), 2.75-2.80 (m, 2H, Lev), 2.63-2.66 (m, 2H, lev), 2.20 (s, 3H, Lev).

2'-O-METHYLCYTIDINE 5'-[O-PHENYL-N—(S-2-METHOXY-1-METHYL-2-OXOETHYL)]PHOSPHORAMIDATE (8)

[0198]



[0199] 3'-O-Levulinoyl-N^4-(4-methoxytrityl)-2'-O-methylcytidine 5'-O-phenyl-N—(S-2-methoxy-1-methyl-2-oxoethyl)]phosphoramidate (8f). Compound 8e (2.58 mmol; 1.62 g) dried on P_2O_5 for 2 days was dissolved in dry pyridine (5 mL) and diphenylphosphite (3.09 mmol; 595 μL) was added under nitrogen. After half an hour, carefully dried L-alanine methyl ester (3.94 mmol; 0.55 g) in a mixture of dry pyridine (1 mL) and MeCN (6 mL) was added. CCl_4 (15 mL) and triethylamine (18.1 mmol; 2.54 mL) was added and the reaction was allowed to proceed for 70 min. Volatiles were removed under reduced pressure and the residue was purified by silica gel chromatography increasing the MeOH content of DCM from 1 to 10% in a stepwise manner. Compound 8f was obtained as a white foam in 70% yield. ^{1}H NMR ($CDCl_3$) mixture of R_P and S_P diastereomers δ 7.02-7.35 (m, 17H, MMTr and Ph), 6.80-6.85 (m, 3H, MMTr and N^4H), 5.99 and 6.02 (2×d, J=3.2 Hz, 1H, H1'), 4.90-5.00 (m, 2H, H3' and H4'), 3.88-4.43 (m, 4H, H5, H2', H5', H5"), 3.80 (s, 3H, MMTr), 3.68-3.75 (m, 1H, H^{α}-Ala, 3.63 and 3.64 (2×s, 3H, MeO-Ala), 3.46 and 3.52 (2×s, 3H, 2'-OMe), 2.74-2.81 (m, 2H, Lev), 2.59-2.64 (m, 2H, Lev), 2.19 and 2.20 (2×s, 3H, Lev), 1.88 (br s, 1H, NH—P), 1.27 and 1.31 (2×d, J=7.1 Hz, Me Ala).

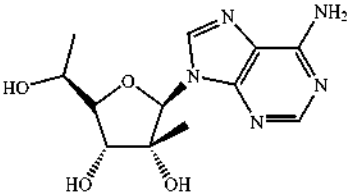
[0200] 2'-O-Methylcytidine 5'-[O-phenyl-N—(S-2-methoxy-1-methyl-2-oxoethyl)]phosphoramidate (8). Compound 8f (1.81 mmol; 1.57 g) was dissolved in a mixture of hydrazine hydrate (7.2 mmol; 350 μL), pyridine (11.5 mL) and AcOH (2.88 mL) and the reaction was allowed to proceed for 5 h. Volatiles were removed under reduced pressure and the residue was dissolved in DCM (50 mL) and washed with water, aq. $NaHCO_3$ and brine. The organic phase was dried on Na_2SO_4, evaporated to dryness and the residue was purified by silica gel chromatography using DCM containing 4-6% MeOH as an eluent.

[0201] The purified product was dissolved 80% aq. AcOH (8 mL) and the mixture was allowed to proceed at 55° C. for 2 h and additionally at 65° C. for 4.5 h. The mixture was evaporated to dryness and the residue was coevaporated twice from water and then purified by silica gel chromatography using gradient elution from 7 to 20% MeOH in DCM. The overall yield from 8 was 50%. ^{1}H NMR ($CDCl_3$) mixture of two diastereomers δ 7.64 and 7.68 (2×d, J=7.4, 1H, H6), 7.26-7.33 (m, 2H, Ph), 7.20-7.24 (m, 2H, Ph), 7.13-7.16 (m, 1H, Ph), 6.32 (br s, 2H, NH_2), 5.90 and 5.94 (2×s, 1H, H1'), 5.69 and 5.82 (2×d, J=7.4, 1H, H5), 4.35-4.55 (m, 2H, H5' and H5"), 4.12-4.18 (m, 2H, H3' and H4'), 3.98-4.08 (m, 2H, α-H-Ala and 3'-OH), 3.72-3.76 (m, 1H, 2'-OMe), 3.67 and 3.68 (2×s, 3H, MeO-Ala), 3.58 and 3.60 (2×s, 3H, 2'-OMe), 2.45 (br s, 1H, NH—P), 1.37 and 1.39 (2×d, J=7.2 Hz, 3H, Me-Ala). ^{13}C NMR ($CDCl_3$) δ174.2 (C=O Ala), 166.0 (C4), 155.9 (C2), 150.5 (Ph), 140.6 (C6), 129.8 (Ph), 125.1 (Ph), 120 (Ph), 95.1 (C5), 88.4 (C1'), 83.4 (C2'), 81.4 (C4'), 68.1 (C3'), 65.1 (C5'), 58.6 (2'-OMe), 52.5 (MeO-Ala), 50.3 (C^{α}-Ala), 20.7 (Me-Ala). ^{31}P NMR δ 3.1 and 3.3. HRMS [M+H]$^+$ obsd. 499.1590, calcd. 499.1583; [M+Na]$^+$ obsd. 521.1438, calcd. 521.1408, [M+K]$^+$ obsd. 537.1149, 537.1147.

Example 9

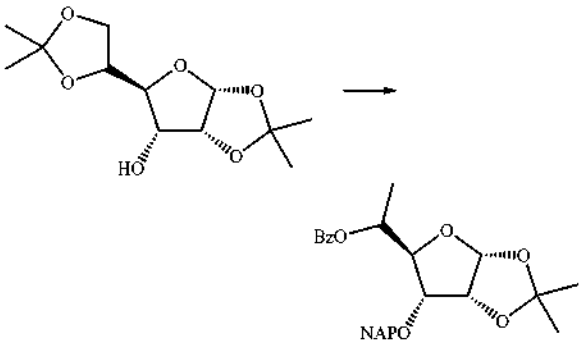
Preparation of 2',5'-C-dimethyladenosine (9)

[0202]



Step 1. Preparation of 5-O-benzoyl-1,2-O-isopropylidene-5-C-methyl-3-O-naphthalenyl-D-ribofuranose

[0203]

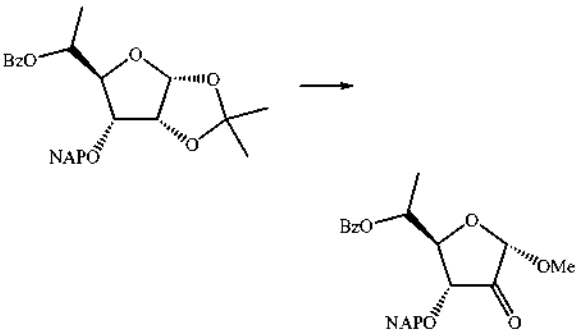


[0204] To a solution of dried 1,25,6-O-di(isopropylidene)-alpha-D-allofuranose (23.83 g, 91.55 mmol) in anhydrous THF (62 mL) was added powdered KOH (36 g, 642.86 mmol), and stirred at room temperature for 30-40 min, then followed by addition of 2-(bromomethyl)naphthalene (21 g), and stirred under nitrogen atmosphere for 4-6 h. The reaction mixture was then quenched with water and extracted with ethyl acetate (3×60 mL). The combined organic phase was dried with sodium sulfate and concentrated into a crude residue (43.38 g), which was treated with a mixture of acetic acid (187 mL) and water (84 mL) at room temperature for 14 h. The reaction mixture was concentrated under a good vacuum below 35° C. to give a crude residue, which was applied to a column of silica gel eluted with hexanes-ethyl acetate (4:1) and dichloromethane-methanol (10:1) to give a pure 3-O-naphthalenyl-1,2;5,6-O-di(isopropylidene)-alpha-D-allofuranose as syrup (36.57 g, 100%).

[0205] To a cold solution of dried 3-O-naphthalenyl-1,2;5,6-O-di(isopropylidene)-alpha-D-allofuranose (36.57 g, 101.3 mmol) in a mixture of 1,4-dioxane (214 mL) and water (534 mL) cooled with ice-bath was added sodium periodate ($NaIO_4$) (32 g, 149.61 mmol) and stirred at the same temperature for 50 min. The reaction mixture was then extracted with ethyl acetate (4×50 mL), and the combined organic phase was dried with anhydrous sodium sulfate, and concentrated into a crude residue, which was dried under a good vacuum for a couple of hours and used in the next reaction without further purification. To a cold solution of the above dried crude residue (33.38 g, 101 mmol) in anhydrous ether (80 mL) cooled with dry ice-acetone to −78° C. was slowly added methylmagnium bromide (100 mL) (3M solution in ether) in portions, and stirred at −78° C. to room temperature overnight under nitrogen. The reaction mixture was then slowly quenched with sat. ammonium chloride solution, and extracted with acetyl acetate (4×60 mL). The combined organic phase was dried with anhydrous sodium sulfate and the filtrate was concentrated into a crude residue of 1,2-O-isopropylidene-5-C-methyl-3-O-naphthalenyl-D-ribofuranose (28.55 g, 83.21 mmol, 82.1%), which was dried under a good vacuum for 2-3 h and treated with benzoyl chloride (12.87 g, 91.53 mmoL) in the presence of DMAP (1.01 g, 8.32 mmol) in anhydrous pyridine (80-100 mL) at room temperature overnight. The reaction mixture was quenched with methanol and concentrated into a crude residue, which was poured into 10% sodium bicarbonate aq. solution and extracted with ethyl acetate (4×50 mL). The combined organic phase was concentrated and co-evaporated with toluene (3×50 mL) into a crude residue, which was applied to a column of silica gel eluted with hexanes-ethyl acetate (100:1, 10:1, and 4:1) to give a pure 5-O-benzoyl-1,2-O-isopropylidene-5-C-methyl-3-O-naphthalenyl-D-ribofuranose (22.58 g, 50.50 mmol, 61%).

Step 2. Preparation of 5-O-benzoyl-2-C,2-O-didehydro-1-O,5-C-dimethyl-3-O-naphthalenyl-D-ribofuranose

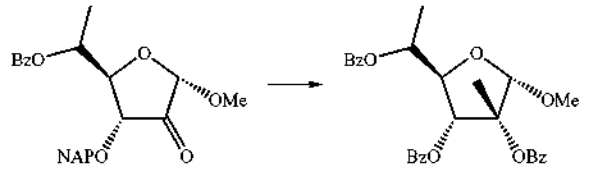
[0206]



[0207] To a solution of dried 5-O-benzoyl-1,2-O-isopropylidene-5-C-methyl-3-O-naphthalenyl-D-ribofuranose (13.58 g, 30.37 mmol) in anhydrous methanol (100 mL) was added 4N HCl in 1,4-dioxane (4.9 mL) and stirred at room temperature for 12 h. The reaction mixture was neutralized with triethylamine to pH=7.0 and concentrated into a crude residue, and poured to 10% sodium bicarbonate aq. solution and extracted with dichloromethane (4×20 mL). The combined organic phase was concentrated and co-evaporated with toluene into a crude residue, which was applied to a column of silica gel eluted with hexanes-ethyl acetate (4:1) to give a pure 5-O-benzoyl-1-O,5-C-dimethyl-3-O-naphthalenyl-D-ribofuranose (12.60 g, 29.93 mmol, 98.5%). To a cold solution of DMSO (12.72 mL, 178.32 mmol) in anhydrous dichloromethane (50 mL) cooled with dry ice-acetone to −75° C. was added trifluoroacetic anhydride (TFAA) (7.6 mL, 53.87 mmol) and stirred at the same temperature for 30 min. 5-O-Benzoyl-1-O,5-C-dimethyl-3-O-naphthalenyl-D-ribofuranose (12.60 g, 29.93 mmol) in anhydrous dichloromethane (10 mL) was added in one portion, then warmed to −20 to −15° C., and stirred at the same temperature for 2 h, and followed by addition of triethylamine (20 mL), and warmed to RT, and stirred at room temperature for 1 h. The reaction mixture was then quenched with water, and extracted with dichloromethane (3×50 mL). The combined organic phase was dried with sodium sulfate, and the filtrate was concentrated into a crude residue, which was applied to a short column of silica gel eluted with hexanes-ethyl acetate (20:1 and 1:1) to give 5-O-benzoyl-2-C,2-O-didehydro-1-O,5-C-dimethyl-3-O-naphthalenyl-D-ribofuranose as amorphous solid. (10.03 g, 23.90 mmol, 80%).

Step 3. Preparation of 2,3,5-O-tribenzoyl-1-O,2,5-C-trimethyl-D-ribofuranose

[0208]



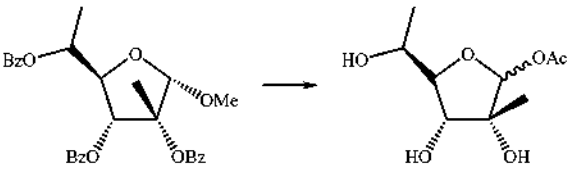
[0209] To a cold solution of dried 5-O-benzoyl-2-C,2-O-didehydro-1-O,5-C-dimethyl-3-O-naphthalenyl-D-ribofuranose (7.76 g, 18.52 mmol) in a mixture of anhydrous tetrahydrofuran (THF) (50 ml) and anhydrous ether (30 mL) cooled with dry ice-acetone to −30 to −15° C. was slowly added methylmagnium bromide (CH_3MgBr) (35 mL) (3.0 M in ether) and stirred at same temperature under nitrogen atmosphere for 6 h, and then at −15° C. to room temperature overnight. The reaction mixture was carefully quenched with sat. ammonium chloride aq. solution, and extracted with ethyl acetate (4×60 mL). The combined organic phase was concentrated and co-evaporated with toluene (3×20 mL) into a crude residue, which was applied to column of silica gel eluted with hexanes-ethyl acetate (20:1) dichloromethane-methanol (10:1) to give a pure 5-O-benzoyl-3-O-naphthalenyl-1-O,2,5-C-trimethyl-D-ribofuranose as syrup (5.32 g, 16.12 mmol, 87%).

[0210] To a solution of dried 5-O-benzoyl-2-C,2-O-didehydro-3-O-naphthalenyl-1-O,2,5-C-trimethyl-D-ribofuranose (10.03 g, 30.39 mmol) and DMAP (1 g, 8.20 mmoL) in anhydrous pyridine (28 mL) was added benzoyl chloride (11.65 g, 9.62 mL, 82.88 mmol) and stirred at room temperature overnight under nitrogen. The reaction mixture was then quenched with methanol and concentrated into a crude residue, which was poured into 10% sodium bicarbonate aq. solution and extracted with ethyl acetate (3×20 mL). The combined organic phase was concentrated and co-evaporated with toluene into a crude residue that was applied to a short column of silica gel eluted with hexanes-ethyl acetate (50:1 and 10:1) to give a pure 2,5-O-dibenzoyl-3-O-naphthalenyl-1-O,2,5-C-trimethyl-D-ribofuranose as amorphous solid (9.17 g, 65%).

[0211] To a solution of 2,5-O-dibenzoyl-3-O-naphthalenyl-1-O,2,5-C-trimethyl-D-ribofuranose (9.17 g, 17.04 mmol) in a mixture of dichloromethane (20 mL) and water (1 mL) was added DDQ (4.45 g, 19.60 mmol) and stirred at room temperature for 6 h. The reaction mixture was diluted with dichloromethane (100 mL) poured into 10% sodium bicarbonate aq. solution, organic phase was separated and water phase was extracted with dichloromethane (3×50 mL). The combined organic phase was washed with sat. sodium bicarbonate aq. solution until all the DDQ was removed. The organic phase was concentrated and co-evaporated with toluene into a crude residue, which was further treated with BzCl (4.88 g, 34.69 mmol) in the presence of DMAP (650 mg) in anhydrous pyridine (20 mL) at room temperature overnight. The reaction mixture was then quenched with methanol and concentrated into a crude residue, which was poured into sat. sodium bicarbonate and extracted with ethyl acetate (4×50 mL). The combined organic phase was dried over anhydrous sodium sulfate and the filtrate was concentrated into a crude residue, which was applied to a short column of silica gel eluted with hexanes-ethyl acetate (30:1, and 10:1) to give a pure 2,3,5-O-tribenzoyl-1-O,2,5-C-trimethyl-D-ribofuranose as amorphous solid (4.21 g, 8.38 mmol, 49.20%).

Step 4: Preparation of 2,3,5-O-tribenzoyl-1-O,2,5-C-trimethyl-D-ribofuranose

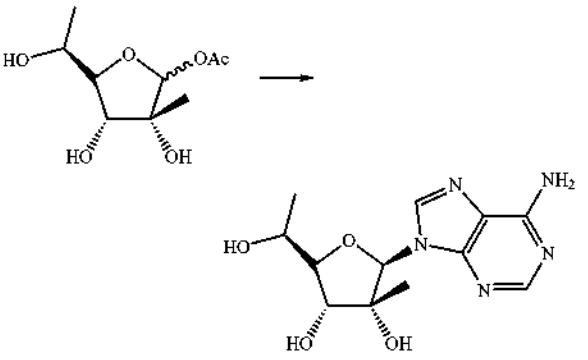
[0212]



[0213] To a cold solution of dried 2,3,5-O-tribenzoyl-1-O,2,5-C-trimethyl-D-ribofuranose (2.43 g, 4.84 mmol) in acetic anhydride (10 mL) cooled with ice-bath was added a cold mixture of acetic anhydride (10 mL) and concentrated sulfuric acid (H_2SO_4) (95-98%) (243 μL) and stirred at the same temperature for 1 h. The reaction mixture was then poured into sat. sodium bicarbonate aq. solution stirred until pH of the mixture is 7 and extracted with ethyl acetate (3×30 mL). The combined organic phase was concentrated and co-evaporated with toluene (3×15 mL) into a crude residue, which was applied to a column of silica gel eluted with hexanes-ethyl acetate (20:1 and 10:1) to give a 1-O-acetyl-2,5-C-dimethyl-2,3,5-O-tribenzoyl-D-ribofuranose (1.6 g, 3.02 mmol, 62%).

Step 5: Preparation of 2',5'-C-dimethyladenosine

[0214]



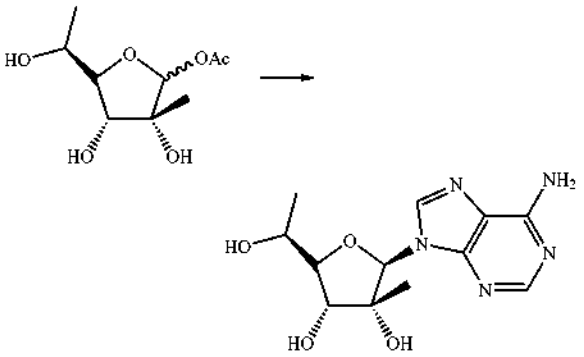
[0215] To a cold solution of N^6-benzoyladenine (99 mg, 0.415 mmol) and 1-O-acetyl-2,5-C-dimethyl-2,3,5-O-tribenzoyl-D-ribofuranose (220 mg, 0.415 mmol) in anhydrous ACN (5 mL) cooled with ice-bath was added TMSOTf (165 μL) and stirred at the same temperature for 1 h. The reaction mixture was then neutralized with triethylamine and concentrated into a crude residue, which was further treated with methanol-ammonia (7N) at room temperature for 4 days. The reaction mixture was then concentrated and co-evaporated with toluene into a crude residue, which was applied to a short

column of silica gel eluted with dichloromethane-methanol (10:1 and 6:1) to give a pure 2',5'-C-dimethyladenosine as amorphous solid.

Example 10

Preparation of 2',5'-diMethylcytidine (10)

[0216]



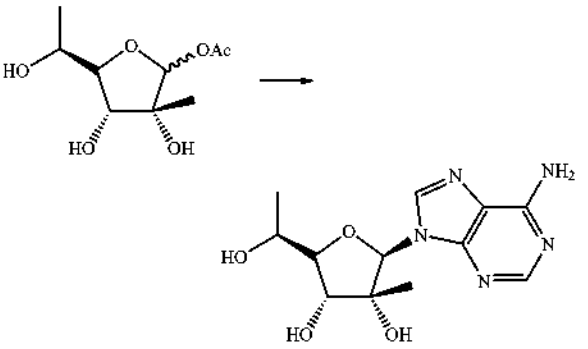
[0217] A stirred suspension of N^4-acetylcytosine (576 mg, 3.76 mmol) and $(NH_4)_2SO_4$ (20 mg) in freshly distilled 1,1,1,3,3,3-hexamethyldisilazane (30 mL) was heated at reflux overnight under nitrogen atmosphere. The clear solution was evaporated under vacuum, and anhydrous toluene (20 mL) was added and subsequently distilled off. The crude bis-(trimethylsilyl) derivative obtained was dissolved in anhydrous acetonitrile (30 mL), and 1-O-acetyl-2,5-C-dimethyl-2,3,5-O-tribenzoyl-D-ribofuranose (1.0 g, 1.88 mmol) was added. The mixture was cold in an ice-water bath under an nitrogen atmosphere, and then TMSOTf (0.5 mL) was added dropwise with vigorous stirring. The resultant homogeneous pale yellow solution was stirred overnight. TLC showed there's still large mount of material. The mixture was cooled in an ice-water bath and another batch of TMSOTf (0.5 ml) was added dropwise. The resultant mixture was stirred overnight further. The reaction was quenched carefully by addition of 10% $NaHCO_3$ (20 mL) and stirred for an additional 15 min. The deposit was filtered and the filtrate was extracted with DCM (60 mL×2). The combined organic phase was washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel chromatography eluting with PE:EA=2:1 to give N^4-acetyl-2,5-C-dimethyl-2,3,5-O-tribenzoylcytidine (660 mg, 56.1%) as foam solid.

[0218] N^4-Acetyl-2,5-C-dimethyl-2,3,5-O-tribenzoylcytidine (660 mg, 1.05 mmol) was dissolved in anhydrous MeOH which was saturated by NH_3. The mixture was heated to 60-70° C. with consistent stirring in a sealed tube for 2 days. The solvent was removed under vacuum and the residue was purified by prep-HPLC to give 2,5-C-dimethylcytidine (120 mg, 41.93% and 22 mg, 7.7%). ^{1}H NMR of 2,5-C-dimethylcytidine (diastereomer 1): (MeOD): δ 7.73-7.75 (d, J=8.0 Hz, 1H), 6.04 (s, 1H), 5.64-5.66 (d, J=8.0 Hz,1H), 4.05-4.07 (dd, J_1=2.4 Hz, J_2=4.8 Hz, 1H), 3.93-3.99 (m, 1H), 3.90 (d, J=2.4 Hz, 1H), 1.23-1.24 (d, J=6.4 Hz, 3H)□1.16 (s, 3H).

Example 11

Preparation of 2',5'-dimethyluridine (11)

[0219]

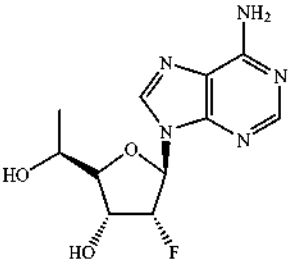


[0220] By a similar procedure as described in example 10, 2,5-C-dimethyluridine was prepared. ^{1}H NMR of 2,5-C-dimethyluridine (diastereomer 2): (MeOD): a 7.73-7.75 (d, J=8.0 Hz, 1H), 6.01 (s, 1H), 5.63-5.65 (d, J=8.0 Hz, 1H), 4.08-4.10 (dd, J_1=2.0 Hz, J_2=5.6 Hz, 1H), 4.06 (d, J=2.0 Hz, 1H), 3.96-4.00 (m, 1H), 1.21-1.22 (d, J=6.4 Hz, 3H)□1.18 (s, 3H).

Example 12

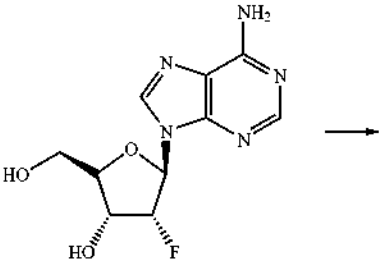
Preparation of 2'-deoxy-2'-fluoro-5'-C-methyladenosine (12)

[0221]

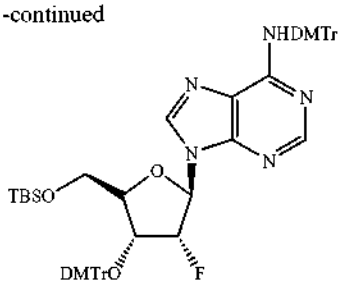


Step 1. Preparation of 3',N^6-bis(4,4'-dimethoxytrityl)-5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluoroadenosine

[0222]



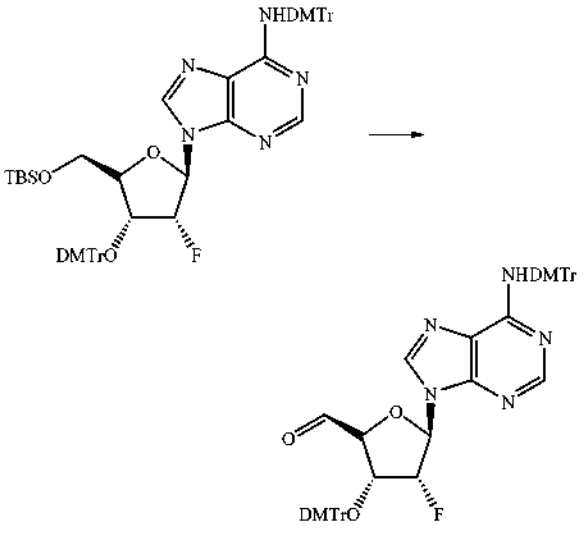
-continued



[0223] A mixture of 0.27 g (1.0 mmol) of 2'-deoxy-2'-fluoroadenosine, DMAP (244 mg, 2.0 mmol) and TBDMS-Cl (1.1 mmol, 181 mg) in anhydrous pyridine (15 mL) was stirred at room temperature overnight and then at 30° C. for 8 hours. DMTr-Cl (1.0 g, 3 mmol) was added and the mixture stirred at 56° C. for 3 days, cooled to 0° C. and quenched with water (1.5 mL). The resulting mixture was stirred at room temperature for 2 hours, diluted with ethyl acetate, washed with brine 3 times, and dried over sodium sulfate. Chromatography on silica gel with 20-35% ethyl acetate in hexane gave 746 mg of 3',N^6-bis(4,4'-dimethoxytrityl)-5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluoroadenosine as white foam.

Step 2. Preparation of 3',N^6-bis(4,4'-dimethoxytrityl)-5'-dehydro-2'-deoxy-2'-fluoroadenosine

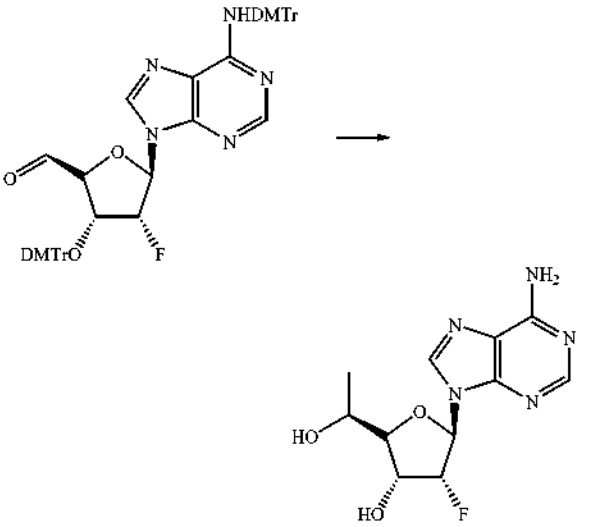
[0224]



[0225] A solution of 3',N^6-bis(4,4'-dimethoxytrityl)-5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluoroadenosine (0.73 g, 0.74 mmol) and TBAF (1.0 M in THF, 1.5 mL) in THF (6 mL) stood at room temperature overnight and then concentrated at room temperature. Chromatography on silica gel with acetone-hexane (2:3) gave the 5'-hydroxy product as white solid, which was dissolved in anhydrous DCM (12 mL). Pyridine (0.9 mL) and Dess-Martin periodinane (0.39 g) were added. The reaction mixture under argon was stirred at 25° C. for 2 hours, diluted with DCM, washed with 10% $Na_2S_2O_3$ 2 times and brine 1 time. Chromatography on silica gel with acetone-hexanes (1:3 to 2:3) gave 606 mg of 3',N^6-bis(4,4'-dimethoxytrityl)-5'-dehydro-2'-deoxy-2'-fluoroadenosine.

Step 3. Synthesis of 2'-deoxy-2'-fluoro-5'(R and S)—C-methyladenosine.

[0226]



[0227] To a solution of 3',N^6-bis(4,4'-dimethoxytrityl)-5'-dehydro-2'-deoxy-2'-fluoroadenosine (600 mg, 0.686 mmol) in THF (7 mL) at 0° C. under argon was added MeMgBr (1.4 M in THF, 2 mL). The reaction mixture was stirred at 0° C. under argon overnight. Additional MeMgBr (1.4 mL) was added and the reaction mixture was stirred at 0° C. for 2 hours and then at room temperature for 30 minutes. After cooling to 0° C., the reaction mixture was quenched very slowly with 10% ammonium sulfate, diluted with ethyl acetate, and washed with 10% ammonium sulfate 2 times and 10% sodium bicarbonate 1 time. Chromatography on silica gel with acetone-hexane (1:3 to 2:3) gave 315 mg of 3',N^6-bis(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-5'(R and S)—C-methyladenosine (216 mg of the upper isomer on TLC and 99 mg of the mixture of the two isomers, both as white solid).

[0228] 3',N^6-Bis(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-5'(R or S)—C-methyladenosine (upper isomer on TLC, 215 mg) was dissolved in 5 mL of THF, 8 mL of AcOH and 5 mL of water. The solution was stirred at 30° C. for 15 hours, concentrated to dryness and co-evaporated with toluene 3 times. Chromatography on silica with 10-12% MeOH in DCM gave 55 mg of 2'-deoxy-2'-fluoro-5'(R or S)—C-methyladenosine as white solid; ^{1}H NMR (DMSO) δ 1.16 (d, J=6.4 Hz, 1H), 3.79-3.85 (m, 2H, H4' and H5'), 4.45 (ddd, $J_{H,H}$=6.4 and 3.2 Hz, $J_{H,F}$=16.4 Hz, 1H, H3'), 5.26 (d, J=6.0 Hz, 1H, OH), 5.40 (ddd, $J_{H,H}$=4.0 and 3.2 Hz, J_{HF}=53.2 Hz, 1H, H2'), 5.68 (d, J=6.0 Hz, 1H, OH), 6.23 (dd, J_{HH}=3.2 Hz, $J_{H,F}$=15.6 Hz, 1H, H1'), 7.38 (s, 2H, NH_2), 8.15 (s, 1H, H8), 8.41 (s, 1H, H2).

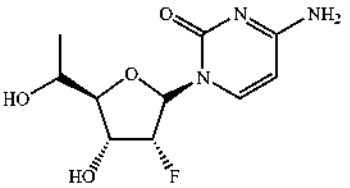
[0229] 3',N^6-Bis(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-5'(R and S)—C-methyladenosine (the upper isomer as the major and lower isomer as the minor, 99 mg) was dissolved in 3 mL of THF, 3 mL of AcOH and 3 mL of water was stirred at room temperature overnight. THF was removed on a rotary evaporator and the remaining solution was heated at 45° C.

for 45 minutes, concentrated, co-evaporated with toluene 3×. Chromatography on silica with 10-12% MeOH in DCM gave 22 mg of 2'-deoxy-2'-fluoro-5'(R and S)—C-methyladenosine.

Example 13

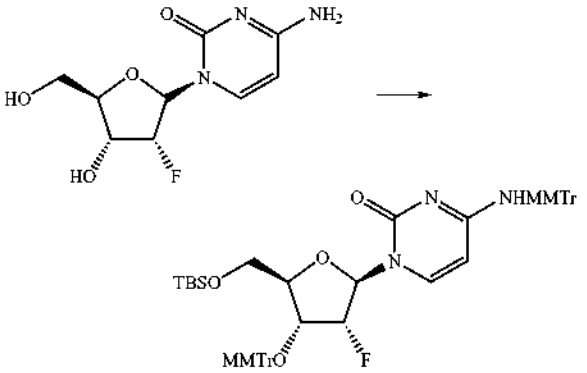
Preparation of 2'-deoxy-2'-fluoro-5'-C-methylcytidine (13)

[0230]



Step 1. Preparation of 3-O—,N^4-bis(4-methoxytrityl)-5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluorocytidine

[0231]

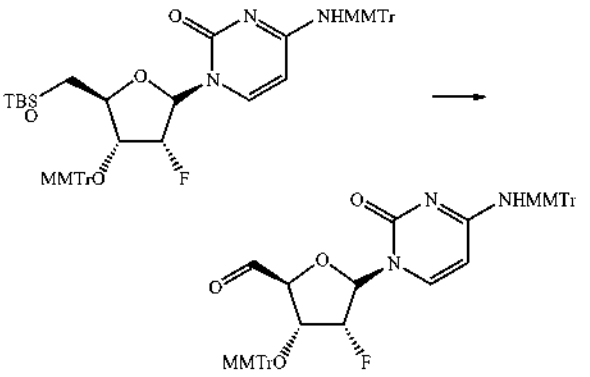


[0232] A solution of 2'-deoxy-2'-fluorocytidine (20.0 g, 81.6 mmol) and TBDMS-Cl (14.8 g, 97.9 mmol) in anhydrous pyridine (200 mL) was stirred at room temperature overnight and then concentrated. The residue was diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give 24 g (82%) of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluorocytidine as a white solid.

[0233] Silver nitrate (7 g, 41.7 mmol) was added to a solution of MMTr-Cl (13 g, 41.7 mmol), 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluorocytidine (5 g, 13.9 mmol) and collidine (19 g, 153 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at room temperature overnight, filtered, and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. Chromatography on silica gel with ethyl acetate-petroleum ether (1:2 to 1:1) gave 11 g (87%) of 3'-O—,N^4-bis(4-methoxytrityl)-5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluorocytidine.

Step 2. Preparation of 3-O—,N^4-bis(4-methoxytrityl)-5'-C,5'-O-didehydro-2'-deoxy-2'-fluorocytidine

[0234]

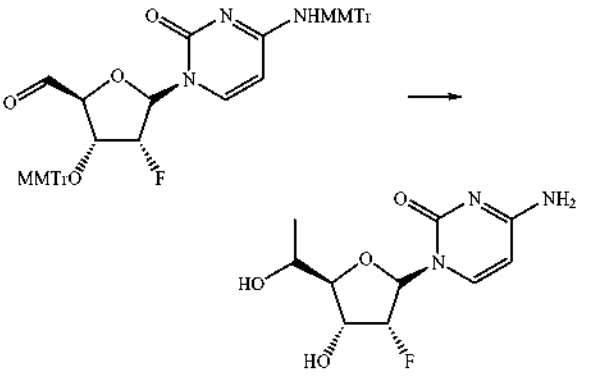


[0235] TBAF (24 mL, 1.0 M in THF) was added dropwise to a solution of 3'-O—,N^4-bis(4-methoxytrityl)-5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluorocytidine (11 g, 12 mmol) in anhydrous THF (100 mL) at 0° C. The solution was stirred at room temperature overnight and then solvent was removed in vacuo at room temperature. The residue was dissolved in ethyl acetate, washed with water and brine, dried over Na_2SO_4, and concentrated. Chromatography on silica gel with acetone/petroleum ether (1:3) gave 9 g (93%) of 3'-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2'-fluorocytidine. Pyridine (6 mL, 15 eq) and Dess-Martin periodinane (2.6 g, 6 mmol) was added to a solution of 3'-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2'-fluorocytidine (4 g, 5 mmol) in anhydrous DCM (30 mL) at 0° C. under N_2. The reaction mixture was stirred at room temperature for 2 hours, diluted with ethyl acetate, washed with 10% $Na_2S_2O_3$ twice and then with brine, dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel with acetone-petroleum ether (1:3 to 2:3) gave 3.5 g (87%) of 3-O—,N^4-bis(4-methoxytrityl)-5'-C,5'-O-didehydro-2'-deoxy-2'-fluorocytidine

(13)

Step 3. Preparartion of 2'-deoxy-2'-fluoro-5'(R and S)'C-methylcytidine

[0236]



[0237] MeMgBr (3.0 M in ether, 15.2 mmol) was added dropwise to a solution of 3-O—,N^4-bis(4-methoxytrityl)-5'-C,5'-O-didehydro-2'-deoxy-2'-fluorocytidine (3 g, 3.8 mmol) in anhydrous THF (50 mL) in an ice-EtOH bath under N_2. The

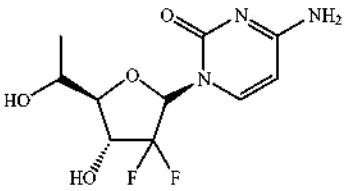
reaction mixture was stirred at room temperature for 5 hours, quenched with sat. NH_4Cl, diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give the crude product. Chromatography on silica gel with acetone-petroleum ether (1:3 to 2:3) gave 1.8 g (58%) of pure 3-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2'-fluoro-5'(R or S)—C-methylcytidine.

[0238] A solution of 3-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2'-fluoro-5'(R or S)—C-methylcytidine (600 mg, 0.75 mmol) in AcOH/H_2O (v/v 4:1, 20 mL) was stirred at 50° C. overnight. The solution was concentrated, diluted with water, extracted with ethyl acetate twice and concentrated to dryness. Chromatography on a reverse-phase HPLC and then on a chiral HPLC gave 2'-deoxy-2'-fluoro-5'(R or S)—C-methylcytidine (30 mg, 16%); ^{1}H NMR (CD_3OD): δ 8.16 (d, J=7.6 Hz, 1H, H6), 5.99 (dd, J=17.6 Hz, 1.2 Hz, 1H, H1'), 5.92 (d, J=7.6 Hz, 1H, H5), 5.06-4.92 (m, 1H, H2'), 4.28 (ddd, $J_{H,H}$=8.4, 4.4 Hz, $J_{H,F}$=21.6 Hz, 1H, H3'), 4.02 (dq, J=4.0, 2.8 Hz, 1H, H5'), 3.87 (dd, J=8.0, 2.0 Hz, 1H, H4'), 1.38 (d, J=6.4 Hz, 3H, Me).

Example 14

Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (14)

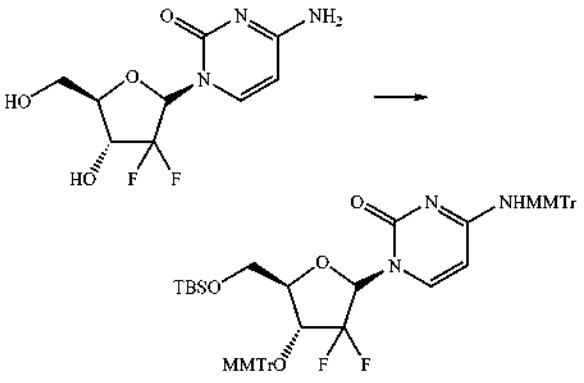
[0239]



(14)

Step 1. Preparation of 3-O—,N^4-bis(4-methoxytrityl)-5'-O-(t-butyldimethylsilyl)-2'-deoxy-2',2'-difluorocytidine

[0240]

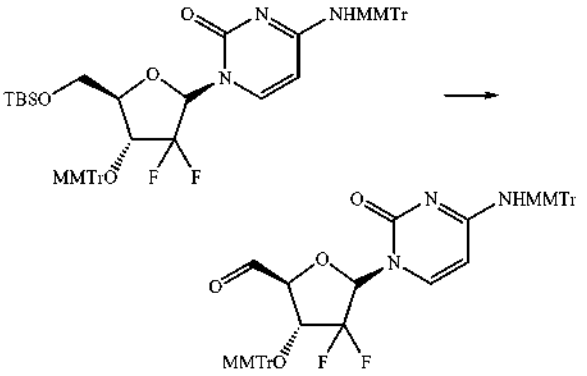


[0241] TBDMS-Cl (10.5 g, 69.3 mmol) was added to a solution of 2'-deoxy-2',2'-difluorocytidine hydrochloride (17.0 g, 57.7 mmol) in anhydrous pyridine (100 mL) at 0° C. under N_2. The reaction mixture was stirred at room temperature overnight, concentrated, diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2',2'-difluorocytidine (21 g, 96%) as a white solid.

[0242] MMTr-Cl (13 g, 41 mmol, 3 eq) was added to a solution of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2',2'-difluorocytidine (5 g, 13.5 mmol) in anhydrous DCM (50 mL), followed by addition of $AgNO_3$ (7 g, 41 mmol) and collidine (19 g, 153 mmol). The reaction mixture was stirred at room temperature overnight under N_2, filtered, washed with saturated $NaHCO_3$ and then with brine. The organic layer was dried over Na_2SO_4 and concentrated. Chromatography on silica gel with ethyl acetate-petroleum ether (1:3 to 1:2) gave 3-O—,N^4-bis(4-methoxytrityl)-5'-O-(t-butyldimethylsilyl)-2'-deoxy-2',2'-difluorocytidine (11 g, 83%).

Step 2. Preparation of 3-O—,N^4-bis(4-methoxytrityl)-5'-C,5'-O-didehydro-2'-deoxy-2',2'-difluorocytidine

[0243]

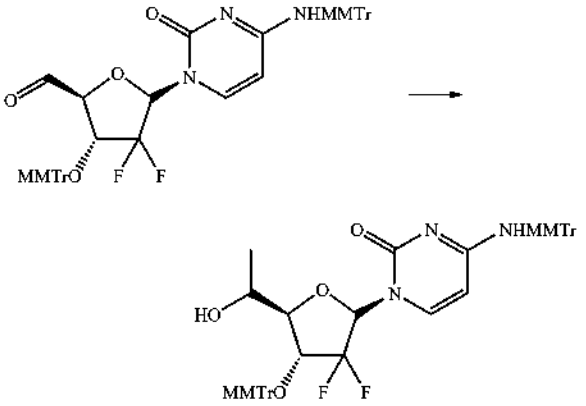


[0244] TBAF (1 M in THF, 21.6 mL) was added dropwise to a solution of 3-O—,N^4-bis(4-methoxytrityl)-5'-O-(t-butyldimethylsilyl)-2'-deoxy-2',2'-difluorocytidine (10.0 g, 10.8 mmol) in anhydrous THF (40 mL) at 0° C. The resulting solution was stirred at room temperature overnight, concentrated, diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel with ethyl acetate-DCM (1:10 to 1:5) gave 3-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2',2'-difluorocytine (4.4 g, 50%).

[0245] TFA (460 uL, 6 mmol) was added to a stirred solution of anhydrous pyridine (960 uL, 12 mmol) in anhydrous DMSO (10 mL) cooled with cold water under N_2. After addition, the TFA/pyridine solution was warmed to R.T. and added to a stirred solution of 3-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2',2'-difluorocytidine (8.1 g, 10 mmol) and DCC (6.2 g, 30 mmol) in anhydrous DMSO (30 mL) cooled with cold water under N_2. The reaction mixture was stirred at R.T. overnight. Cooled with cold water, quenched with water (20 mL) and stirred at R.T. for 1 h and diluted with EA. Precipitate was filtered and washed with EA. The combined EA solution was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified by silica gel column (PE/EA=1/1 to 1/3) to give 3-O—,N^4-bis(4-methoxytrityl)-5'-C,5'-O-didehydro-2'-deoxy-2',2'-difluorocytidine (6.2 g, 76%).

Step 3. Preparation of 3-O—,N^4-bis(4-methoxytrityl)-5'-dehydro-2'-deoxy-2',2'-difluorocytidine

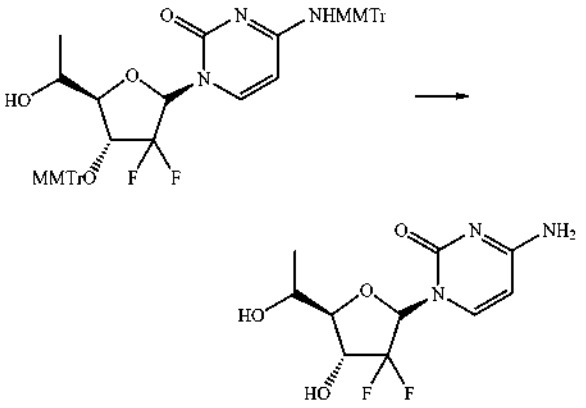
[0246]



[0247] MeMgBr (3.0M in ether, 10 mL, 30 mmol) was added dropwise to a solution of the crude 3-O—,N^4-bis(4-methoxytrityl)-5'-dehydro-2'-deoxy-2',2'-difluorocytidine (6.0 g, 7.4 mmol) in anhydrous THF (30 mL) in an ice-EtOH bath under N_2. The reaction mixture was stirred at room temperature overnight, quenched with saturated NH_4Cl, diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel with ethyl acetate-petroleum ether (1:3 to 1:1) gave 3.6 g of 3-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2',2'-difluoro-5'-C-methylcytidine (59%).

Step 4. Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine.

[0248]

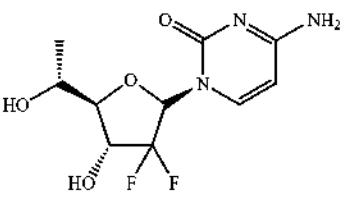


[0249] A solution of 3-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2',2'-difluoro-5'(R and S)—C-methylcytidine (3 g, 3.65 mmol) in AcOH/H_2O (20 mL, v/v 4:1) was stirred at 50° C. overnight. After removal of solvents the residue was diluted with water, extracted with ethyl acetate twice and concentrated. Chromatography on a reverse-phase HPLC gave 0.3 g (30%) of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine as white solid; ^1H NMR (CD_3OD) δ 7.93 (d, J=7.6 MHz, 1H, H6), 6.24 (t, $J_{H,F}$=8.0 Hz, 1H, H1'), 5.95 (d, J=7.6 MHz, 1H, H5), 4.26 (dt, $J_{H,H}$=8.4 Hz, $J_{H,F}$=12.4 Hz, 1H, H3'), 4.03 (dq, J=4.0, 2.7 Hz, 1H, H5'), 3.74 (dd, J=8.4, 2.8 Hz, 1H, H4'), 1.37 (d, J=6.4 MHz, 3H).

Example 15

2'-DEOXY-2',2'-DIFLUORO-5'(R)—C-METHYL-CYTIDINE (15)

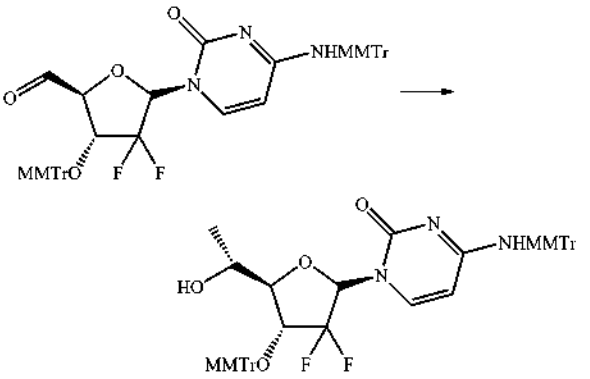
[0250]



(15)

Step 1. Preparation of 3-O,N^4-bis(4-methoxytrityl)-2'-deoxy-2',2'-difluoro-5'(R)- methylcytidine

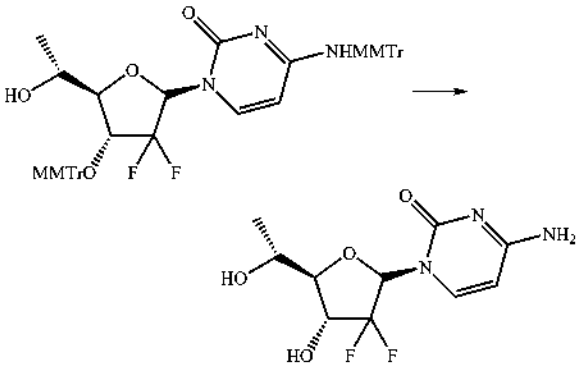
[0251]



[0252] MeMgBr (1.4 M in THF, 2.6 mL, 3.6 mmol) was added dropwise to a solution of the crude 3-O—,N^4-bis(4-methoxytrityl)-5'-dehydro-2'-deoxy-2',2'-difluorocytidine (580 mg, 0.72 mmol) in anhydrous THF (8 mL) at 0° C. under argon. The reaction mixture was stirred at room temperature for 3 h, cooled with ice, quenched with aqueous $(NH_4)_2SO_4$, diluted with ethyl acetate, washed with aqueous $(NH_4)_2SO_4$ solution four times and then with brine, dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel with ethyl acetate-hexanes (55:45 to 70:30) gave 317 mg of 3-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine and 44 mg of 3-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2',2'-difluoro-5'(R)—C-methylcytidine.

Step 2. Preparation of 2'-deoxy-2',2'-difluoro-5'(R)—C-methylcytidine

[0253]

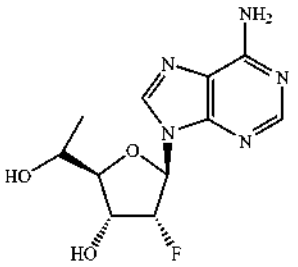


[0254] A solution of 3-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2',2'-difluoro-5'(R)—C-methylcytidine (44 mg 0.53 mmol) in AcOH/H_2O (3 mL, v/v 4:1) was stirred at 40° C. overnight. After removal of solvents the residue was co-evaporated with toluene two times. Chromatography on silica gel with 10-15% MeOH in DCM gave 9 mg of 2'-deoxy-2',2'-difluoro-5'(R)—C-methylcytidine as white solid;

Example 16

Preparation of 2'-deoxy-2'-fluoro-5'(S)—C-methyladenosine (16)

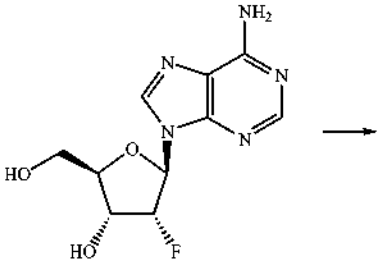
[0255]



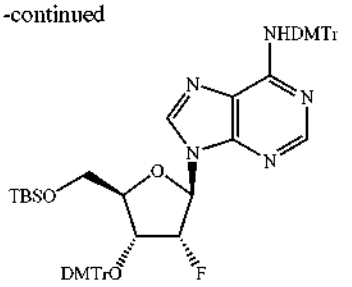
(16)

Step 1. Preparation of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-3'-O,N^6-bis(4,4'-dimethoxytrityl)-2'-fluoroadenosine

[0256]



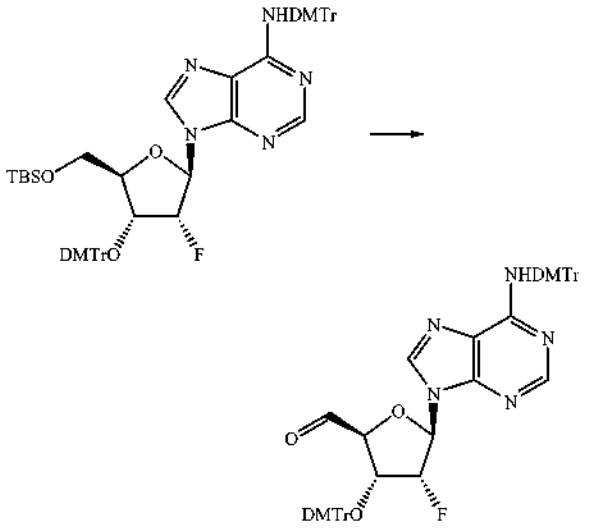
-continued



[0257] A mixture of 0.27 g (1.0 mmol) of 2'-deoxy-2'-fluoroadenosine, DMAP (244 mg, 2.0 mmol) and TBDMS-Cl (1.1 mmol, 181 mg) in anhydrous pyridine (15 mL) was stirred at RT overnight and then at 30° C. for 8 h. DMTr-Cl (1.0 g, 3 mmol) was added and the mixture stirred at 56° C. for 3 days, cooled to 0° C. and quenched with water (1.5 mL). The resulting mixture was stirred at RT for 2 h, diluted with ethyl acetate, washed with brine 3×, and dried over sodium sulfate. Chromatography on silica gel with 20-35% ethyl acetate in hexane gave 746 mg of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-3'-O,N^6-bis(4,4'-dimethoxytrityl)-2'-fluoroadenosine

Step 2. Preparation of 2'-deoxy-5'-C,5'-O-didehydro-3'-O,N^6-bis(4,4'-dimethoxytrityl)-2'-fluoroadenosine

[0258]

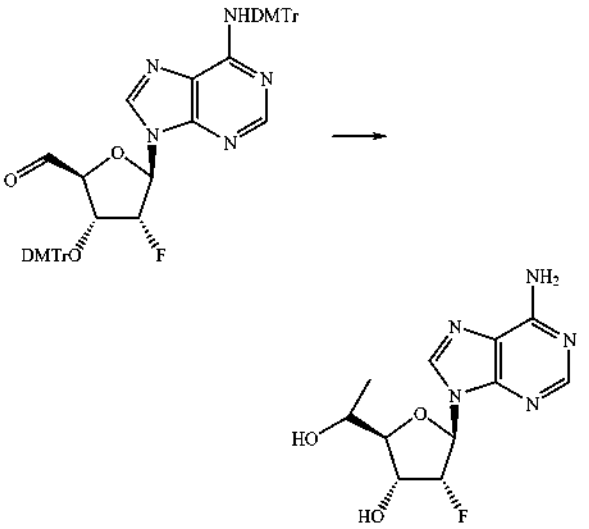


[0259] A solution of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-3',N^6-di(4,4'-dimethoxytrityl)-2'-fluoroadenosine (0.73 g, 0.74 mmol) and TBAF (1.0 M in THF, 1.5 mL) in THF (6 mL) stood at RT overnight and then concentrated at RT. Chromatography on silica gel with acetone-hexane (2:3) gave the 5'-hydroxy product as white solid, which was dissolved in anhydrous DCM (12 mL). Pyridine (0.9 mL) and Dess-Martin periodinane (0.39 g) were added. The reaction mixture under argon was stirred at 25° C. for 2 h, diluted with DCM, washed with 10% $Na_2S_2O_3$ 2× and brine 1×. Chromatography on silica gel with acetone-hexanes (1:3 to 2:3) gave 606 mg of

2'-deoxy-5'-C,5'-O-didehydro-3'-O,N^6-bis(4,4'-dimethoxytrityl)-2'-fluoroadenosine as white foam.

Step 3. Preparation of 2'-deoxy-2'-fluoro-5'(R and S)—C-methyladenosine

[0260]



[0261] To a solution of 2'-deoxy-5'-C,5'-O-didehydro-3'-O,N^6-bis(4,4'-dimethoxytrityl)-2'-fluoroadenosine (600 mg, 0.686 mmol) in THF (7 mL) at 0° C. under argon was added MeMgBr (1.4 M in THF, 2 mL). The reaction mixture was stirred at 0° C. under argon overnight. More MeMgBr (1.4 mL) was added and the reaction mixture was stirred at 0° C. for 2 h and then at RT for 30 min. After cooling to 0° C., the reaction mixture was quenched very slowly with 10% ammonium sulfate, diluted with ethyl acetate, and washed with 10% ammonium sulfate 2× and 10% sodium bicarbonate 1×. Chromatography on silica gel with acetone-hexane (1:3 to 2:3) gave 315 mg of 3'-O,N^6-bis(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-5'(R and S)—C-methyladenosine (216 mg of 5'(S)-isomer and 99 mg of the mixture of the 5'(S)-isomer and 5'(R)-isomer, both as white foam.

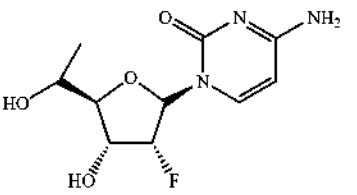
[0262] 3'-O,N^6-bis(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-5'(S)—C-methyladenosine (upper spot on TLC, 215 mg) was dissolved in 5 mL of THF, 8 mL of AcOH and 5 mL of water. The solution was stirred at 30° C. for 15 h, concentrated to dryness and co-evaporated with toluene 3×. Chromatography on silica with 10-12% MeOH in DCM gave 55 mg of 2'-deoxy-2'-fluoro-5'(S)—C-methyladenosine as white solid; ^{1}H NMR (DMSO) δ 1.16 (d, J=6.4 Hz, 1H), 3.79-3.85 (m, 2H, H4' and H5'), 4.45 (ddd, $J_{H,H}$=6.4 and 3.2 Hz, $J_{H,F}$=16.4 Hz, 1H, H3'), 5.26 (d, J=6.0 Hz, 1H,OH), 5.40 (ddd, $J_{H,H}$=4.0 and 3.2 Hz, J_{HF}=53.2 Hz, 1H, H2'), 5.68 (d, J=6.0 Hz, 1H,OH), 6.23 (dd, $J_{H,H}$=3.2 Hz, $J_{H,F}$=15.6 Hz, 1H, H1'), 7.38 (s, 2H, NH_2), 8.15 (s, 1H, H8), 8.41 (s, 1H, H2).

[0263] 2'-Deoxy-3',N^6-di(4,4'-dimethoxytrityl)-2'-fluoro-5'(R and S)—C-methyladenosine (the upper isomer as the major and lower isomer as the minor, 99 mg) was dissolved in 3 mL of THF, 3 mL of AcOH and 3 mL of water was stirred at RT overnight. THF was removed on a rotary evaporator and the remaining solution was heated at 45° C. for 45 min, concentrated, co-evaporated with toluene 3×. Chromatography on silica with 10-12% MeOH in DCM gave 22 mg of 2'-deoxy-2'-fluoro-5'(R and S)—C-methyladenosine as white solid.

Example 17

Preparation of 2'-deoxy-2'-fluoro-5'-C-methylcytidine (17)

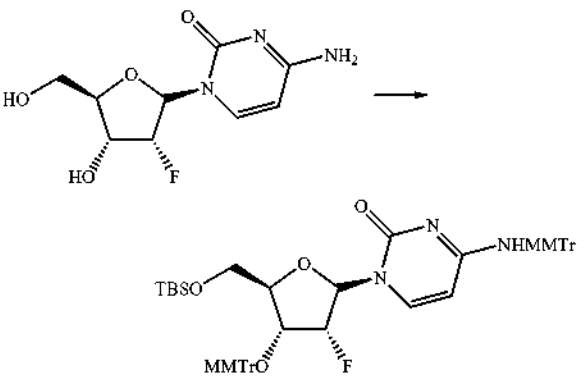
[0264]



(17)

Step 1. Preparation of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-3-O,N^4-di(4-methoxytrityl)-2'-fluorocytidine

[0265]

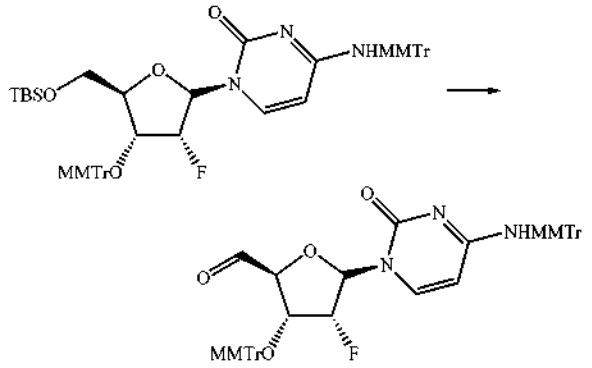


[0266] A solution of 2'-deoxy-2'-fluorocytidine (20.0 g, 81.6 mmol) and TBDMS-Cl (14.8 g, 98.2 mmol) in anhydrous pyridine (200 mL) was stirred at RT overnight and then concentrated. The residue was diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give 24 g (82%) of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluorocytidine as a white solid.

[0267] Silver nitrate (7 g, 41.2 mmol) was added to a solution of MMTr-Cl (13 g, 42.2 mmol), 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluorocytidine (5 g, 13.9 mmol) and collidine (19 g, 157 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at RT overnight, filtered, and washed with sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. Chromatography on silica gel with ethyl acetate-petroleum ether (1:2 to 1:1) gave 11.5 g (91%) of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-3'-O—, N^4-di(4-ethoxytrityl)-2'-fluorocytidine.

Step 2. Preparation of 2'-deoxy-5'-C,5'-O-didehydro-3-O—,N^4-di(4-methoxytrityl)-2'-fluorocytidine

[0268]

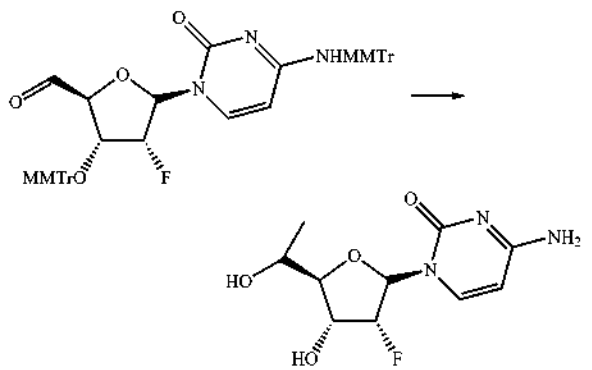


[0269] TBAF (24.4 mL, 1.0 M in THF) was added dropwise to a solution of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-3'-O—,N^4-di(4-methoxytrityl)-2'-fluorocytidine (11 g, 12.2 mmol) in anhydrous THF (100 mL) at 0° C. The solution was stirred at RT overnight and then solvent was removed in vacuo at RT. The residue was dissolved in ethyl acetate, washed with water and brine, dried over Na_2SO_4, and concentrated. Chromatography on silica gel with acetone/petroleum ether (1:3) gave 9 g (93%) of 2'-deoxy-3'-O—,N^4-di(4-methoxytrityl)-2'-fluorocytidine.

[0270] Pyridine (6 mL) and Dess-Martin periodinane (2.6 g, 6.1 mmol) was added to a solution of 2'-deoxy-3'-O—,N^4-di(4-methoxytrityl)-2'-fluorocytidine (4 g, 5.0 mmol) in anhydrous DCM (30 mL) at 0° C. under N_2. The reaction mixture was stirred at RT for 2 h, diluted with ethyl acetate, washed with 10% $Na_2S_2O_3$ twice and then with brine, dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel with acetone-petroleum ether (1:3 to 2:3) gave 3.5 g (87%) of 2'-deoxy-5'-C,5'-O-didehydro-3-O—,N^4-di(4-methoxytrityl)-fluorocytidine.

Step 3. Preparation of 2'-deoxy-2'-fluoro-5'(R and S)—C-methylcytidine

[0271]



[0272] MeMgBr (3.0 M in ether, 5.1 mL) was added dropwise to a solution of 2'-deoxy-5'-C,5'-O-didehydro-3-O—,N^4-di(4-methoxytrityl)-2'-fluorocytidine (3 g, 3.8 mmol) in anhydrous THF (50 mL) in an ice-EtOH bath under N_2. The reaction mixture was stirred at RT for 5 h, quenched with sat. NH_4Cl, diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give a crude product (one isomer was dominant). Chromatography on silica gel with acetone-petroleum ether (1:3 to 2:3) gave 1.8 g (58%) of 2'-deoxy-3-O—,N^4-di(4-methoxytrityl)-2'-fluoro-5'-C-methylcytidine.

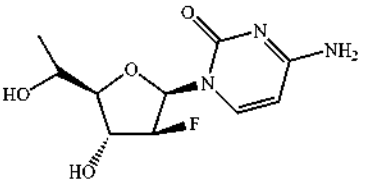
[0273] A solution of 2'-deoxy-3-O—,N^4-di(4-methoxytrityl)-2'-fluoro-5'-C-methylcytidine (600 mg, 0.75 mmol) in AcOH/H_2O (v/v 4:1, 20 mL) was stirred at 50° C. overnight. The solution was concentrated, diluted with water, extracted with ethyl acetate twice and concentrated to dryness. Chromatography on a reverse-phase HPLC and then by SFC separation gave 30 mg (16%) of 2'-deoxy-2'-fluoro-5'(S)—C-methylcytidine as white solid; ^1H NMR (CD_3OD): δ 8.16 (d, J=7.6 Hz, 1H, H6), 5.99 (dd, J=17.6 Hz, 1.2 Hz, 1H, H1'), 5.92 (d, J=7.6 Hz, 1H, H5), 5.06-4.92 (m, 1H, H2'), 4.28 (ddd, $J_{H,H}$=8.4, 4.4 Hz, $J_{H,F}$=21.6 Hz, 1H, H3'), 4.02 (dq, J=4.0, 2.8 Hz, 1H, H5'), 3.87 (dd, J=8.0, 2.0 Hz, 1H, H4'), 1.38 (d, J=6.4 Hz, 3H, Me).

Example 18

Preparation of 2'-deoxy-2'-fluoro-5'-C-methylarabinocytidine (18)

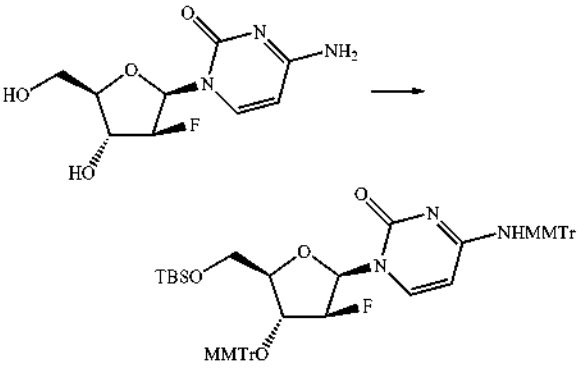
[0274]

(18)



Step 1. Preparation of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-3'-O,N^4-di(4-methoxytrityl)-2'-fluoroarabinocytidine

[0275]



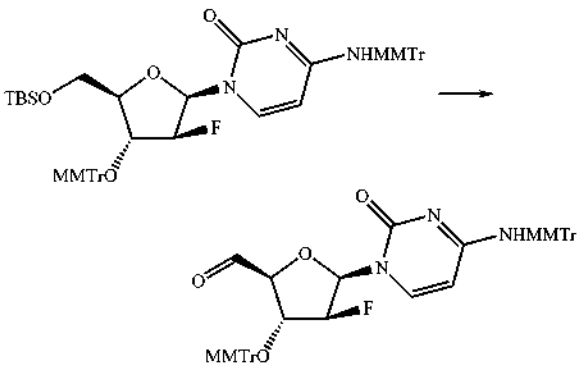
[0276] TBSCl (738 mg, 4.9 mmol) was added into a solution of 2'-deoxy-2'-fluoroarabinocytidine (1.0 g, 4.08 mmol) in anhydrous pyridine (10 mL) at 0° C. under N_2, and stirred

at RT overnight. TLC showed the reaction was completed. Then the pyridine was evaporated under reduced pressure. The residue was diluted with EA, washed with water and followed by brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluoroarabinocytidine (1.3 g, 89%) as a white solid.

[0277] MMTrCl (3.38 g, 10.8 mmol) was added into a solution of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2'-fluoroarabinocytidine (1.3 g, 3.6 mmol) in anhydrous DCM (15 mL), $AgNO_3$ (1.82 g, 10.8 mmol) and collidine (5.4 ml, 39.6 mmol) was added thereto. The reaction mixture was stirred at RT overnight under N_2 and TLC showed the reaction was well. Then the reaction mixture was filtered and washed with sat. $NaHCO_3$ solution and followed by brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give the residue which was purified by silica gel (hexane/EA=2/1 to 1/1) to give 5'-O-(t-butyldimethylsilyl)-2'-deoxy-3'-O,N^4-di(4-methoxytrityl)-2'-fluoroarabinocytidine (2.3 g, 71%).

Step 2. Preparation of 2'-deoxy-5-C,5'-O-didehydro-3'-O,N^4-di(4-methoxytrityl)-2'-fluoroarabinocytidine

[0278]

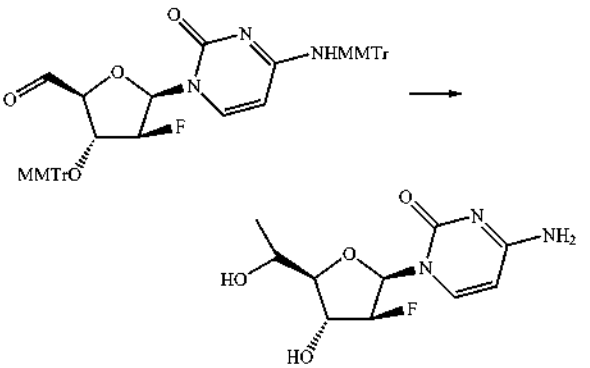


[0279] TBAF (5.08 ml, 1M in THF, 5.08 mmol) was added dropwise into a solution of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-3'-O,N^4-di(4-methoxytrityl)-2'-fluoroarabinocytidine (2.3 g, 2.54 mmol) in anhydrous THF (20 mL) at 0° C. and stirred at RT overnight. TLC showed the reaction was completed. Then the solvent was removed in vacuo at RT. EA was added to the residue and washed with water, followed by brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the residue which was purified by silica gel (heaxne/EA=1:3) to give 2'-deoxy-3'-O,N^4-di(4-methoxytrityl)-2'-fluoroarabinocytidine (1.7 g, 85%).

[0280] Pyridine (2.55 mL, 32.3 mmol) and Dess-Martin (1.1 g, 1.2 eq) was added into a solution of 2'-deoxy-3'-O,N^4-di(4-methoxytrityl)-2'-fluoroarabinocytidine (1.7 g, 2.15 mmol) in anhydrous CH_2Cl_2 (15 mL) at 0° C. under N_2. The reaction mixture was stirred at RT for 2 h and TLC showed the reaction was completed. Then the reaction mixture was diluted with EA. The organic layer was washed with 10% $Na_2S_2O_3$ twice, followed by water and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the residue which was purified by silica gel (hexane/EA=1/3) to give 2'-deoxy-5-C,5'-O-didehydro-3'-O,N^4-di(4-methoxytrityl)-2'-fluoroarabinocytidine (1.15 g, 68%).

Step 3. Preparation of 2'-deoxy-2'-fluoro-5'-C-methylarabinocytidine

[0281]



[0282] MeMgBr (4.17 mL, 5.84 mmol) was added dropwise into a solution of 2'-deoxy-5- C,5'-O-didehydro-3'-O, N^4-di(4-methoxytrityl)-2'-fluoroarabinocytidine (1.15 g, 1.46 mmol, 1 eq) in anhydrous THF (25 mL) which was cooled by ice-EtOH bath under N_2. The reaction mixture was stirred at RT for 5 h and TLC showed the reaction was completed. Then the reaction mixture was quenched with sat. NH_4Cl. EA was added to the mixture for extracting. The organic layer was washed with water and followed by brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the residue which was purified by silica gel (hexanes/EA=1/1 to 1/3) to give 2'-deoxy-3'-O,N^4-di(4-methoxytrityl)-2'-fluoro-5'-C-methylarabinocytidine (1.0 g, 85%).

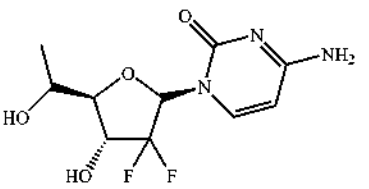
[0283] A solution of 2'-deoxy-3'-O,N^4-di(4-methoxytrityl)-2'-fluoro-5'-C-methylarabinocytidine (200 mg, 0.24 mmol) in AcOH/H_2O (v/v=4:1, 10 mL) was stirred at 50° C. overnight. TLC showed the reaction was completed. The solvent was evaporated in vacuo and the residue was diluted with water, extracted with EA twice to remove some impurity. The water layer was concentrated in vacuo to give the residue which was purified by Chromatography on silica with 5-12% MeOH in DCM gave give 2'-deoxy-2'-fluoro-5'-C-methylarabinocytidine (61mg). ^1H NMR (DMSO-d_6): 1.14 (d, J=8.0 Hz, 3H), 3.53 (t, J=5.2 Hz, 3H), 3.74 (br s, 1H), 4.11-4.35 (m, 1H), 4.79-5.00 (m, 2H), 5.71-5.82 (m, 2H), 6.01, 6.07 (each d, J=3.6 Hz, 1H), 7.57 & 7.74 (each dd, J=1.6, 7.6 Hz, 1H).

Example 19

Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (19)

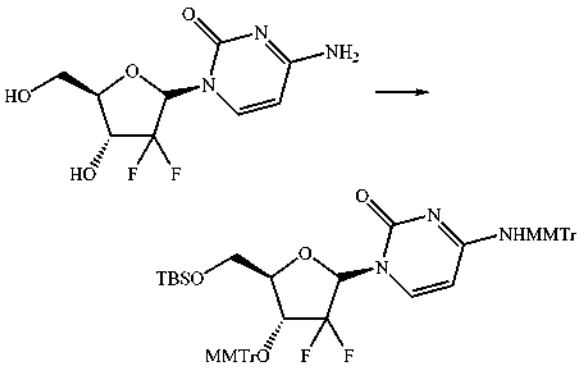
[0284]

(19)



Step 1. Preparation of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2',2'-difluoro-3'-O,N^4-di(4-methoxytrityl) cytidine

[0285]

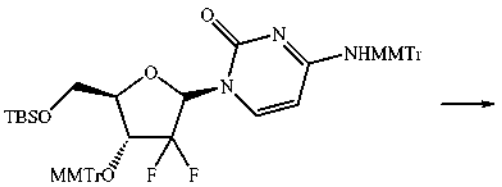


[0286] To an ice-cold solution of 2'-deoxy-2',2'-difluorocytidine (51.0 g, 170.7 mmol) in anhydrous pyridine (500 mL) was added TBSCl (32 g, 208 mmol) in small portions under N_2. The reaction mixture was stirred at RT overnight. The solvent was removed under vacuum and the residue was diluted with EA (1000 mL), washed with water and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give crude 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2',2'-difluorocytidine (63 g, 96%) as a white solid which was used without further purification.

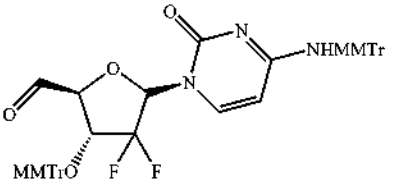
[0287] To a mixture of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2',2'-difluorocytidine (60 g, 160 mmol), $AgNO_3$ (77.8 g, 510 mmol) and collidine (159.8 g, 1.32 mol) in anhydrous DCM (800 mL) was added MMTrCl (156.8 g, 510 mmol) in small portions under N_2. The reaction mixture was stirred at RT overnight. The reaction mixture was filtered through a Buchner Funnel and the filtrate was washed with sat. $NaHCO_3$ solution and followed by brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give the residue which was purified by silica gel column (PE/EA=3/1 to 2/1) to give crude 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)cytidine (200 g).

Step 2. Preparation of 2'-deoxy-5'-C,5'-O-didehydro-2',2'-difluoro-3'-O—,N^4-bis(4-methoxytrityl)cytidine

[0288]



-continued

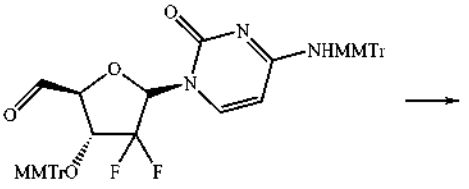


[0289] To an ice-cold solution of 5'-O-(t-butyldimethylsilyl)-2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl) cytidine (200 g, 210 mmol) in anhydrous THF (322 mL) was added TBAF (1 M solution in THF, 330 mmol) dropwise under N_2. The reaction mixture was stirred at RT overnight. The solvent was removed and the residue was dissolved in EA (800 mL). The solution was washed with water and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give a residue which was purified by silica gel column (CH_2Cl_2/EA=10/1 to 5/1) to give the 2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)cytidine (128 g, 73%); ^1H NMR (400 MHz) ($CDCl_3$): δ 7.45-7.39 (m, 4H), 7.35-6.91 (m, 29H), 6.76 (dd, J=8.8 Hz, 2.4 Hz, 4H), 6.24 (t, J=8.0 Hz, 1H), 4.93 (d, J=8.0 Hz, 1H), 4.20 (dd, J=15.2 Hz, 9.2 Hz, 1H), 3.72 (d, J=4.0 Hz, 6H), 3.27 (d, J=13.2 Hz, 1H), 2.84 (d, J=12.4 Hz, 1H).

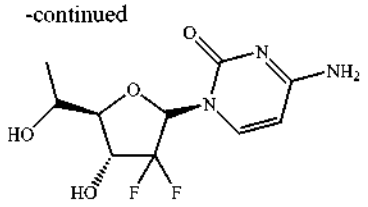
[0290] To a solution of pyridine (2.85 g, 36 mmol) in anhydrous DMSO (30 mL) at 10° C. was added dropwise TFA (2.05 g, 18 mmol). The mixture was stirred at RT until a clear solution formed. The solution was added dropwise into a solution of 2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)cytidine (24.2 g, 30 mmol) and DCC (18.6 g, 90 mmol) in anhydrous DMSO at 10° C. The mixture was stirred at RT for 12 hours as checked by TLC. The mixture was quenched with water (200 mL) and stirred for 1 hour at 10° C. The precipitate was removed by filtration and the filtrate was extracted by EtOAc (1000 ml). The combined organic layer was washed by brine (200 mL) and dried by anhydrous Na_2SO_4. The solution was concentrated and the residue was purified by column (silica gel, EtOAc: Petro ether=1/1 to 2/1) to give 2'-deoxy-5'-C,5'-O-didehydro-2',2'-difluoro-3'-O—,N^4-bis(4-methoxytrityl)cytidine (21 g, 88%) which was used in the next step without any further purification.

Step 3. Preparation of 2'-deoxy-2',2'-difluoro-5'-C-methylcytidine

[0291]



-continued



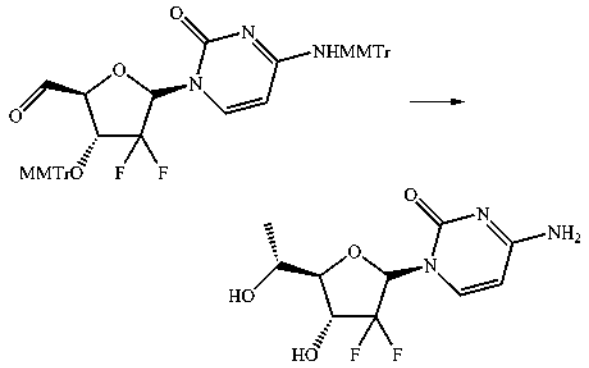
[0292] To an ice-EtOH bath cold solution of 2'-deoxy-5'-C, 5'-O-didehydro-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)cytidine (21 g, 26.08 mmol) in anhydrous THF (200 mL) was added MeMgBr (3 M solution in ether, 31.3 mL, 78.23 mmol) dropwise under N_2. The reaction mixture was stirred at RT overnight. The mixture was quenched by sat. NH_4Cl and extracted with EA (500 mL×3). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by silica gel column (EA: PE=10/1 to 3/2) to give the 2'-deoxy-2',2'-difluoro-3'-O—, N^4-di(4-methoxytrityl)-5'-C-methylcytidine (13 g, 61%, major:minor=93:7); ^1H NMR (400 MHz) ($CDCl_3$): δ 7.41-7.05 (m, 27H), 6.77-6.74 (m, 4H), 6.22 (t, J=8.8 Hz, 1H), 4.91 (d, J=7.6 Hz, 1H), 4.20-4.15 (m, 1H), 3.74-3.69 (m, 6H), 3.03-3.00 (m, 1H), 0.98 (d, J=7.2 Hz, 3H).

[0293] 2'-Deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'-C-methylcytidine (4.1 g, 5 mmol) was dissolved in 50 mL AcOH/H_2O (v/v=4:1). The mixture was stirred at 50° C. overnight. The solvent was removed under vacuum and the residue was diluted with water (30 mL), extracted with EA (20 mL×2) to remove some impurity. 2'-Deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (1.2 g, 87%) was obtained after column separation. ^1H NMR (400 Hz) (MeOD): δ 7.93 (d, J=7.6 Hz, 1H), 6.24 (t, J=7.6 Hz, 1H), 5.95 (d, J=7.6 Hz, 1H), 4.30-4.22 (m, 1H), 4.05-4.00 (m, 1H), 3.74 (dd, J=8.4 Hz, 2.8 Hz, 1H), 1.37 (d, J=6.4 Hz, 3H).

Example 20

Preparation of 2'-deoxy-2',2'-difluoro-5'(R)—C-methylcytidine (20)

[0294]



[0295] To an ice-EtOH bath cold solution of 2'-deoxy-5'-C, 5'-O-didehydro-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)cytidine (6.0 g, 7.4 mmol) in anhydrous THF (30 mL) was added MeMgBr (3M solution in ether) (10 mL, 30 mmol) dropwise under N_2. After addition, the reaction mixture was stirred at RT overnight. Then the reaction was quenched by sat. NH_4Cl. The mixture was extracted with EA (100 mL×2). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified by silica gel column (PE/EA=3/1 to 1/1) to give 2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(R and S)—C-methylcytidine (3.6 g, 58.8%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.48-7.08 (m, 26H), 6.80-6.84 (m, 4H), 6.28 (t, J=8.8 Hz, 1H), 4.99 (d, J=7.6 Hz, 1H), 4.25-4.20 (m, 1H), 3.81-3.79 (m, 7H), 3.77 (s, 3H), 3.12-3.07 (m, 1H), 1.05 (d, J=6.8 Hz, 3H).

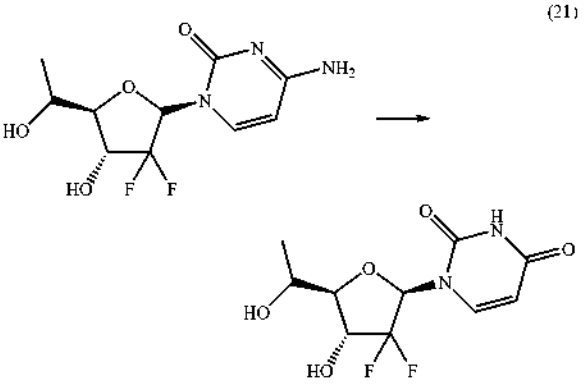
[0296] 2'-Deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(R and S)—C-methylcytidine (3 g, 3.65 mmol) was dissolved in 20 mL AcOH/H_2O (v/v=4:1). The mixture was stirred at 50° C. overnight. The solvent was removed under vacuum and the residue was diluted with water (10 mL) and washed with EA (10 mL×2). The aqueous layer was lyophilized and the residue was purified by prep. SFC to give 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (300 mg, 29.7%) and 2'-deoxy-2',2'-difluoro-5'(R)—C-methylcytidine (80 mg, 7.9%), both as white solid. 5'(R)-isomer: ^1H NMR (400 Hz, CD_3OD): δ 7.89 (d, J=7.6 Hz, 1H), 6.19 (t, J=7.6 Hz, 1H), 5.91 (d, J=7.6 Hz, 1H), 4.17-4.25 (m, 1H), 3.97-3.99 (m, 1H), 3.69 (dd, J=8.4 Hz, 2.8 Hz, 1H), 1.32 (d, J=6.4 Hz, 3H). ESI-MS: m/z 555 $[2M+H]^+$, 278 $[M+H]^+$.

Example 21

Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methyluridine (21)

[0297]

(21)



[0298] A solution of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (1 g, 3.6 mmol), acetic anhydride (2.2 g, 21.6 mmol), 4-(dimethylamino)pyridine (DMAP, 12 mg, 0.1 mmol), and pyridine (20 mL) was stirred until disappearance of the starting material. The mixture was quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with EA and the organic layer was washed with brine dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by flash chromatography to give 1.35 g of 2'-deoxy-3',5'-diacetyl-2',2'-difluoro-5'(S)—C-methylcytidine at 93% yield.

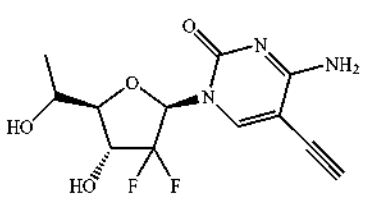
[0299] A solution of 2'-deoxy-3',5'-diacetyl-2',2'-difluoro-5'(S)—C-methylcytidine (1 g, 2.48 mmol) in DME (30 mL) and H_2O (20 mL) was heated in a sealed flask for 9 h at 125° C. Volatiles were evaporated, and chromatography of the residue gave 600 mg of 2'-deoxy-3',5'-diacetyl-2',2'-difluoro-

5'(S)—C-methyluridine (67%) as a colorless solid, which was dissolved in 20 mL saturated NH_3/MeOH solution. The mixture was stirred at 0° C. overnight. The solvent was removed under vacuum. Purification by flash chromatography on silica gel gave 440 mg (95%) of 2'-deoxy-2',2'-difluoro-5'(S)—C-methyluridine; ^{1}H NMR (400 Hz) (DMSO-d6): δ 11.55(s, 1H), 7.86(d, J=8 Hz, 1H), 6.26 (d, J=4.8 Hz, 1H), 6.03 (t, J=7.8 Hz, 1H), 5.22 (d, J=5.2 Hz, 1H), 4.17-4.13 (m, 1H), 3.85-3.81 (m, 1H), 3.65 (dd, J=8.4 Hz, 2.8 Hz, 1H), 1.18 (d, J=6.8 Hz, 3H).

Example 22

Preparation of 2'-deoxy-2',2'-difluoro-5-ethynyl-5'(S)—C-methylcytidine (22)

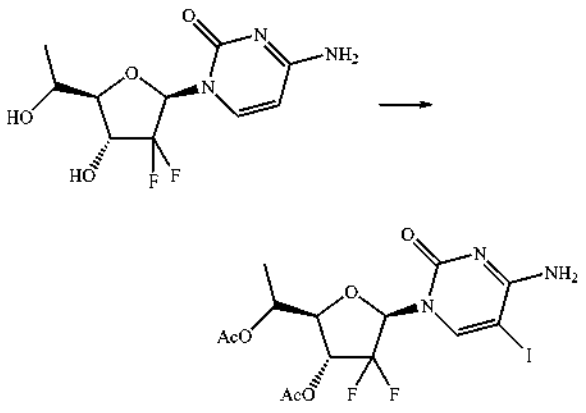
[0300]



(22)

Step 1. Preparation of 2'-deoxy-3',5'-O-diacetyl-2',2'-difluoro-5-iodo-5'(S)—C-methylcytidine

[0301]

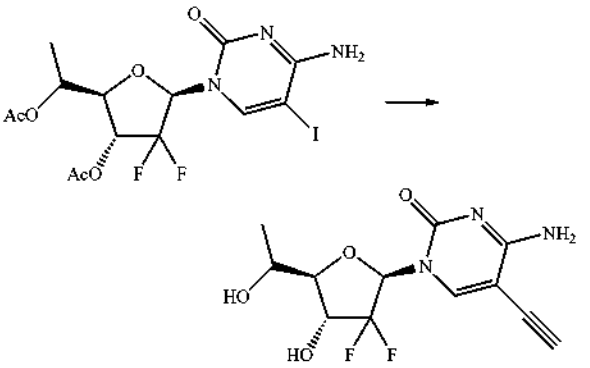


[0302] A solution of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (1 g, 3.6 mmol), acetic anhydride (2.2 g, 21.6 mmol), 4-(dimethylamino)pyridine (DMAP, 12 mg, 0.1 mmol), and pyridine (20 mL) was stirred until disappearance of the starting material. The mixture was quenched with a saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with diethyl ether and the organic layers were washed with water, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by flash chromatography to give 1.35 g of 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-3',5'-O,N^4-triacetylcytidine in 93% yield.

[0303] 2'-Deoxy-2',2'-difluoro-5'(S)—C-methyl-3',5'-O, N^4-triacetylcytidine (1.5 g, 3.7 mmol) was dissolved into a solution of I_2 (3 g, 11.8 mmol) in methanol (300 mL). The reaction was refluxed and monitored by TLC. Upon completion, a small quantity of sodium thiosulfate was added to quench the reaction. The solvent was removed and the residue was purified by column chromatography on silica gel to give 500 mg of 2'-deoxy-3',5'-O-diacetyl-2',2'-difluoro-5-iodo-5'(S)—C-methylcytidine in 27% yield.

Step 2. Preparation of 2'-deoxy-2',2'-difluoro-5-ethynyl-5'-C-methylcytidine

[0304]



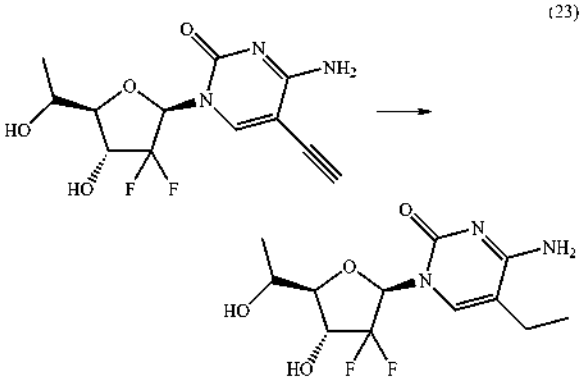
[0305] A solution of 2'-deoxy-3',5'-O-diacetyl-2',2'-difluoro-5-iodo-5'(S)—C-methylcytidine (500 mg, 1.03 mmol), acetic anhydride (648 mg, 6.1 mmol), 4-(dimethylamino) pyridine (DMAP, 12 mg, 0.1 mmol), and pyridine (20 mL) was stirred until disappearance of the starting material. The mixture was quenched with a saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with diethyl ether and the organic layers were washed with water, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by flash chromatography to give 500 mg of 2'-deoxy-2',2'-difluoro-5-iodo-5'(S)—C-methyl-3',5'-O—, N^4-triacetylcytidine in 92% yield.

[0306] To a nitrogen degas sed solution of triethylamine (303 mg, 3 eq) in CH_3CN (30 mL) were added ethynyltrimethylsilane (196 mg, 2 eq), 2'-deoxy-2'-deoxy-2',2'-difluoro-5-iodo-5'(S)—C-methyl-3',5'-O—,N^4-triacetylcytidine (500 mg, 1 eq), $Pd(PPh_3)_2Cl_2$ (8.4 mg, 0.012 eq), and CuI (2.3 mg, 0.012 eq), and the mixture was stirred at 25° C. for 12 h. After removal of the solvent, the residue was filtered, concentrated, and purified by flash chromatography on silica gel eluting with PE:EtOAc (2:1) to give 200 mg (42%) of 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-3',5'-O—,N^4-triacetyl-5-(trimethylsilylethynyl)cytidine as a white solid, which was dissolved in 20 mL saturated NH_3/MeOH solution. The mixture was stirred at RT overnight. The solvent was removed under vacuum. Purification by flash chromatography on silica gel gave 110 mg (91%) of 2'-deoxy-2',2'-difluoro-5-ethynyl-5'-C-methylcytidine; ^{1}H NMR (400 Hz) (MeOD-d4): δ 8.34 (s, 1H), 6.18 (t, J=7.6 Hz, 1H), 4.26-4.19 (m, 1H), 4.00-3.98 (m, 1H), 3.84 (s, 1H), 3.72 (dd, J=8.4 Hz, 2.8 Hz, 1H), 1.34 (d, J=6.8 Hz, 3H).

Example 23

Preparation of 2'-deoxy-2',2'-difluoro-5-ethyl-5'(S)—C-methylcytidine (23)

[0307]



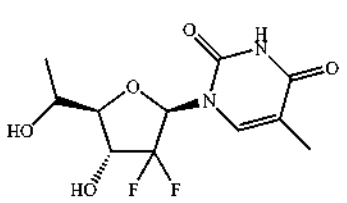
(23)

[0308] To a solution of 2'-deoxy-2',2'-difluoro-5-ethynyl-5'(S)—C-methylcytidine (50 mg) in EA (50 mL) was added Pd/C (50 mg) at 25° C. Then the mixture was stirred under H_2 atmosphere at 1 atm for 4 h. The solvent was removed under vacuum. Purification by flash chromatography on silica gel gave 40 mg of 2'-deoxy-2',2'-difluoro-5-ethyl-5'-C-methylcytidine (79%); ^{1}H NMR (400 Hz) (MeOD-d4): δ 7.80 (s, 1H), 6.21 (t, J=7.6 Hz, 1H), 4.29-4.21 (m, 1H), 4.03-3.95 (m, 1H), 3.71 (dd, J=8.8 Hz, 2.8 Hz, 1H), 2.35(q, 2H), 1.34(d, J=6.8 Hz, 3H), 1.17 (t, J=7.4 Hz, 3H).

Example 24

Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylthymidine (24)

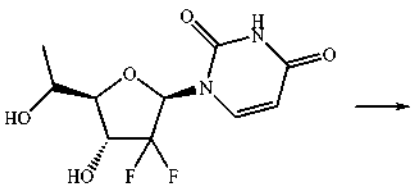
[0309]



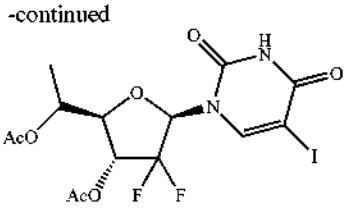
(24)

Step 1. Preparation of 2'-deoxy-3',5'-diacetyl-2',2'-difluoro-5-iodo-5'-C-methyluridine

[0310]



-continued

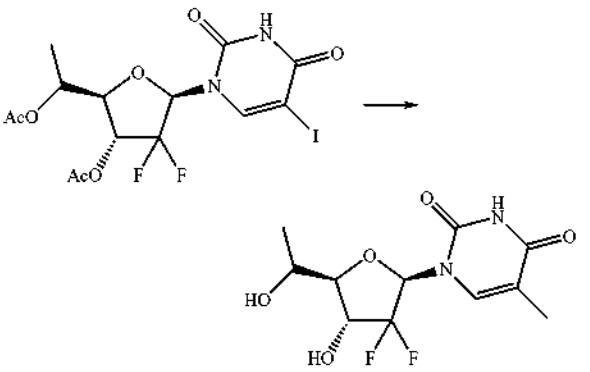


[0311] A solution of 2'-deoxy-2',2'-difluoro-5'(S)—C-methyluridine (200 mg, 0.72 mmol), acetic anhydride (466 mg, 4.3 mmol), 4-(dimethylamino)pyridine (DMAP, 12 mg, 0.1 mmol), and pyridine (20 mL) was stirred until disappearance of the starting material. The mixture was quenched with a saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with diethyl ether and the organic layers were washed with water, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by flash chromatography to give 236 mg of 2'-deoxy-3',5'-diacetyl-2',2'-difluoro-5'(S)—C-methyluridine in 91% yield.

[0312] A mixture of 230 mg (0.64 mmol) of 2'-deoxy-3',5'-diacetyl-2',2'-difluoro-5'(S)—C-methyluridine, 210 mg (0.83 mmol) of I_2, and 766 mg of CAN in 25 mL of MeCN was stirred at ambient temperature. When iodination was complete (as monitored by TLC), solvent was evaporated under reduced pressure. The resulting residue was treated with a cold mixture of ethyl acetate (15 mL), 5% $NaHSO_3$/H_2O (5 mL), and saturated NaCl/H_2O (5 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc. The crude products were purified by silica gel column chromatogrphy to give 245 mg of 2'-deoxy-3',5'-diacetyl-2',2'-difluoro-5-iodo-5'(S)—C-methyluridine in 80% yield.

Step 2. Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylthymidine

[0313]



[0314] To a mixture of 2'-deoxy-3',5'-diacetyl-2',2'-difluoro-5-iodo-5'(S)—C-methyluridine (245 mg, 0.5 mmol) and $Pd(PPh_3)Cl_2$ (40 mg) in anhydrous THF (30 mL) was refluxed under Ar atmosphere for 10 min. Then $AlMe_3$ was added dropwise by a syringe through septum and the solution was refluxed overnight. After cooled to RT, water (20 mL) was added to the reaction and the mixture was extracted with DCM. The extract was dried and evaporated under reduced

pressure. The residue was purified by Prep-TLC to give 2'-deoxy-3',5'-diacetyl-2',2'-difluoro-5(S)'-C-methylthymidine (50 mg) at 26% yield.

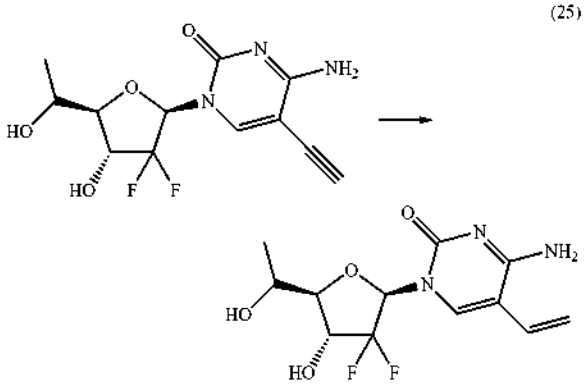
[0315] 2'-Deoxy-3',5'-diacetyl-2',2'-difluoro-5'(S)—C-methylthymidine (50 mmol) was dissolved in 20 mL of saturated NH_3/MeOH solution. The mixture was stirred at 0° C. overnight. The solvent was removed under vacuum. Purification by flash chromatography on silica gel gave 30 mg of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylthymidine (77%); ^{1}H NMR (400 Hz) (DMSO-d6): 67.57(s, 1H), 6.11 (t, J=8 Hz, 1H), 4.30-4.22 (m, 1H), 4.02-3.96 (m, 1H), 3.70 (dd, J=8.4 Hz, 2.8 Hz, 1H), 1.87 (s, 3H), 1.33 (d, J=6.4 Hz, 3H).

Example 25

Preparation of 2'-deoxy-2',2'-difluoro-5-vinyl-5'(S)—C-methylcytidine (25)

[0316]

(25)



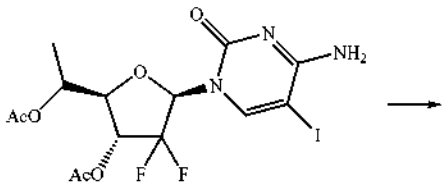
[0317] To a solution of 2'-deoxy-2',2'-difluoro-5-ethynyl-5'-C-methylcytidine (30 mg, 1 eq) in EA (50 mL) was added Lindlar Pd (30 mg) at 25° C. Then the mixture was stirred under H_2 atmosphere at 1 atm for 4 h. The solvent was removed under vacuum. Purification by flash chromatography on silica gel gave 22 mg of 2'-deoxy-2',2'-difluoro-5-vinyl-5'(S)—C-methylcytidine (73%). ^{1}H NMR (400 Hz) (MeOD-d4): δ 8.24 (s, 1H), 6.51 (m, 1H), 6.21 (t, J=14.5 Hz, 1H), 5.61 (dd, J=17.2 Hz, 1.2 Hz, 1H), 5.27 (dd, J=10.8 Hz, 1.2 Hz, 1H), 4.32-4.24 (m, 1H), 4.04-4.00 (m, 1H), 3.74 (dd, J=8.8 Hz, 2.8 Hz, 1H), 1.35(d, J=6.8 Hz, 3H).

Example 26

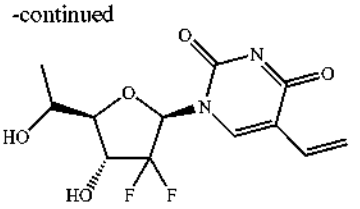
Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-5-vinyluridine (26)

[0318]

(26)



-continued



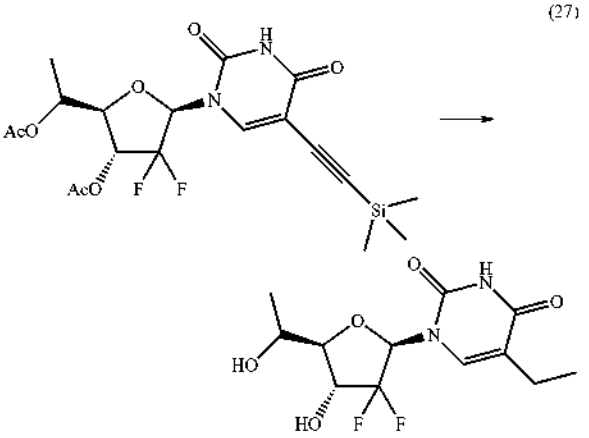
[0319] To a solution of $PdCl_2(PPh_3)_2$ (15.8 mg, 0.022 mmol) in acetonitrile (10 mL) was added 2'-deoxy-3',5'-O-diacetyl-2',2'-difluoro-5-iodo-5'(S)—C-methyluridine (110 mg, 0.23 mmol) and ethenyltributylstannane (143 mg, 0.45 mmol). The mixture was heated up to reflux and stirred overnight, filtered through celite and evaporated under reduced pressure to remove solvent. The oily residue was purified by silica gel column chromatography to give 50 mg of 2'-deoxy-3',5'-O-diacetyl-2',2'-difluoro-5-vinyl-5'(S)—C-methyluridine in 50% yield, which was dissolved in 20 mL of saturated NH_3/MeOH solution. The mixture was stirred at 0° C. overnight. The solvent was removed under vacuum. Purification by flash chromatography on silica gel gave 20 mg of product, which was further purified by flash chromatography on silica gel to give 7 mg 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-5-vinyluridine (18%); ^{1}H NMR (400 Hz) (DMSO-d6): δ8.17(s, 1H), 6.50 (dd, J=17.6 Hz, 11.2 Hz, 1H), 6.16 (t, J=7 Hz, 1H), 6.16 (t, J=7 Hz, 1H), 5.97 (d, J=17.6 Hz, 1H), 5.20 (d, J=11.2 Hz, 1H), 4.35-4.27 (m, 1H), 4.04-3.98 (m, 1H), 3.75 (d, J=8.8 Hz, 1H), 1.34 (d, J=6.4 Hz, 3H).

Example 27

Preparation of 2'-deoxy-2',2'-difluoro-5-ethyl-5'(S)—C-methyluridine (27)

[0320]

(27)



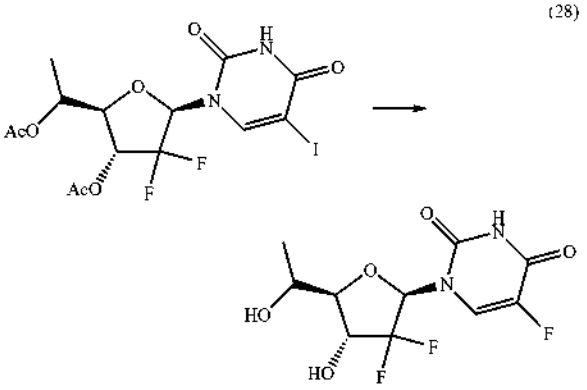
[0321] 2'-Deoxy-3',5'-O-diacetyl-2',2'-difluoro-5-(trimethylsilylethynyl)-5'(S)—C-methyluridine (79 mg, 0.18 mmol) was dissolved in Sat. NH_3/MeOH (20 mL). The mixture was stirred at 25° C. for 20 h. The solvent was removed and the crude dissolved in MeOH (10 mL). Pd/C (5%, 10 mg) was added and the mixture was stirred at 25° C. under H_2 (1 atm) for 30 h. Then the catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was puri-

fied by columnon silica gel (DCM/MeOH=1:10) to give 2'-deoxy-2',2'-difluoro-5-ethyl-5'(S)—C-methyluridine (19 mg, 36% over 2 steps); ^{1}H NMR (400 Hz, D_2O): δ 7.41 (s, 1H), 6.02 (t, J=8 Hz, 1H), 4.15-4.23 (m, 1H), 3.93 (dd, J1=4.4 Hz, J2=6.4 Hz, 1H), 3.68 (dd, J1=4.4 Hz, J2=8.4 Hz, 1H), 2.15 (q, J=7.2 Hz, 2H), 1.17 (d, J=6.4 Hz, 3H), 0.91 (t, J=7.2 Hz, 3H); LCMS (ESI): 307 $[M+H]^+$.

Example 28

Preparation of 2'-deoxy-5'(S)—C-methyl-2',2',5-trifluorouridine (28)

[0322]



(28)

[0323] 2'-Deoxy-3',5'-O-diacetyl-2',2'-difluoro-5-iodo-5'(S)—C-methyluridine (320 mg, 0.66 mmol), hexamethylditin (429 mg, 1.32 mmol), bis(triphenylphosphine)palladium dichloride (46 mg, 0.066 mmol), and 1,4-dioxane (20 mL) were stirred at 80° C. for 2 h. Upon completion, the solvent was removed at 45° C. under reduced pressure and the residue was purified on Prep-TLC to provide 245 mg of 2'-deoxy-3',5'-O-diacetyl-2',2'-difluoro-5-(trimethylstannyl)-5'(S)—C-methyluridine in 71% yield.

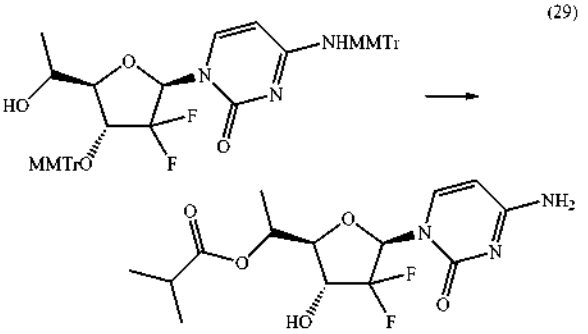
[0324] To a dried round-bottomed flask (25 mL), 2'-deoxy-3',5'-O-diacetyl-2',2'-difluoro-5-(trimethylstannyl)-5'(S)—C-methyluridine (245 mg, 0.47 mmol), MeCN (15 mL) and Selectfluor (177 mg, 0.51 mmol) were added. The mixture was stirred at 55° C. for 10 h, during which the progress of the reaction was followed by TLC, until the starting material had been consumed (~10 h). The solvent was removed and the crude mixture was purified by column chromatography to give 50 mg of 2'-deoxy-3',5'-O-diacetyl-5'(S)—C-methyl-2',2',5-trifluorouridine (30%).

[0325] 2'-Deoxy-3',5'-O-diacetyl-5'(S)—C-methyl-2',2',5-trifluorouridine (50 mg, mmol) was dissolved in 20 mL saturated NH_3/MeOH solution. The mixture was stirred at 0° C. overnight. The solvent was removed under vacuum. Purification by flash chromatography on silica gel gave 30 mg of 2'-deoxy-5'(S)—C-methyl-2',2',5-trifluorouridine (77%); ^{1}H NMR (400 Hz) (MeOD-d4): 88.25(d, J=6.8 Hz 1H), 6.10 (t, J=6.8 Hz, 1H), 4.31-4.22 (m, 1H), 4.02-3.97 (m, 1H), 3.72 (dd, J=8.4 Hz, 2.4 Hz, 1H), 1.33 (d, J=6.4 Hz, 3H); ^{19}F NMR (400 Hz) (MeOD-d4): δ-121.15(t, J=44.7 Hz, 2F), δ-169.89 (s, 1F).

Example 29

Preparation of 2'-deoxy-2',2'-difluoro-5'-O-isobutyryl-5'(S)—C-methylcytidine (29)

[0326]



(29)

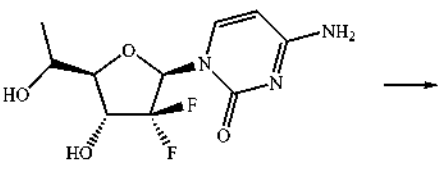
[0327] To a mixture of 2'-deoxy-2',2'-difluoro-3'-O,N^4-di(4'-methoxytrityl)-5'(S)—C-methylcytidine (1.64 g, 2 mmol), isobutyric acid (211 mg, 2.4 mmol) and DMAP (0.12 g, 1 mmol) in DCM (20 mL) was added EDCI (1.15 g, 6 mmol). The mixture was stirred at RT for 16 hours under N_2 as checked by TLC. Then the mixture was washed with Sat. $NaHCO_3$ aq. solution and followed by brine. The organic layer was dried over Na_2SO_4 and concentrated. The solvent was removed and the residue was purified by column (PE: EA=1:1) to give 2'-deoxy-2',2'-difluoro-3'-O,N^4-di(4'-methoxytrityl)-5'-O-isobutyryl-5'(S)—C-methylcytidine (1.2 g, 67%).

[0328] 2'-Deoxy-2',2'-difluoro-3'-O,N^4-di(4'-methoxytrityl)-5'-O-isobutyryl-5'(S)—C-methylcytidine (900 mg) was dissolved in 80% HOAc (20 mL). The mixture was stirred at 60° C. overnight as checked by TLC. The solvent was removed under reduced pressure and the residue was purified by prep. HPLC (HCOOH system) to give 2'-deoxy-2',2'-difluoro-5'-O-isobutyryl-5'(S)—C-methylcytidine as white solids (120 mg, 35%); 1H NMR (D_2O, 400 M Hz) δ 7.46 (d, J=7.6 Hz, 1H), 6.24 (t, J=8.0 Hz, 1H), 6.15 (d, J=7.6 Hz, 1H), 5.28 (dt, J1=6.4 Hz, J2=11.2 Hz, 1H), 4.31 (dt, J1=8 Hz, J2=12.8 Hz, 1H), 4.15 (dd, J1=4.4 Hz, J2=8.0 Hz, 1H), 2.67-2.74 (m, 1H), 1.42 (d, J=6.8 Hz, 3H), 1.18-1.21 (m, 6H); ESI-LCMS: m/z 348 $[M+H]^+$, 370 $[M+Na]^+$, 717 $[2M+Na]^+$.

Example 30

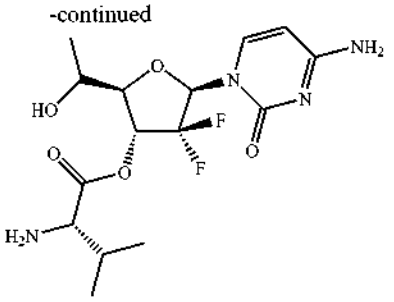
Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-3'-O-(L-valinyl)cytidine (30)

[0329]



(30)

-continued



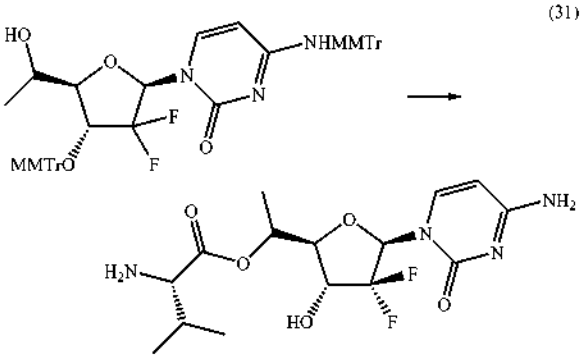
[0330] (N-t-Butoxycarbonyl)-L-valine (0.78 g, 3.6 mmol) and CDI (0.58 g, 3.6 mmol) were suspended in anhydrous THF (15 mL). The mixture was stirred at RT for 1 hour and then warmed to 50° C. Stirring was continued for 30 mins. Then the mixture was cooled to RT and the solution was added into a solution of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (0.90 g, 3.25 mmol), DMAP (37 mg, 0.3 mmol) and TEA (10 mL) in anhydrous DMF (20 mL) dropwise at RT After addition the mixture was stirred at RT for 20 h and then concentrated under reduced pressure to remove THF and TEA. Then the solution was diluted in EA and washed with brine. The organic layer was dried over Na_2SO_4. The solvent was concentrated and the residue was purified by column (pure EA) to afford 3'-O-(N-t-butoxycarbonyl)-L-valinyl)-2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine as a white foam (1.04 g, 67%), which was dissolved in a solution of HCl in EA (4 N, 150 mL). The mixture was stirred at RT for 10 hrs. The solvent was removed to afford crude product which was further purified by neutral prep. HPLC to afford 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-3'-O-(L-valinyl)cytidine as white solids (410 mg, 50%); 1H NMR (400 MHz, D_2O) δ 7.80 (d, J=7.6 Hz, 1H), 6.29 (t, J=8.8 Hz, 1H), 6.13 (d, J=7.6 Hz, 1H), 5.49-5.56 (m, 1H), 4.24-4.27 (m, 2H), 4.07-4.13 (m, 1H), 2.40-2.48 (m, 1H), 1.31 (d, J=6.4 Hz, 3H), 1.07 (dd, J1=7.2 Hz, J2=11.2 Hz, 6H); ESI-MS: 753 $[2M+H]^+$, 377 $[M+H]^+$.

Example 31

Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-5'-O-(L-valinyl)cytidine (31)

[0331]

(31)



[0332] To a mixture of 2'-deoxy-2',2'-difluoro-3'-O,N^4-di(4-methoxytrityl)-5'(S)—C-methylcytidine (2.0 g, 2.43 mmol), EDCI (933 mg, 4.86 mmol) and DMAP (179 mg, 1.46 mmol) in anhydrous DCM (20 mL) was added N-Boc-L-Val (529 mg, 2.43 mmol) under N_2. The reaction mixture was stirred at RT for 2 h. The reaction mixture was washed with sat. $NaHCO_3$ solution and followed by brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give the residue which was purified by silica gel column (PE/EA=3/1 to 2/1) to give 5'-O—(N-(t-butoxycarbonyl)-L-valinyl)-2'-deoxy-2',2'-difluoro-3'-O,N^4-di(4-methoxytrityl)-5'(S)—C-methylcytidine (1.4 g, 56%).

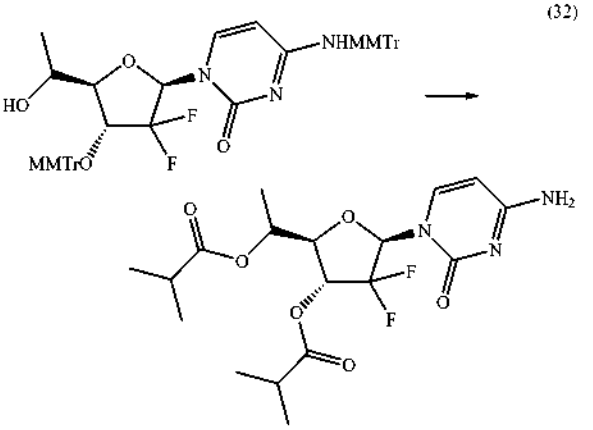
[0333] To a solution of 5'-O—(N-(t-butoxycarbonyl)-L-valinyl)-2'-deoxy-2',2'-difluoro-3'-O,N^4-di(4-methoxytrityl)-5'(S)—C-methylcytidine (1.1 g, 1.08 mmol) in EA (5 mL) was added 4 N HCl/EA (15 mL). The reaction mixture was stirred at RT overnight, concentrated into a residue which was purified by prep. HPLC (HCOOH system) to give 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-5'-O-(L-valinyl)cytidine (55 mg, 13.6%) as a white solid; ^1H NMR (400 Hz) (D_2O): δ 8.29 (S, 0.8H), 7.47 (d, J=7.6 Hz, 1H), 5.99 (t, J=8.0 Hz, 1H), 5.92 (d, J=7.6 Hz, 1H), 5.32-5.25 (m, 1H), 4.25-4.17 (m, 1H), 3.98-3.93 (m, 2H), 2.25-2.17 (m, 1H), 1.29 (t, J=6.4 Hz, 3H), 0.88 (d, J=6.8 Hz, 1H), 0.85 (d, J=6.8 Hz, 1H).

Example 32

Preparation of 2'-deoxy-2',2'-difluoro-3',5'-O-dhisobutyryl)-5'(S)—C-methylcytidine (32)

[0334]

(32)



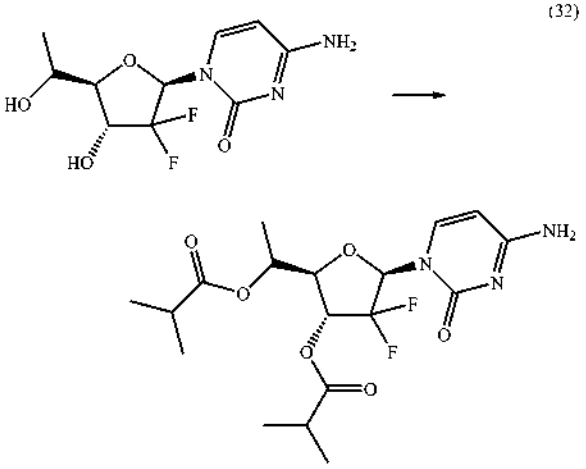
[0335] 2'-Deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'-C-methylcytidine (5 g, 6.08 mmol) was dissolved in 30 mL of AcOH/H_2O (v/v=4:1). The mixture was stirred at RT for 5 h. The solvent was removed under vacuum and the residue was purified by silica gel column (PE/EA=1/1 to 1/5) to give 2'-deoxy-2',2'-difluoro-N^4-(4-methoxytrityl)-5'(S)—C-methylcytidine (2.1g, 60%). ^1H NMR (400 Hz) (DMSO): δ 8.56 (s, 1H), 7.62 (d, J=8.0 Hz, 1H), 7.26-7.09 (m, 13H), 6.80 (d, J=8.8 Hz, 1H), 6.25 (d, J=7.6 Hz, 1H), 6.13 (d, J=6.8 Hz, 1H), 5.91 (t, J=8.4 Hz, 1H), 5.05-5.03 (m, 1H), 4.08-4.04 (m, 1H), 3.77-3.75 (m, 1H), 3.68 (s, 3H), 3.51 (dd, J=8.0, 2.8 Hz, 1H), 1.12 (d, J=6.8 Hz, 1H).

[0336] To a mixture of 2'-deoxy-2',2'-difluoro-N^4-(4-methoxytrityl)-5'(S)—C-methylcytidine (2.0 g, 3.64 mmol), EDCI (1.4 g, 7.28 mmol) and DMAP (270 mg, 2.18 mmol) in anhydrous DCM (20 mL) was added isobutyric acid (961 mg,

10.92 mmol) under N_2. The reaction mixture was stirred at RT for 3 h. The reaction mixture was washed with sat. $NaHCO_3$ solution and followed by brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give the residue which was purified by silica gel column (PE/EA=3/1 to 2/1) to give 2'-deoxy-2',2'-difluoro-3',5'-O-di(isobutyryl)-N^4-(4-methoxytrityl)-5'(S)—C-methylcytidine (1.8 g, 71.7%), which was dissolved in 20 mL AcOH/H_2O (v/v=4:1). The mixture was stirred at 50° C. overnight. The solvent was removed under vacuum and the residue was purified by prep. HPLC to give the 2'-deoxy-2',2'-difluoro-3',5'-O-di(isobutyryl-5'(S)—C-methylcytidine (480 mg, 48%). ^{1}H NMR (400 Hz) (MeOD): δ 7.84 (d, J=7.6 Hz, 1H), 6.27 (t, J=8.8 Hz, 1H), 6.15 (d, J=7.6 Hz, 1H), 5.36-5.28 (m, 1H), 5.25-5.18 (m, 1H), 4.30 (dd, J=6.8, 4.4 Hz, 1H), 2.75-2.67 (m, 1H), 2.65-2.54 (m, 1H), 1.33 (d, J=6.8 Hz, 1H), 1.21-1.15 (m, 12H).

Alternative method.

(32)



[0337] 2'-Deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (0.8 g, 2.89 mmol) was dissolved in 20 mL of DMF-DMA. The mixture was stirred at RT for 2 h. The solvent was removed under vacuum to give the crude 2'-deoxy-2',2'-difluoro-N^4—(N,N-dimethylaminomethylene)-5'-C-methylcytidine (998 mg) which was used for next step with no further purification.

[0338] To a mixture of 2'-deoxy-2',2'-difluoro-N^4—(N,N-dimethylaminomethylene)-5'-C-methylcytidine (950 mg, 2.86 mmol), EDCI (1.1 g, 5.72 mmol) and DMAP (210 mg, 1.72 mmol) in anhydrous DMF (10 mL) was added isobutyric acid (756 mg, 8.58 mmol) under N_2. The reaction mixture was stirred at RT for 5 h. The reaction was complex. Then EDCI (1.1 g, 5.72 mmol), DMAP (210 mg, 1.72 mmol) and isobutyric acid (756 mg, 8.58 mmol) was added into the solution and stirred at RT overnight. The reaction mixture was diluted with EA, washed with water and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated to give a crude 2'-deoxy-2',2'-difluoro-3',5'-O-di(isobutyryl)-N^4—(N,N-dimethylaminomethylene)-5'(S)—C-methylcytidine (750 mg) which was used for the next step with no further purification.

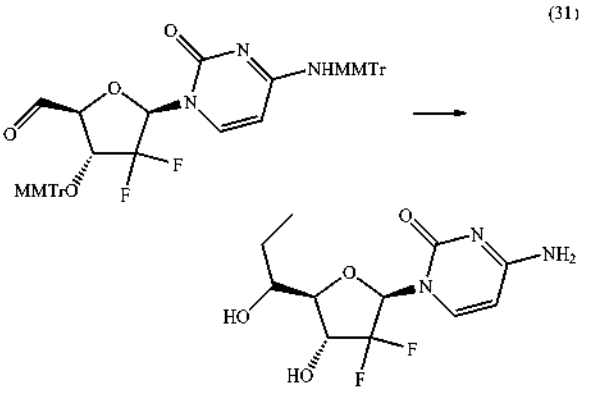
[0339] 2'-Deoxy-2',2'-difluoro-3',5'-O-di(isobutyryl)-N^4—(N,N-dimethylaminomethylene)-5'(S)—C-methylcytidine (700 mg, 1.48 mmol) was dissolved in 10 mL AcOH/H_2O (v/v=4:1). The mixture was stirred at 50° C. overnight. The solvent was removed under vacuum and the residue was purified by prep. HPLC to give the 2'-deoxy-2',2'-difluoro-3',5'-O-di(isobutyryl)-5'(S)—C-methylcytidine (220 mg, 35.6%). ^{1}H NMR (400 Hz) (MeOD): δ 7.62 (d, J=7.6 Hz, 1H), 6.32 (t, J=8.8 Hz, 1H), 5.96 (d, J=7.6 Hz, 1H), 5.30-5.25 (m, 1H), 5.24-5.17 (m, 1H), 4.25 (dd, J=6.8, 4.0 Hz, 1H), 2.73-2.66 (m, 1H), 2.64-2.57 (m, 1H), 1.34 (d, J=7.2 Hz, 1H), 1.22-1.17 (m, 12H).

Example 33

Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-ethylcytidine (33)

[0340]

(31)



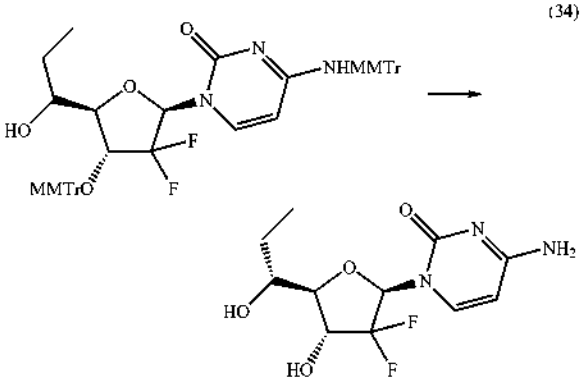
[0341] To an ice-EtOH bath cold solution of 2'-deoxy-5'-C,5'-O-didehydro-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)cytidine (3 g, 3.72 mmol) in anhydrous THF (10 mL) was added EtMgBr (3 M solution in ether) (5 mL, 15 mmol) dropwise under N_2. The reaction mixture was stirred at RT overnight. The mixture was quenched by sat. NH_4Cl. The product was extracted with EA (50 mL×2). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified by silica gel column (PE/EA=3/1 to 1/1) to give the 2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(S)—C-ethylcytidine (1.7 g, 54.6%).

[0342] 2'-Deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(S)—C-ethylcytidine (1.3 g, 1.56 mmol) was dissolved in 15 mL AcOH/H_2O (v/v=4:1). The mixture was stirred at 50° C. overnight. The solvent was removed under vacuum and the residue was diluted with water (3 mL), extracted with EA (2 mL×2) to remove some impurity. The water layer was subjected to prep. HPLC separation to give 2'-deoxy-2',2'-difluoro-5'(S)—C-ethylcytidine (42 mg, 5%); ^{1}H NMR (400 Hz) (MeOD): δ 7.91 (d, J=7.6 Hz, 1H), 6.18 (t, J=8.0 Hz, 1H), 5.90 (d, J=7.6 Hz, 1H), 4.28-4.22 (m, 1H), 3.78 (dd, J=8.4 Hz, 2.4 Hz, 1H), 3.69-3.66 (m, 1H), 1.70-1.64 (m, 2H), 1.03 (t, J=7.2 Hz, 3H).

Example 34

Preparation of 2'-deoxy-2',2'-difluoro-5'(R)—C-ethylcytidine (34)

[0343]



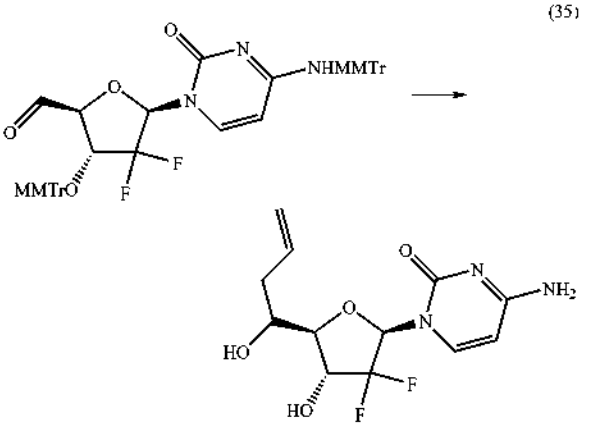
[0344] To an ice-cold suspension of CrO_3 (478 mg, 4.79 mmol) in anhydrous DCM (15 mL) was added anhydrous pyridine (0.77 mL, 9.57 mmol) and Ac_2O (0.45 mL, 4.79 mmol) under N_2. The mixture was stirred at RT for about 10 min until the mixture became homogeneous. The mixture was cooled to 0° C. and a solution of 2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(S)—C-ethylcytidine (1.0 g, 1.2 mmol) in anhydrous DCM (5 mL) was added. The resultant mixture was stirred at RT overnight. The reaction was complete detected by HPLC. The reaction mixture was diluted with EA (100 mL), washed with $NaHCO_3$ solution twice and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give a residue which was purified by silica gel column (EA/PE=1/2) to give the desired 2'-deoxy-5'-C,5'-O-didehydo-2',2'-difluoro-3'-O—,N^4-bis(4-methoxytrityl)-5'-C-ethylcytidine (505 mg, 50.6%).

[0345] To an ice-cold solution of 2'-deoxy-5'-C,5'-O-didehydo-2',2'-difluoro-3'-O—,N^4-bis(4-methoxytrityl)-5'-C-ethylcytidine (505 mg, 0.605 mmol) in 95% EtOH (10 mL) was added $NaBH_4$ (46 mg, 1.21 mmol) under N_2. The reaction mixture was stirred at RT for 7 h. The solvent was evaporated. The residue was diluted with EA (30 mL), washed with sat. $NaHCO_3$ and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated to give the residue which was purified by prep. TLC to give 2'-deoxy-2',2'-difluoro-3'-O—,N^4-bis(4-methoxytrityl)-5'-C-ethylcytidine (320 mg, 63.1%), which was dissolved in 10 mL AcOH/H_2O (v/v=4:1). The mixture was stirred at 50° C. overnight. The solvent was removed under vacuum and the residue was diluted with water (3 mL), extracted with EA (2 mL×2) to remove some impurity. Concentrated into a residue which was purified by silica gel column eluting with DCM/MeOH=10:1 to give the product (80 mg, S:R=3:7). 60 mg was subjected to SFC separation to give 2'-deoxy-2',2'-difluoro-5'(R)—C-ethylcytidine (17 mg, S:R=7:93). ^1H NMR (400 Hz) (MeOD): δ 7.89 (d, J=7.6 Hz, 0.07H), 7.91 (d, J=7.6 Hz, 1H), 6.20 (t, J=8.0 Hz, 1H), 5.89 (d, J=7.6 Hz, 1H), 4.33-4.25 (m, 1H), 3.84-3.79 (m, 2H), 1.66-1.51 (m, 2H), 1.01 (t, J=7.2 Hz, 3H).

Example 35

Preparation of 5'(S)—C-allyl-2'-deoxy-2',2'-difluorocytidine (35)

[0346]

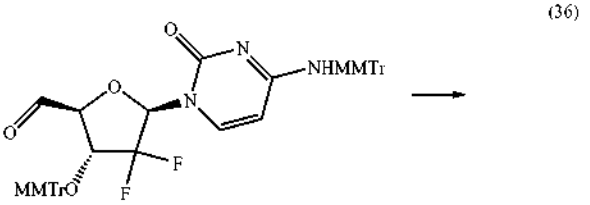


[0347] To an ice-EtOH bath cold solution of 2'-deoxy-5'-C,5'-O-didehydro-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)cytidine (3 g, 3.72 mmol) in anhydrous THF (10 mL) was added AllylMgBr (1M solution in THF) (15 mL, 15 mmol) dropwise under N_2. The reaction mixture was stirred at RT overnight. The mixture was quenched by sat. NH_4Cl. The product was extracted with EA (50 mL×2). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified by silica gel column (PE/EA=3/1 to 1/1) to give 5'-C-allyl-2'-deoxy-5'-C,5'-O-didehydro-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl) cytidine (1.1 g, 34.9%) which was dissolved in 15 mL AcOH/H_2O (v/v=4:1). The mixture was stirred at 50° C. overnight. The solvent was removed under vacuum and the residue was diluted with water (3 mL), extracted with EA (2 mL×2) to remove some impurity. The aqueous layer was subjected to SFC separation to give 5'(S)—C-allyl-2'-deoxy-2',2'-difluorocytidine (5 mg, 1.3%); ^1H NMR (400 Hz) (D_2O): δ 7.50 (d, J=7.6 Hz, 1H), 5.94 (t, J=7.6 Hz, 1H), 5.81 (d, J=7.6 Hz, 1H), 5.70-5.59 (m, 1H), 4.98-4.90 (m, 2H), 4.17-4.08 (m, 1H), 3.75-3.68 (m, 2H), 2.25-2.15 (m, 2H) and 3'-O—,N^4-bis(4-methoxytrityl)-5'(R)-C-allyl-2'-deoxy-2',2'-difluorocytidine (5 mg, 1.3%).

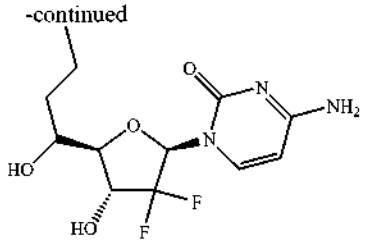
Example 36

Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-propylcytidine (36)

[0348]



-continued



[0349] To an ice-EtOH bath cold solution of 2'-deoxy-5'-C, 5'-O-didehydro-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)cytidine (3 g, 3.72 mmol) in anhydrous THF (10 mL) was added PrMgBr (2M solution in THF) (8 mL, 16 mmol) dropwise under N_2. The reaction mixture was stirred at RT overnight. The mixture was quenched by sat. NH_4Cl. The product was extracted with EA (50 mL×2). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified by silica gel column (PE/EA=3/1 to 1/1) to give 2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'-propylcytidine (1.3 g, 41.1%) as a mixture.

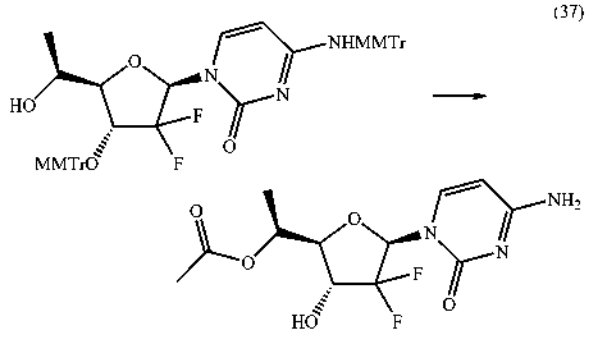
[0350] 2'-Deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'-propylcytidine (1.3 g, 1.53 mmol) was dissolved in 15 mL AcOH/H_2O (v/v=4:1). The mixture was stirred at 50° C. overnight. The solvent was removed under vacuum and the residue was diluted with water (3 mL), extracted with EA (2 mL×2) to remove some impurity. The water layer was subjected to SFC separation to give 2'-deoxy-2',2'-difluoro-5'(S)-propylcytidine (30 mg, 6%) was obtained after HPLC separation. ^1H NMR (400 Hz) (MeOD): δ 7.58 (dd, J=7.6 Hz, 3.6 Hz, 1H), 6.04-5.99 (m, 1H), 5.89 (dd, J=7.6 Hz, 3.6 Hz, 1H), 4.23-4.15 (m, 1H), 3.75-3.73 (m, 2H), 1.47-1.21 (m, 6H), 0.78-0.74 (m, 3H) and 3'-O—,N^4-bis(4-methoxytrityl)-2'-deoxy-2',2'-difluoro-5'(R)-propylcytidine (5 mg, 1%).

Example 37

Preparation of 5'-O-acetyl-2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (37)

[0351]

(37)



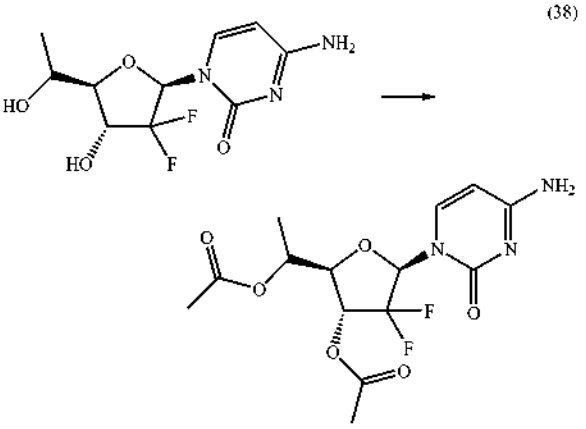
[0352] 2'-Deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(S)—C-ethylcytidine (1 g, 1.2 mmol), EDCI (1 g, 5.2 mmol) and DMAP (1 g, 8.2 mmol) in DCM (100 mL) was added acetic acid (0.5 g, 8.3 mmol) in portions at 0° C. under ice-water, then stirred at room temperature about 10° C. for 1 hour. Then the reaction mixture was washed with water (100 mL) and extracted with DCM (50 mL) twice. The organic layer was concentrated to afford the crude desired 5'-O-acetyl-2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(S)—C-methylcytidine which was used for next-step without purification. 5'-O-Acetyl-2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(S)—C-methylcytidine was dissolved in AcOH:H_2O (50 mL, 80%). The reaction mixture was stirred at 60° C. overnight. Then concentrated and purified by Prep. HPLC to obtain 5'-O-acetyl-2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (210 mg) as white solid; ^1HNMR (CD_3OD, 400 MHz) δppm: 7.65 (d, J=7.6 Hz, 1H), 6.23 (t, J=8.4 Hz, 1H), 5.95 (d, J=7.6 Hz, 1H), 5.22 (m, 1H), 4.09 (m, 1H), 3.90 (dd, J1=4.8 Hz, J2=6.4 Hz, 1H), 2.08 (s, 3H), 1.37 (d, J=6.4 Hz, 3H). ESI-LCMS: m/z 320 $[M+H]^+$, 639 $[2M+H]^+$.

Example 38

Preparation of 2'-deoxy-3',5'-O-diacetyl-2',2'-difluoro-5'(S)—C-methylcytidine (38)

[0353]

(38)



[0354] To a stirred solution of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (1 g, 3.6 mmol) in DMF (10 mL) was added DMF-DMA (10 mL). The mixture was stirred at 60° C. for 2 hours as checked with LCMS. The solvent was then removed under reduced pressure to give 2'-deoxy-2',2'-difluoro-N^4—(N,N-dimethylaminomethylene)-5'(S)—C-methylcytidine (1.1 g).

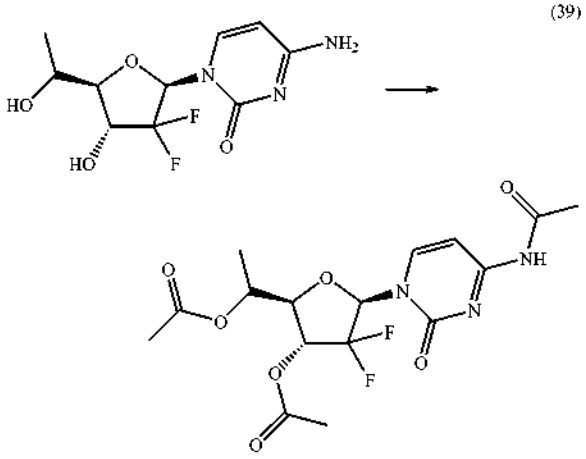
[0355] To a stirred solution of 2'-deoxy-2',2'-difluoro-N^4—(N,N-dimethylaminomethylene)-5'(S)—C-methylcytidine (0.5 g crude) in pyridine (20 mL) were added DMAP (122 mg, 1 mmol) and acetyl anhydride (1.02 g, 10 mmol). The mixture was stirred at RT for 4 hours as checked with LCMS. Then the solvent was removed under reduced pressure to give 2'-deoxy-3',5'-O-diacetyl-2',2'-difluoro-N^4—(N,N-dimethylaminomethylene)-5'(S)—C-methylcytidine (1.8 g), which was dissolved in 50 mL 80% HOAc and stirred at 50° C. for 4 hours as checking with LCMS. The solvent was removed and the residue was purified by prep. HPLC (HCOOH system) to give 2'-deoxy-3',5'-O-diacetyl-2',2'-difluoro-5'(S)—C-methylcytidine as white solid (280 mg, 43% for 3 steps); 1H NMR (CD_3OD, 400 M Hz) δ 7.65 (d, J=7.6 Hz, 1H), 6.32 (t, J=8.8 Hz, 1H), 5.98 (d, J=7.6 Hz, 1H), 5.23-5.31 (m, 1H),

5.18-5.22 (m, 1H), 4.22 (dd, J1=4 Hz, J2=6.4 Hz, 1H), 2.16 (s, 3H), 2.09 (s, 3H), 1.34 (d, J=6.4 Hz, 3H); ESI-LCMS: m/z 362 $[M+H]^+$, 723 $[2M+H]^+$.

Example 39

Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-3',5'-O,N^4-triacetylcytidine (39)

[0356]



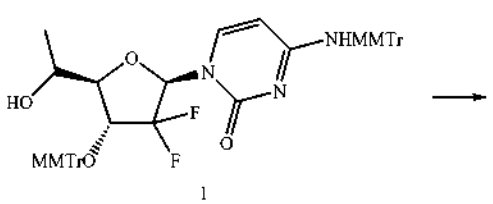
(39)

[0357] To a stirred solution of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (100 mg, 0.36 mmol) in pyridine (5 mL) were added Ac_2O (153 mg, 1.44 mmol) and DMAP (15 mg, 0.12 mmol). The mixture was stirred at RT for 3 hours as checked with LCMS. The mixture was then diluted with EA and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by prep. TLC (PE:EA=2:1) to afford 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-3',5'-O,N^4-triacetylcytidine as white solid (80 mg, 55%). 1H NMR ($CDCl_3$, 400 M Hz) δ 9.41 (br s, 1H), 7.90 (dd, J1=2.0 Hz, J2=7.2 Hz, 1H), 7.53 (d, J=7.2 Hz, 1H), 6.46 (dd, J1=6 Hz, J2=10.4 Hz, 1H), 5.17-5.24 (m, 2H), 4.14 (dd, J1=4.0 Hz, J2=5.6 Hz, 1H), 2.28 (s, 3H), 2.18 (s, 3H), 2.12 (s, 3H), 1.38 (d, J=6.8 Hz, 3H); ESI-MS: m/z 404$[M+H]^+$, 807 $[2M+H]^+$.

Example 40

Preparation of 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-5'-O-propionylcytidine (40)

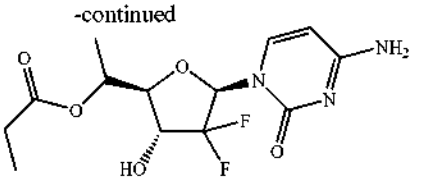
[0358]



(40)

1

-continued



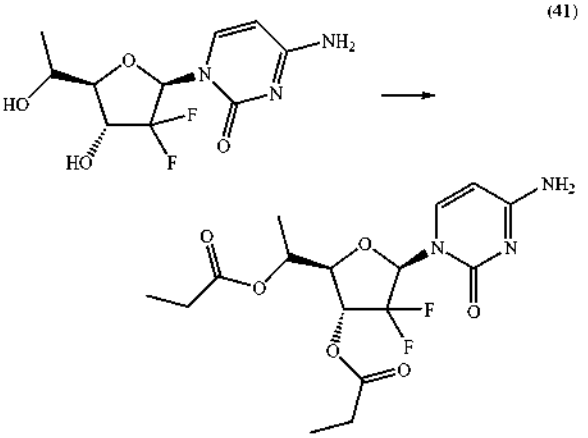
[0359] 2'-Deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(S)—C-methylcytidine (1 g, 1.2 mmol), EDCI (1 g, 5.2 mmol) and DMAP (1 g, 8.2 mmol) in DCM (100 mL) was added propionic acid (0.5 g, 6.8 mmol) in portions at 0° C., then stirred at room temperature (about 10° C.) for 1 hour. Then the reaction mixture was washed with water (100 mL) and extracted with DCM (50 mL) twice. The organic layer was concentrated to afford the 2'-deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(S)—C-methyl-5'-O-propionylcytidine which was used for next-step without purification.

[0360] 2'-Deoxy-2',2'-difluoro-3'-O—,N^4-di(4-methoxytrityl)-5'(S)—C-methyl-5'-O-propionylcytidine was dissolved in AcOH:H_2O (50 mL, 80%). The reaction mixture was stirred at 60° C. overnight. Then concentrated and purified by Prep. HPLC to obtain the desired 2'-deoxy-2',2'-difluoro-5'(S)—C-methyl-5'-O-propionylcytidine (180 mg) as white solids. ^{1}HNMR (CD_3OD, 400 MHz) δ ppm: 7.65 (d, J=7.6 Hz, 1H), 6.23 (t, J=8.4 Hz, 1H), 5.94 (d, J=7.6 Hz, 1H), 5.19-5.25 (m, 1H), 4.04-4.12 (m, 1H), 3.91 (dd, J1=4.8 Hz, J2=7.6 Hz, 1H), 2.39 (q, J=7.6 Hz, 2H), 1.36 (d, J=6.4 Hz, 3H), 1.13 (m, J=7.6 Hz, 3H). ESI-LCMS: m/z 334 $[M+H]^+$, 667 $[2M+H]^+$.

Example 41

Preparation of 2'-deoxy-2',2'-difluoro-3',5'-O-dipropionyl-5'(S)—C-methylcytidine (41)

[0361]



(41)

[0362] To a stirred solution of 2'-deoxy-2',2'-difluoro-5'(S)—C-methylcytidine (1 g, 3.6 mmol) in DMF (10 mL) was added DMF-DMA (10 mL). The mixture was stirred at 60° C. for 2 hours as checked with LCMS. The solvent was then

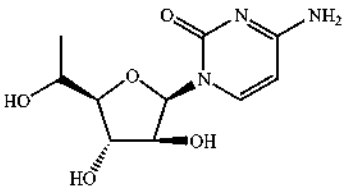
removed under reduced pressure to give crude 2'-deoxy-2',2'-difluoro-N^4—(N,N-dimethylaminomethylene)-5'(S)—C-methylcytidine (1.1 g).

[0363] To a stirred solution of 2'-deoxy-2',2'-difluoro-N^4—(N,N-dimethylaminomethylene)-5'(S)—C-methylcytidine (0.5 g crude) in pyridine (20 mL) were added DMAP (12 mg, 0.1 mmol) and propionyl anhydride (1.3 g, 10 mmol). The mixture was stirred at RT for 4 hours as checked with LCMS. Then the solvent was removed under reduced pressure to give crude 2'-deoxy-2',2'-difluoro-N^4—(N,N-dimethylaminomethylene)-3',5'-O-dipropionyl-5'(S)—C-methylcytidine (1.9 g).

[0364] Crude 2'-deoxy-2',2'-difluoro-N^4—(N,N-dimethylaminomethylene)-3',5'-O-dipropionyl-5'(S)—C-methylcytidine (1.9 g) was dissolved in 50 mL 80% HOAc and stirred at 50° C. for 4 hours. The solvent was removed and the residue was purified by prep. HPLC (HCOOH system) to give 2'-deoxy-2',2'-difluoro-3',5'-O-dipropionyl-5'(S)—C-methylcytidine as white solid (205 mg, 29% for 3 steps); 1H NMR (CD_3OD, 400 M Hz) δ 7.65 (d, J=7.6 Hz, 1H), 6.31 (t, J=8.8 Hz, 1H), 5.97 (d, J=7.6 Hz, 1H), 5.25-5.31 (m, 1H), 5.19-5.24 (m, 1H), 4.23 (dd, J1=4 Hz, J2=6.8 Hz, 1H), 2.48 (q, J=7.6 Hz, 2H), 2.40 (q, J=7.6 Hz, 2H), 1.34 (d, J=6.4 Hz, 3H), 1.15 (t, J=7.6 Hz, 3H), 1.13 (t, J=7.6 Hz, 3H); ESI-LCMS: m/z 390 $[M+H]^+$, 412 $[M+Na]^+$, 779 $[2M+H]^+$.

Example 42

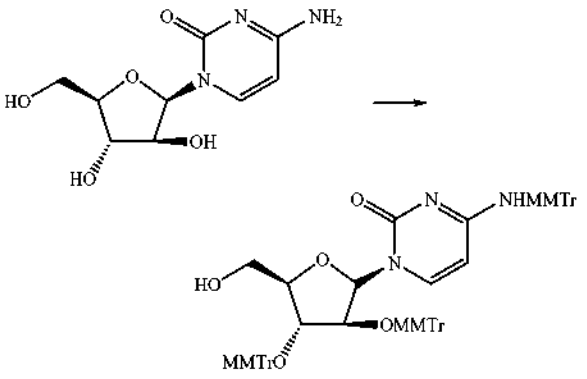
[0365] Preparation of 5'(S)—C-methylarabinocytidine (42)



(42)

Step 1. Preparation of 2',3'-O,N^4-tri(4-methoxytrityl) arabinocytidine

[0366]



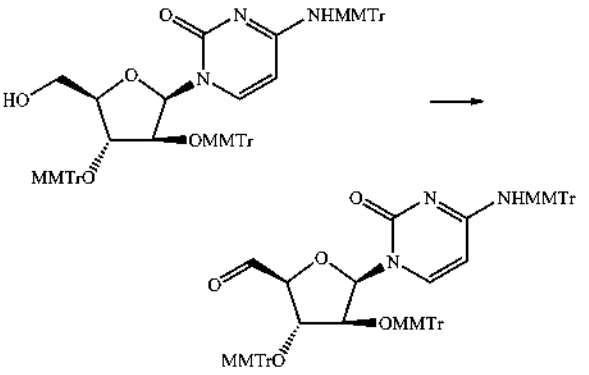
[0367] To an ice-cold solution of arabinocytidine (20.0 g, 82.2 mmol) in anhydrous pyridine (200 mL) was added TBSCl (14.9 g, 98.7 mmol) in small portions under N_2. The reaction mixture was stirred at RT overnight. The solvent was removed under vacuum and the residue was diluted with EA (300 mL), washed with water and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give 5'-O-(t-butydimethylsilyl)arabinocytidine (25.1 g, 85.4%) as a white solid which was used without further purification.

[0368] To a mixture of 5'-O-(t-butydimethylsilyl)arabinocytidine (15.0 g, 41.96 mmol), $AgNO_3$ (43.5 g, 252 mmol) and collidine (61 g, 503.5 mmol) in anhydrous DCM (300 mL) was added MMtrCl (77.7 g, 252 mmol) in small portion under N_2. The reaction mixture was stirred at RT for two days under N_2. The reaction mixture was filtered through a Buchner Funnel. The filtrate was washed with sat. $NaHCO_3$ solution and followed by brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give the residue which was purified by silica gel column (PE/EA=2/1) to give 5'-O-(t-butydimethylsilyl)-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (33.5 g, 67.9%).

[0369] To an ice-cold solution of 5'-O-(t-butydimethylsilyl)-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (12.0 g, 10.2 mmol) in anhydrous THF (80 mL) was added TBAF (1 M solution in THF) (20.5 mL, 20.5 mmol) dropwise under N_2. The reaction mixture was stirred at RT overnight. The solvent was removed to give a residue. The residue was dissolved in EA (200 mL) and washed with water and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give a residue which was purified by silica gel column (PE/EA=6/1 to 2/1) to give 2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (9.8 g, 90.5%).

Step 2. Preparation of 5'-dehydro-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine

[0370]

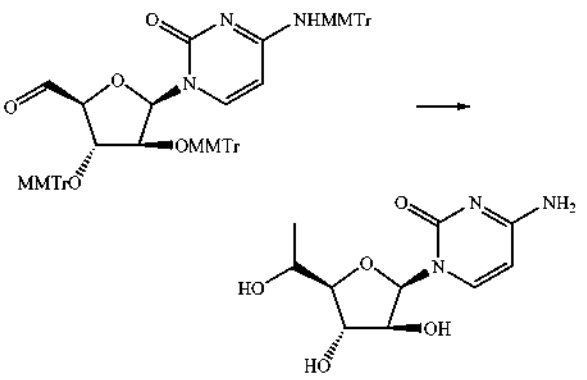


[0371] To an ice-cold mixture of anhydrous pyridine (2.0 mL) and Dess-Martin (3.2 g, 7.55 mmol) in anhydrous DCM (20 mL) was added a solution of 2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (4.0 g, 3.77 mmol) in 10 mL anhydrous DCM under N_2. The reaction mixture was stirred at RT overnight. The reaction mixture was diluted with EA (100 mL), washed with 10% $Na_2S_2O_3$ solution twice and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give

5'-C,5'-O-didehydro-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (3.8 g, 95%) which was used without further purification.

Step 3. Preparation of 5'-C-methylarabinocytidine

[0372]



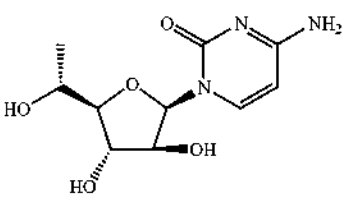
[0373] To an ice-EtOH cold solution of 5'-C,5'-O-didehydro-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (2.0 g, 1.89 mmol) in anhydrous THF (10 mL) was added MeMgBr (3 M solution in ether) (3.2 mL, 9.43 mmol) dropwise under N_2. The reaction mixture was stirred at RT for 5 h. The reaction was complete detected by HPLC. The mixture was cooled to 0° C. and quenched by sat. NH_4Cl. The product was extracted with EA (100 mL×2). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to give the crude 5'(S)—C-methyl-2',3'-O,N^4-tri(4-methoxytrityl) arabinocytidine (1.4 g, 68.9%).

[0374] 5'-C-Methyl-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (700 mg, 0.65 mmol) was dissolved in 10 mL of AcOH/H_2O (v/v=4:1). The mixture was stirred at 50° C. overnight. The solvent was removed under vacuum and the residue was diluted with water (3 mL), extracted with EA (2 mL×2) to remove some impurity. The water layer was subjected to prep-HPLC separation. 5'(S)—C-Methylarabinocytidine (40 mg, 23.5%) was obtained after HPLC separation. ^1H NMR (400 Hz) (MeOD): δ 7.96 (d, J=7.6 Hz, 1H), 6.20 (d, J=4.0 Hz, 1H), 5.91 (d, J=7.2 Hz, 1H), 4.20 (dd, J=4.0 Hz, 2.4 Hz, 1H), 4.10 (t, J=2.8 Hz, 1H), 4.01-4.07 (m, 1H), 3.75 (dd, J=4.0 Hz, 3.2 Hz, 1H), 1.33 (d, J=6.4 Hz, 3H).

Example 43

Preparation of 5'(R)—C-methylarabinocytidine (43)

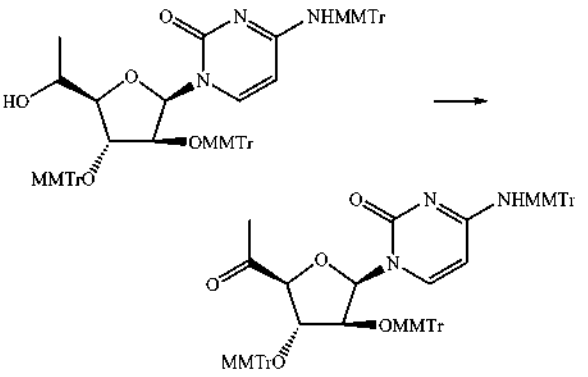
[0375]



(43)

Step 1. Preparation of 5'-C,5'-O-didehydro-5'-C-methyl-2',3'-O,N^4-methoxytrityl)arabinocytidine

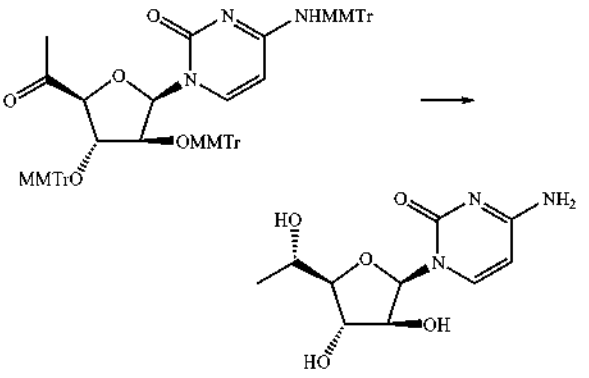
[0376]



[0377] To an ice-cold suspension of CrO_3 (279 mg, 2.79 mmol) in anhydrous DCM (5 mL) was added anhydrous pyridine (0.45 mL, 5.59 mmol) and Ac_2O (0.28 mL, 2.79 mmol) under N_2. The mixture was stirred at RT for about 10 min until the mixture became homogeneous. The mixture was cooled to 0° C. and a solution of 5'(S)—C-methyl-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (1.0 g, 0.93 mmol) in anhydrous DCM (5 mL) was added. The resultant mixture was stirred at RT overnight. The reaction was complete as detected by HPLC. The reaction mixture was diluted with EA (100 mL), washed with $NaHCO_3$ solution twice and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give a residue which was purified by silica gel column (acetone/PE=1/2) to give 5'-C,5'-O-didehydro-5'-C-methyl-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (548 mg, 55%).

Step 2. Preparation of 5'-dehydro-5'(R)—C-methylarabinocytidine

[0378]



[0379] To an ice-cold solution of 5'-C,5'-O-didehydro-5'-C-methyl-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (540 mg, 0.505 mmol) in 95% EtOH (10 mL) was added $NaBH_4$ (39 mg, 1.01 mmol) under N_2. The reaction mixture was stirred at RT for 7 h. The reaction was complete detected

by HPLC. The solvent was evaporated. The residue was diluted with EA (30 mL), washed with sat. $NaHCO_3$ and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated to give the crude 5'-C-methyl-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (480 mg, 88%).

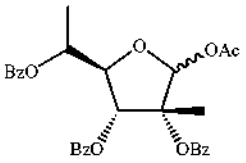
[0380] 5'-C-methyl-2',3'-O,N^4-tri(4-methoxytrityl)arabinocytidine (480 mg, 0.45 mmol) was dissolved in 10 mL AcOH/H_2O (v/v=4:1). The mixture was stirred at 50° C. overnight. The solvent was removed under vacuum and the residue was diluted with water (3 mL), extracted with EA (2 mL×2) to remove some impurity. The water layer was sent to prep. HPLC separation. 5'(R)—C-methylarabinocytidine (30 mg, 26%) was obtained after HPLC separation. ^1H NMR (400 Hz) (MeOD): δ 7.84 (d, J=7.6 Hz, 1H), 6.18 (d, J=3.6 Hz, 1H), 5.91 (d, J=7.6 Hz, 1H), 4.28 (t, J=2.0 Hz, 1H), 4.17 (dd, J=3.6 Hz, 1.6 Hz, 1H), 4.03-4.09 (m, 1H), 3.75 (dd, J=5.2 Hz, 2.4 Hz, 1H), 1.32 (d, J=6.4 Hz, 3H).

Example 44

Preparation of 1-O-acetyl-2,5(S)—C-dimethyl-2,3,5-O-tribenzoyl-D-ribofuranose (44)

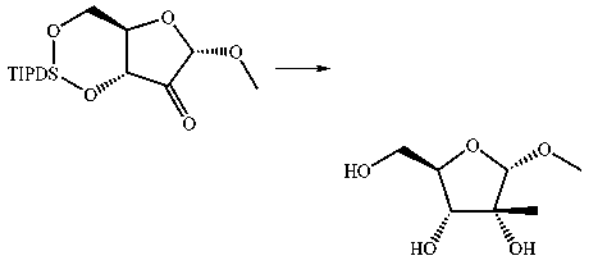
[0381]

(44)



Step 1. Preparation of 1(α)-O,2-C-dimethyl-D-ribofuranose

[0382]

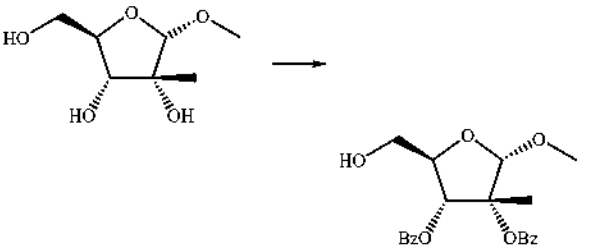


[0383] To a solution of 2-C,2-O-didehydro-1(α)-O-methyl-3,5-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-D-ribofuransone (prepared according to a published procedure, 46.0 g, 113.9 mmol) in THF (300 mL) cooled with dry ice was added CH_3MgBr in ether (3.0 M, 113.9 mL, 341.6 mmol) dropwise under N_2. The mixture was warmed to RT and stirred for 2 h. The mixture was quenched by saturated NH_4Cl. The product was extracted with EA (200×2). The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give 1(α)-O,5-C-dimethyl-3,5-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-D-ribofuransone as syrup (42.1 g, 88.0%) which was used without further purification.

[0384] To a solution of 1(α)-O,2-C-dimethyl-3,5-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-D-ribofuransone (42.1 g, 100.2 mmol) in anhydrous THF (200 mL) was added TBAF (52.6 g, 200.5 mmol) in small portion. The mixture was stirred at RT overnight. The solvent was removed and the residue was purified by silica gel column. (EA/MeOH=100/1) to give 1(α)-O,2-C-dimethyl-D-ribofuransone as syrup (16.5 g, 92.4%); 1HNMR (400 MHz) (MeOD):δ4.56 (s, 1H), 3.87-3.90 (m, 1H), 3.60-3.77 (m, 2H), 3.52 (d, J=6.0 Hz), 3.43(s, 3H), 1.25 (s, 3H).

Step 2. Preparation of 2,3-O-dibenzoyl-1(α)-O,2-C-dimethyl-D-ribofuranose

[0385]



[0386] To an ice-cold of solution of 1(α)-O,2-C-dimethyl-D-ribofuransone (11.0 g, 61.8 mmol) in anhydrous pyridine (100 mL) was added TBSCl (11.2 g, 74.2 mmol) in small portion under N_2. The reaction mixture was stirred at RT for 4 h. The mixture was cooled in an ice bath and BzCl (17.4 g, 124 mmol) was added. The mixture was stirred at RT overnight. The solvent was diluted with EA (300 mL) and washed with saturated $NaHCO_3$. the organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give crude syrup. The cured syrup was purified by flash chromatography (PE/EA=20/1 to 10/1) to give 3-O-benzoyl-5-O-(t-butyldimethylsilyl)-1(α)-O,2-C-dimethyl-D-ribofuransone (21.4 g, 87.2%); 1HNMR (400 MHz) ($CDCl_3$):δ8.05 (d, J=7.2 Hz, 2H), 7.56 (t, J=7.2 Hz, 1H), 7.44 (t, J=6.4 Hz, 2H), 4.98 (d, J=4.0 Hz, 1H), 4.58 (s, 1H), 4.18 (m, 1H), 3.90-3.93 (m, 1H), 3.47 (s, 1H), 3.11 (s, 1H), 1.47 (s, 3H), 0.91 (s, 9H), 0.10 (d, J=2.0 Hz, 3H).

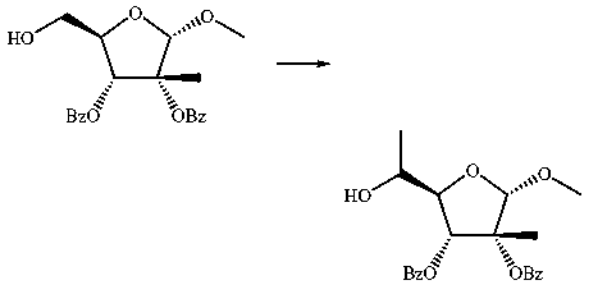
[0387] To an ice-cold mixture of TEA (54.6 g, 540 mmol) and DMAP (6.6 g, 54.0 mmol) in anhydrous DCM (200 mL) was added BzCl (15.2 g, 108.0 mmol) followed by 3-O-benzoyl-5-O-(t-butyldimethylsilyl)-1(α)-O,2-C-dimethyl-D-ribofuransone (21.4 g, 54.0 mmol). The reaction mixture was stirred at RT for 2 days. The mixture was diluted with DCM (200 mL) and then washed with water and saturated $NaHCO_3$. The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give crude syrup. The cured syrup was purified by flash chromatography (PE/EA=50/1 to 20/1) to give 5-O-(t-butyldimethylsilyl)-2,3-O-dibenzoyl-1(α)-O,2-C-dimethyl-D-ribofuransone as syrup (24.5 g, 90.7%); 1HNMR (400 MHz) ($CDCl_3$):δ8.11 (m, 2H), 7.75-7.77 (m, 2H), 7.54-7.57 (m, 1H), 7.38-7.45 (m, 3H), 7.13-7.15 (m, 1H), 5.35 (d, J=2.8 Hz, 1H), 5.28 (s, 1H), 4.27 (m, 1H), 3.95-3.97 (m, 1H), 3.40 (s, 1H), 1.77 (s, 3H), 0.92 (s, 9H), 0.12 (d, J=2.0 Hz, 3H).

[0388] To an ice-cold solution of 5-O-(t-butyldimethylsilyl)-2,3-O-dibenzoyl-1(α)-O,2-C-dimethyl-D-ribofuransone (24.5 g, 49.0 mmol) in THF (200 mL) was added a 1 M

solution of TBAF in THF (58.8 mL, 58.8 mmol) dropwise. The mixture was stirred at this temperature for 2 h. HOAc was added to the mixture to neutralize the solution to light acid. After that, the solvent was removed and the residue was purified by flash chromatography (PE/EA=20/1 to 8/1) to give 2,3-O-dibenzoyl-1(α)-O,2-C-dimethyl-D-ribofuranose as syrup (15.2 g, 80.4%); 1HNMR (400 MHz) ($CDCl_3$):δ8. 11-8.13 (dd, J_1=5.2 Hz, J_2=7.2 Hz, 2H), 7.79-7.82 (dd, J_1=0.8 Hz, J_2=8.0 Hz, 2H), 7.57-7.61 (t, J=7.2 Hz, 1H), 7.41-7.46 (m, 3H), 7.17-7.21 (t, J=8.0 Hz, 2H), 5.30 (s, 1H), 5.28 (d, J=4.4 Hz, 1H), 4.34 (q, J=4.0 Hz, 1H), 3.94-4.04 (m, 2H), 3.43 (s, 3H), 2.29 (w, 1H), 1.79 (s,3H).

Step 3. Preparation of 2,3-O-dibenzoyl-5-C-methyl-1 (α)-O,2-C-dimethyl-D-ribofuranose

[0389]

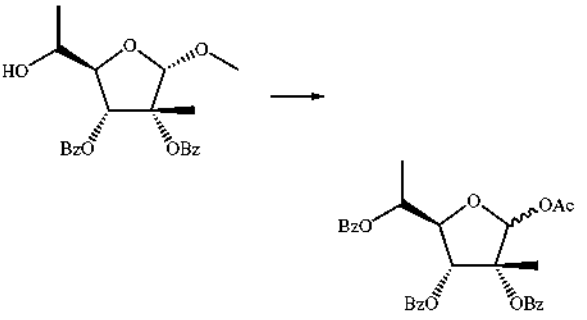


[0390] To a suspension of Dess-Martin reagent (15.7 g, 37.0 mmol) in anhydrous DCM (200 mL) cooled with an ice-bath was added a solution of 2,3-O-dibenzoyl-1(α)-O,2-C-dimethyl-D-ribofuranose (11.0 g, 28.5 mmol) in anhydrous DCM (50 mL) under N_2. The reaction mixture was stirred at RT overnight. The mixture was diluted with ether (500 mL) and washed with saturated $Na_2S_2O_3$ (22.51 g, 142.3 mmol). The organic layer was separated, dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated in vacuum under a temperature lower than 30° C. to give 2,3-O-dibenzoyl-5-C,5-O-didehydro-1(α)-O,2-C-dimethyl-D-ribofuranose as a white foam (9.5 g, 96.4%).

[0391] $TiCl_4$ (10.85 mL, 18.75 g, 98.86 mmol) was added dropwise to anhydrous ether (310 mL) cooled to −78° C. To the resultant yellow etherate was slowly added 3.0 M CH_3MgBr in ether (33.0 mL, 32.9 mmol), the reaction mixture was then allowed to warmed to −30° C., whereupon a solution of 2,3-O-dibenzoyl-5-C,5-O-didehydro-1(α)-O,2-C-dimethyl-D-ribofuranose (9.5 g, 24.7 mmol) in 30 mL of ether was added dropwise. After 4 h at −30 to −10° C., TLC analysis showed complete conversion, and the reaction was separated and the aqueous phase extracted with 3×150 mL of ether. The combined organic layer was washed with water, dried over anhydrous $MgSO_4$, filtered and concentrated to a syrup. The syrup was purified by silica gel column (PE/EA=20/1 to10/1) to give 2,3-O-dibenzoyl-1(α)-O,2,5-C-trimethyl-D-ribofuranose as a foam solid (7.1 g, 71.7%); 1HNMR (400 MHz) ($CDCl_3$):δ8.13 (d, J=4.4 Hz, 2H), 7.09 (d, J=4.4 Hz, 2H), 7.36-7.47 (m, 5H), 7.11 (t, J=1.6 Hz), 5.40 (d, J=4.0 Hz, 1H), 5.28 (d, J=8.4 Hz, 1H), 4.10-4.15 (m, 2H), 3.42 (s, 3H), 2.36 (w, 1H), 1.77 (s, 3H), 1.27 (d, J=8.4 Hz, 3H).

Step 4. Preparation of 1-O-acetyl-2,3,5-O-tribenzoyl-2,5-C-dimethyl-D-ribofuranose

[0392]



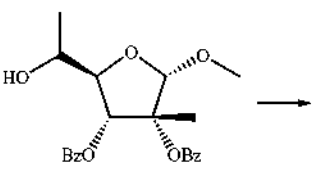
[0393] To an ice-cold solution of 2,3-O-dibenzoyl-1(α)-O, 2,5-C-trimethyl-D-ribofuranose (2.0 g, 5.0 mmol) in anhydrous pyridine (20 mL) was added a BzCl (1.05 g, 7.5 mmol) under N_2. The mixture was stirred at RT overnight. The solvent was removed and the residue was purified by sillca gel column (PE/EA=50/1 to 30/1). 2,3,5-O-Tribenzoyl-1(α)-O, 2,5-C-trimethyl-D-ribofuranose was obtained as a white foam solid (1.5 g, 60%); 1HNMR (400 MHz) ($CDCl_3$):δ8.18 (d, J=7.2 Hz, 2H), 8.06 (d, J=7.2 Hz, 2H), 7.36-7.71 (m, 9H), 7.11 (t, J=7.6 Hz, 2H), 5.60 (m, 1H), 5.56 (d, J=5.2 Hz, 1H), 4.44 (t, J=4.8 Hz, 1H), 3.43 (s, 1H), 1.79 (s, 1H), 1.48 (d, J=6.4 Hz, 3H).

[0394] To a solution of 2,3,5-O-tribenzoyl-1(α)-O,2,5-C-trimethyl-D-ribofuranose (1.5 g, 3.0 mmol) in HOAc (10 mL) and Ac_2O (1 mL) cooled with a water bath was added 0.5 mL conc. H_2SO_4 dropwise. The mixtrure was stirred at RT for 3 h. The mixture was poured into ice-water and the precipitate was collected by filtration. The solid cake was dissolved in EA (50 mL) and washed with saturated $NaHCO_3$ (30 mL×2). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under vacuum to give 1-O-acetyl-2,5(S)—C-dimethyl-2,3,5-O-tribenzoyl-D-ribofuranose as white foam solid (1.4 g, 88.6%); 1HNMR (400 MHz) ($CDCl_3$):δ7.87-8.02 (m, 5H), 7.06-7.63 (m, 10H), 6.73+6.55 (s, 1H), 5.84 (d, J=8.0 Hz, 0.5H), 5.64 (d, J=3.6 Hz, 0.5H), 5.30-5.51 (m, 1H), 4.41-4.50 (m, 1H), 1.94+1.86 (s, 3H), 1.76+1.71 (s, 3H), 1.41+1.33 (d, J=6.8 Hz, 3H).

Example 45

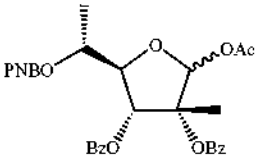
Preparation of 1-O-acetyl-2,5(R)—C-dimethyl-2,3, 5-O-tribenzoyl-D-ribofuranose (45)

[0395]



(45)

-continued



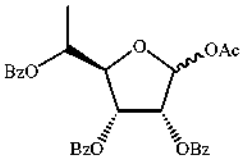
[0396] To an ice-cold solution of 2,3-O-dibenzoyl-1(α)-O, 2,5-C-trimethyl-D-ribofuranose (2.0 g, 5.0 mmol), PNBA (3.3 g, 19.9 mmol) and Ph_3P (5.2 g, 19.9 mmol) in anhydrous THF (50 mL) was added DEAD (3.48 g, 19.9 mmol) dropwise under N_2. The resultant mixture was stirred at RT overnight. The solvent was removed and the residue was purified by silica gel column (PE/EA=30/1 to 20/1). 2,3-O-dibenzoyl-1(α)-O,2,5-C-trimethyl-5-O-(4-nitrobenzoyl)-D-ribofuranose was obtained as a white foam solid (1.4 g, 51.0%); 1HNMR (400 MHz) ($CDCl_3$): δ 8.13-8.21 (m, 4H), 8.11-8.13 (dd, J_1=0.8 Hz, J_2=8.0 Hz, 2H), 7.70-7.73 (dd, J_1=0.8 Hz, J_2=8.0 Hz, 2H), 7.61 (t, J=7.2 Hz, 1H), 7.38-7.45 (m, 3H), 7.13 (t, J=8.0 Hz, 2H), 5.59 (m, 1H), 5.33 (s, 1H), 5.27 (d, J=5.2 Hz, 1H), 4.47 (t, J=5.2 Hz, 1H), 3.44 (s, 3H), 1.80 (s, 3H), 1.51-1.56 (dd, J_1=6.4 Hz, J_2=12.8 Hz, 3H).

[0397] To a water-cold solution of 2,3-O-dibenzoyl-1(α)-O,2,5-C-trimethyl-5-O-(4-nitrobenzoyl)-D-ribofuranose (1.4 g, 2.5 mmol) in HOAc (10 mL) and Ac_2O (1 mL) was added 0.5 mL conc. H_2SO_4 dropwise. The mixtrure was stirred at RT for 3 h. The mixture was poured into ice-water and the precipitate was collected by filtration. The solid cake was re-dissolved in EA (50 mL) and washed with saturated $NaHCO_3$ (30 mL×2). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under vacuum to give 1-O-acetyl-2,3-O-dibenzoyl-2,5(R)—C-dimethyl-5-O-(4-nitrobenzoyl)-D-ribofuranose as white foam solid (1.3 g, 89.6%); 1HNMR (400 MHz) ($CDCl_3$):δ7.91-8.20 (m, 7H), 7.12-7.67 (m, 7H), 6.75+6.56 (s, 1H), 5.75 (d, J=8.4 Hz, 0.5H), 5.57 (m, 0.5H), 5.37 (m, 1H), 4.43-4.50 (m, 1H), 2.16+1.97 (s, 3H), 1.78+1.73 (s, 3H), 1.48+1.38 (d, J=6.8 Hz, 3H).

Example 46

Preparation of 1-O-acetyl-5-C-methyl-2,3,5-O-tribenzoyl-D-ribofuranose (46)

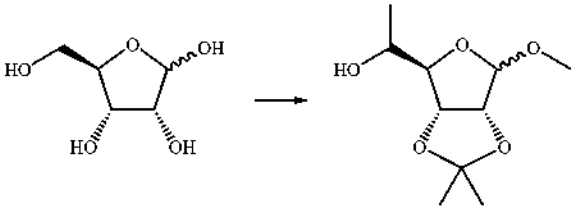
[0398]



(46)

Step 1. Preparation of 1-O,5-C-dimethyl-2,3-O-isopropylidene-D-ribofuranose

[0399]



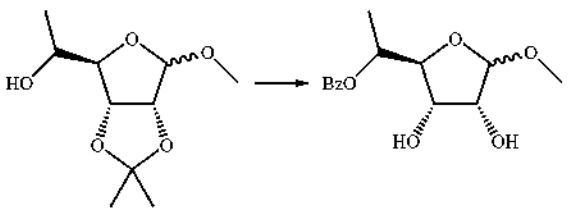
[0400] To D-ribose (200 g, 1.33 mol) in acetone (760 mL) and MeOH (760 mL) was added concentrated HCl (20 mL), and the solution was allowed to reflux for 18 h. The reaction was cooled, neutralized with pyridine, poured into H_2O (2 L), and extracted with Et_2O (3×400 mL). The combined organic layers were washed with saturated aqueous $CuSO_4$ (300 mL), dried with $MgSO_4$ and then evaporated. The residue was distilled to afford 2,3-O-isopropylidene-1-O-methyl-D-ribofuranose as colorless oil (180.5 g, 56.5%).

[0401] To an ice-cold suspension of Dess-Martin preiodinane (487.3 g, 1.15 mol) in anhydrous DCM (800 L) was a solution of 2,3-O-isopropylidene-1-O-methyl-D-ribofuranose (180.5 g, 883.85 mmol) in anhydrous DCM (200 mL) dropwise under N_2. The resultant mixture was stirred at RT overnight and then diluted with Et_20 (2 L). The mixture was washed with saturated aqueous Na_2SO_3 (3×600 mL). The organic layer was separated, dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated in vacuum to give 5-C,5-O-didehydro-2,3-O-isopropylidene-1-O-methyl-D-ribofuranose as syrup which was used for the next step without further purification (161.7 g, 90.48%).

[0402] To a solution of 5-C,5-O-didehydro-2,3-O-isopropylidene-1-O-methyl-D-ribofuranose (161.7 g, 799.70 mmol) in anhydrous THF (3.0 L) was added a 3M solution of MeMgBr in ether (800 mL, 2.40 mol) at 50° C. under N_2. After the addition, the reaction mixture was warmed to 0° C. during a 4 h period. The mixture was quenched with saturated aqueous NH_4Cl and the product was extracted with EA (2×2.0 L). The combined organic layer was dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated in vacuum to give a syrup which was purified by silica gel column (PE/EA=30/1 to 10/1) to give 1-O,5-C-dimethyl-2,3-O-isopropylidene-D-ribofuranose as colorless syrup (120.3 g, 69.4%).

Step 2. Preparation of 5-O-benzoyl-1-O,5-C-dimethyl-D-ribofuranose

[0403]



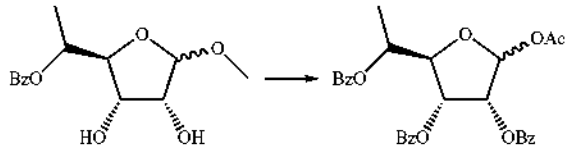
[0404] To an ice-cold solution of 1-O,5-C-dimethyl-2,3-O-isopropylidene-D-ribofuranose (20.0 g, 92.06 mmol) and DMAP (1.12 g, 9.21 mmol) in anhydrous pyridine (150 mL) was added BzCl (19.41 g, 138.1 mmol) dropwise under N_2.

The reaction mixture was stirred at RT overnight. EA (300 mL) was added to the mixture and then washed with water (200 mL) and saturated aqueous $NaHCO_3$ (200 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give a residue which was purified by column (PE/EA=30/1 to 10/1) to give 5-O-benzoyl-1-O,5-C-dimethyl-2,3-O-isopropylidene-D-ribofuranose as syrup (20.7 g, 69.7%).

[0405] 5-O-Benzoyl-1-O,5-C-dimethyl-2,3-O-isopropylidene-D-ribofuranose (20.7 g, 67.14 mmol) was added to a solution of TFA (180 mL) and H_2O (20 mL) at 0° C. The resultant mixture was stirred at 0° C. for 3 h. TLC showed no starting material remained. The solvent was removed under vaccum at 0° C. The residue was dissolved in DCM (200 mL) and washed with saturated aqueous $NaHCO_3$ (2×150 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give syrup 5-O-benzoyl-1-O,5-C-dimethyl-D-ribofuranose, which was used without further purification (12.0 g).

Step 3. Preparation of 1-O-acetyl-2,3,5-O-tribenzoyl-5-C-methyl-D-ribofuranose

[0406]



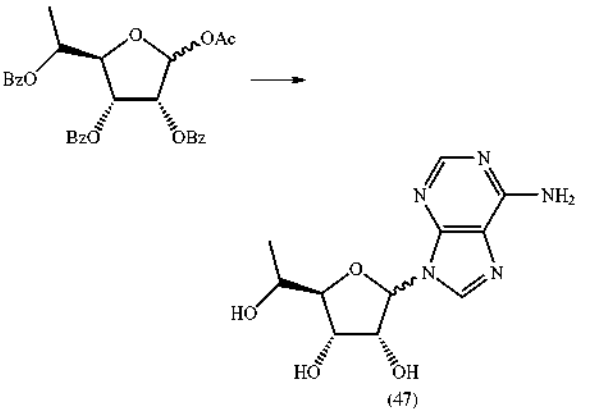
[0407] The crude 5-O-benzoyl-1-O,5-C-dimethyl-D-ribofuranose (12.0 g, 42.51 mmol) was dissolved in anhydrous pyridine and cooled with an ice-bath. DMAP (0.52, 4.25 mmol) and BzCl (14.9 g, 106.27 mmol) was added to the mixture and then stirred at RT overnight. EA (300 mL) was added to the mixture and then washed with water (200 mL) and saturated aqueous $NaHCO_3$ (200 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give a residue which was purified by column (PE/EA=30/1 to 10/1) to give 1-O,5-C-dimethyl-2,3,5-O-tribenzoyl-D-ribofuranose as syrup (15.5 g, 74.34%).

[0408] To an water-cold (10° C.) mixture of 1-O,5-C-dimethyl-2,3,5-O-tribenzoyl-D-ribofuranose (15.5 g, 31.6 mmol) in HOAc (50 mL) and Ac_2O (5 mL) was added concentrated H_2SO_4 (2.5 mL) dropwise. The resultant mixture was stirred at RT for 5 h and then poured in ice-water. The precipitate was collected by filtration. The collected solid was dissolved in EA (100 mL) and washed with saturated aqueous $NaHCO_3$ (100 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give a residue which was purified by column (PE/EA=30/1 to 20/1) to give 1-O-acetyl-5-C-methyl-2,3,5-O-tribenzoyl-D-ribofuranose as foam solid (10.5 g, 64.02%).

Example 47

Preparation of 5'(S)—C-methyladenosine (47)

[0409]

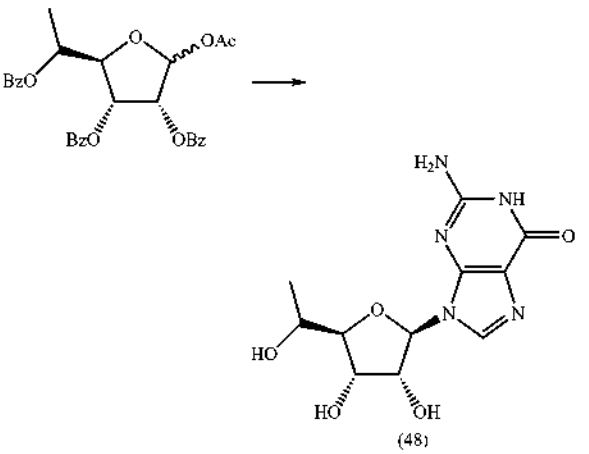


(47)

[0410] To an ice-cold solution of 1-O-acetyl-5(S)—C-methyl-2,3,5-O-tribenzoyl-D-ribofuranose (8.0 g, 15.43 mmol) and adenine (3.13 g, 23.14 mmmol) in anhydrous MeCN (100 mL) was added a 1 M solution of $SnCl_4$ in anhydrous MeCN (38.6 mL, 38.6 mmol) dropwise under N_2. The mixture was stirred at RT overnight and then quenched by aqueous $NaHCO_3$. The product was extracted by EA (2×100 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give a residue which was purified by silica gel column to give 8.0 g of 2',3',5'-O-tribenzoyl-5'-C-methyladenosine, which was dissolved in methanol (100 mL) and saturated with NH_3 for 1 h at 0° C., and then stirred at RT overnight. The solvent was removed under reduced pressure and the residue was re-dissolved in 400 mL saturated aqueous NH_3. The mixture was stirred at RT overnight and the solvent was removed. The residue was purified by prep-HPLC to give 5'(S)—C-methyladenosine (1.5 g, 34.5%).

Example 48

[0411] Preparation of 5'(S)—C-methylguanosine (48)



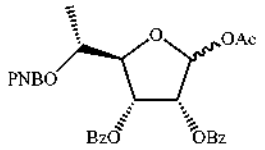
(48)

[0412] Under an argon atmosphere, a mixture of N^2-acetylguanine (10.65 g, 81.00 mmol), dry pyridine (50 mL), and HMDS (300 mL) was heated to reflux for 2 h to obtain a clear solution. The solvent was removed carefully under vacuum, and the residue was dried under high vacuum for 1 h. To the flask containing persilylated N^2-acetylguanosine was added anhydrous toluene (100 mL) and 1-O-acetyl-5(S)—C-methyl-2,3,5-O-tribenzoyl-D-ribofuranose (10.5 g, 20.25 mmol). To the resulting mixture was added TMSOTf (18.0 g, 81.00 mmol) slowly with vigorous stirring at room temperature. After stiffing under argon atmosphere under reflux for 6 h, the reaction mixture was cooled to room temperature and quenched with saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with DCM (2×150 mL). The combined organic layers was washed with brine, and dried over anhydrous $MgSO_4$. The $MgSO_4$ was filtered off, and the solvent was removed by evaporation under vacuum to give light yellow foam (13.1 g). 8.0 g of the foam solid was purified by prep-HPLC to give 4.4 g (95% purity) of N^2-acetyl-5'(S)—C-methyl-2,3,5-O-tribenzoylguanosine, which was dissolved in methanol (100 mL) saturated with NH_3 and stirred at RT overnight. The solvent was removed under reduced pressure and the residue was re-dissolved in 400 mL of saturated aqueous NH_3. The mixture was stirred at RT overnight and the solvent was concentrated to about 150 mL. The precipitate was collected by filtration and dried under vacuum to give 5'(S)—C-methylguaonosine as a white solid (550 mg, 30.7%); ^1H NMR (400 MHz) (MeOD): δ 7.88 (s, 1H), 5.76 (d, J=7.2Hz, 1H), 4.64 (t, J=6.0 Hz, 1H), 4.30 (d, J=4.4 Hz, 1H), 4.03-4.01 (m, 1H), 3.93 (s, 1H), 1.24 (d, J=6.4 Hz, 3H).

Example 49

Preparation of 1-O-acetyl-2,3-O-dibenzoyl-5(R)—C-methyl-5-O-(4-nitrobenzoyl)-D-ribofuranose (49)

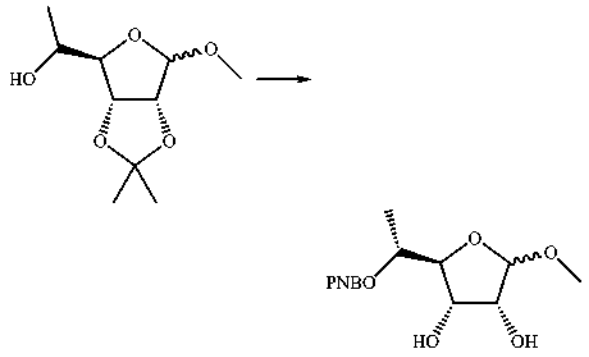
[0413]



(49)

Step 1. Preparation of 1-O,5-C-dimethyl-5-O-(4-nitrobenzoyl)-D-ribofuranose

[0414]

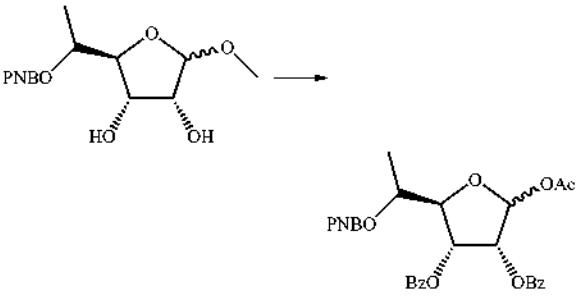


[0415] To an ice-cold solution of 1-O,5-C-dimethyl-2,3-O-isopropylidene-D-ribofuranose (25.0 g, 114.55 mmol), PNBA (76.57 g, 458.19 mmol) and Ph_3P (120.18 g, 458.19 mmol) in anhydrous THF (600 mL) was added DEAD (79.79 g, 458.19 mmol) dropwise under N_2. The reaction mixture was stirred at RT overnight. The solvent was removed and the residue was purified by silica gel column (PE/EA=50/1 to 20/1) to give 1-O,5-C-dimethyl-2,3-O-isopropylidene-5-O-(4-nitrobenzoyl)-D-ribofuranose as a light yellow syrup (21.3 g, 50.62%).

[0416] 1-O,5-C-Dimethyl-2,3-O-isopropylidene-5-O-(4-nitrobenzoyl)-D-ribofuranose (16.3 g, 44.37 mmol) was added to a solution of TFA (90 mL) and H_2O (10 mL) at 0° C. The resultant mixture was stirred at 0° C. for 3 h. TLC showed no starting material remained. The solvent was removed under vaccum at 0° C. The residue was dissolved in DCM (150 mL) and washed with saturated aqueous $NaHCO_3$ (2×150 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give a syrup which was purified by silica gel column (PE/EA=15/1 to 5/1). 1-O,5-C-dimethyl-5-O-(4-nitrobenzoyl)-D-ribofuranose was obtained as syrup (7.0 g, 48.2%).

Step 2. Preparation of 1-O-acetyl-2,3-O-dibenzoyl-5-C-methyl-5-O-(4-nitrobenzoyl)-D-ribofuranose

[0417]



[0418] 1-O,5-C-Dimethyl-5-O-(4-nitrobenzoyl)-D-ribofuranose (7.0 g, 21.39 mmol) was dissolved in anhydrous pyridine (50 mL) and cooled with an ice-bath. DMAP (0.26, 2.14 mmol) and BzCl (7.52 g, 53.47 mmol) was added to the mixture and then stirred at RT overnight. EA (200 mL) was added to the mixture and then washed with water (100 mL) and saturated aqueous $NaHCO_3$ (100 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuum to give a residue which was purified by column (PE/EA=30/1 to 10/1) to give 2,3-O-dibenzoyl-1-O,5-C-dimethyl-5-O-(4-nitrobenzoyl)-D-ribofuranose as syrup (9.2 g, 80.33%).

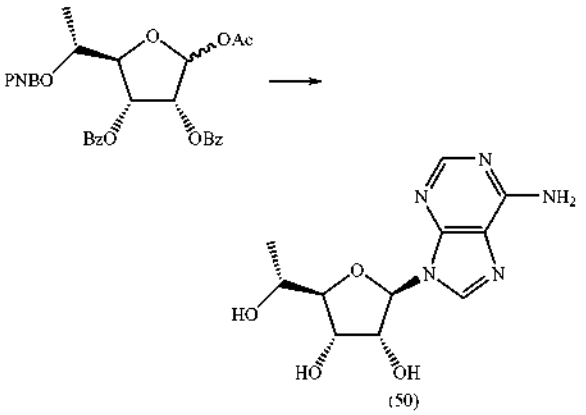
[0419] To a solution of 2,3-O-dibenzoyl-1-O,5-C-dimethyl-5-O-(4-nitrobenzoyl)-D-ribofuranose (9.2 g, 17.18 mmol) in HOAc (30 mL) and Ac_2O (3 mL) cooled with water bath was added 1.5 mL of concentrated H_2SO_4 dropwise. The mixture was stirred at RT for 3 h. The mixture was poured into ice-water and the precipitate was collected by filtration. The solid cake was re dissolved in EA (50 mL) and washed with saturated $NaHCO_3$ (30 mL×2). The organic layer was separated, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under vacuum to give 1-O-acetyl-2,3-O-

dibenzoyl-5(R)—C-methyl-5-O-(4-nitrobenzoyl)-D-ribofuranose as white foam solid (8.1 g, 83.7%). ^{1}H NMR (400 MHz) ($CDCl_3$): δ 8.83-8.18 (m, 4H), 8.05-7.96 (m, 2H), 7.86-7.82 (m, 2H), 7.86-7.82 (m, 2H), 7.59-7.49 (m, 2H), 7.45-7.40 (m, 2H), 6.75 (d, J=4.4 Hz, 0.3H), 6.42 (s, 0.7H), 5.86-5.83 (m, 0.7H), 5.74-70 (m, 1H), 5.54-4.92 (m, 1.3H), 4.64-4.61 (m, 1H), 2.20 (s, 2H), 2.16 (s, 1H), 1.54 (d, J=6.8Hz, 1H),1.49 (d, J=6.4 Hz, 2H).

Example 50

Preparation of 5'(R)—C-methyladenosine (50)

[0420]



(50)

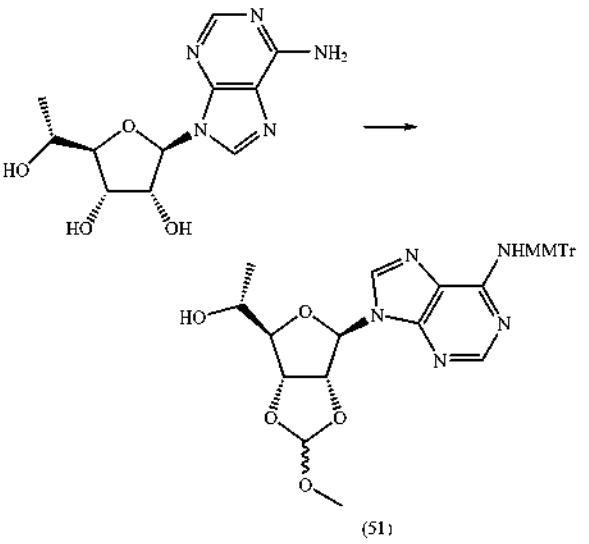
[0421] A mixture of N^6-benzoyladenosine (2.39 g, 20 mmol) and N,O-bis(trimethylsilyl)acetamide (9.78 mL, 40 mmol) in anhydrous acetonitrile (50 mL) under argon was heated under reflux for 1 h and cooled to rt. A solution of 1-O-acetyl-2,3-O-dibenzoyl-5(R)—C-methyl-5-O-(4-nitrobenzoyl)-D-ribofuranose (1.5 g, 2.89 mmol) in anhydrous acetonitrile (50 mL) was added, followed by addition of trimethylsilyl trifluoromethanesulfonate (1.36 g, 7.5 mmol). The resulting mixture was heated under reflux overnight, cooled with ice, diluted with ethyl acetate, washed with aqueous sodium bicarbonate, dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel withl0-15% ethyl acetate in DCM gave 3.62 g of 2',3'-O-dibenzoyl-5'(R)—C-methyl-5'-O-(4-nitrobenzoyl)adeno sine.

[0422] 2',3'-O-Dibenzoyl-5'(R)—C-methyl-5'-O-(4-nitrobenzoyl)adenosine (3.62 g) in methanol (300 mL) and 28% aqueous ammonia (30 mL) was stirred at RT overnight. The solvent was removed and the residue was re-dissolved in 28% aqueous NH_3 (250 mL). The mixture was stirred at rt for 2 days and the solvent was removed. Precipitation from MeOH/DCM gave 0.59 g of 5'(R)—C-methyladenosine as a white solid. The filtrate was concentrated and chromatographed on silica gel with 10-14% MeOH in DCM to give 0.68 g of 5'(R)—C-methyladenosine as a white solid. Total yield was 1.27 g. ^{1}H NMR (CD_3OD): δ 8.31 (s, 1H), 8.18 (s, 1H), 5.95 (d, J=6.4 Hz, 1H), 4.73 (q, J_1=5.2 Hz, J_2=6.8 Hz, 1H), 4.27 (dd, J_1=2.4 Hz, J_2=5.2 Hz, 1H), 4.01 (t, J=2.4 Hz, 1H), 3.97-3.91 (m, 1H), 1.25 (d, J=6.4 Hz, 3H).

Example 51

Preparation of 2',3'-methoxymethylidene-5'-O,N^6-(4'-methoxytrityl)-5'(R)-methyladenosine (51)

[0423]



(51)

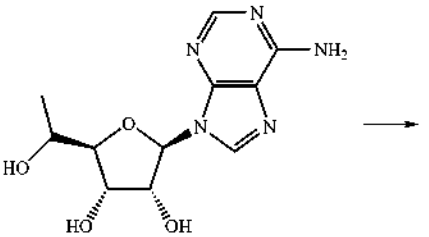
[0424] A mixture of 5'(R)—C-methyladenosine (890 mg, 3.17 mmol), trimethyl orthoformate (9 mL) and p-toluenesulfonic acid monohydrate (904 mg, 4.75 mmol) in 1,4-dioxane (11.2 mL) was stirred at rt for 24 h, cooled with ice and quenched by adding triethylamine (1 mL) and concentrated. Chromatography on silica gel with 5-6% MeOH in DCM gave 716 mg of 2',3'-O-methoxymethylidene-5'(R)—C-methyladenosine.

[0425] A solution of 2',3'-O-Methoxymethylidene-5'(R)—C-methyladenosine (715 mg, 2.21 mmol) and 4-methoxytrityl chloride (1.03 g, 3.32 mmol) in pyridine (14 mL) was stirred at 50° C. for 20 h, diluted with ethyl acetate, washed with brine three times. Solvent was evaporated and the residue was chromatographed on silica gel with 25-55% ethyl acetate in hexanes to give 352 mg of 5'-O,N^6-di(4'-methoxytrityl)-2',3'-methoxymethylidene-5'(R)-methyladenosine and 634 mg of 2',3'-methoxymethylidene-5'-O,N^6-(4'-methoxytrityl)-5'(R)-methyladenosine as foam solid.

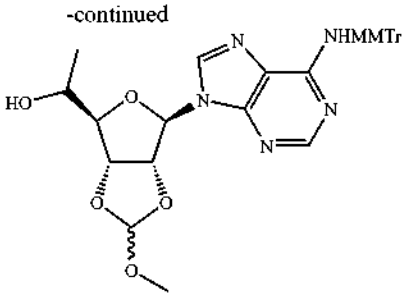
Example 52

Preparation of 2',3'-methoxymethylidene-5'-O,N^6-(4'-methoxytrityl)-5'(S)-methyladenosine (52)

[0426]



-continued



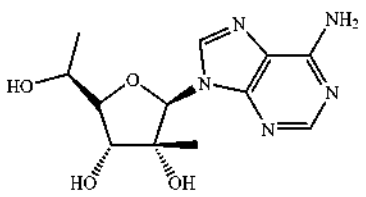
(52)

[0427] By a similar procedure as described for example 48-2, 377 mg of 5'-O,N^6-di(4'-methoxytrityl)-2',3'-methoxymethylidene-5'(S)-methyladenosine and 750 mg of 2',3'-methoxymethylidene-5'-O,N^6-(4'-methoxytrityl)-5'(S)-methyladenosine as foam solid were prepared from 5'(S)—C-methyladenosine.

Example 53

Preparation of 2',5'(R and S)—C-dimethyladenosine (53)

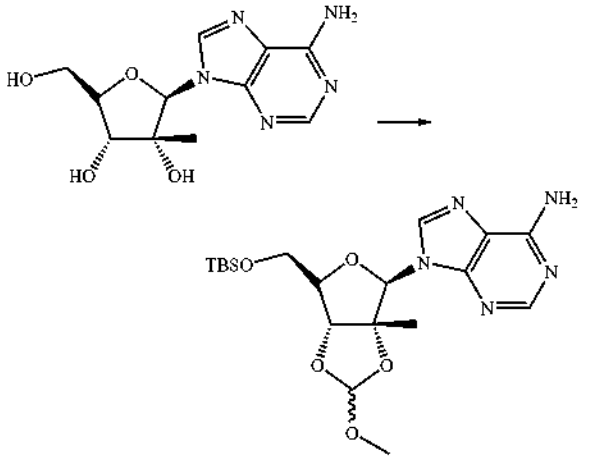
[0428]



(53)

Step 1. Preparation of 5'-O-(t-butyldimethylsilyl)-2',3'-O-(methoxymethylene)-2'-C-methyladenosine

[0429]

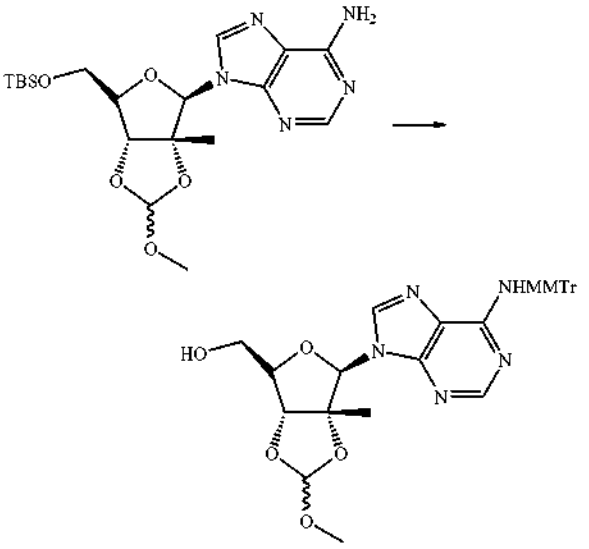


[0430] To a solution of dried 2'-C-methyladenosine (720 mg, 2.56 mmol) and trimethyl orthoformate (7.22 mL) in anhydrous 1,4-dioxane (9 mL) was added p-toluenesulfonic acid (374 mg), and stirred at room temperature under nitrogen atmosphere overnight. The reaction mixture was neutralized with methanol-ammonia (7N) to pH of 5-6 and concentrated into a crude residue, which was re-dissolved with methanol-dichloromethane (2:1, 10 mL) and stirred at room temperature overnight. The above reaction mixture was then concentrated into a crude residue, which was applied to a short column of silica gel eluted with dichloromethane-methanol (10:1) to give a pure compound 2',3'-O-(methoxymethylene)-2'-C-methyladenosine as amorphous solid (720 mg, 87%). Two-isomer: ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 8.36 (s, 1H), 8.20 (s, 1H), 8.15 (s, 1H), 7.31 (s, 2H, NH_2), 6.41 (s, 1H), 6.22 (s, 0.3H), 6.15 (s, 0.3H), 5.40-5.37 (m, 1.05H, H-1'), 4.64 (d, 0.3H), 4.59 (d, 0.93H, J=4.10 Hz), 4.28 (dd, 0.93H), 4.20 (dd, 0.3H), 3.80-3.76 (dt, 1.1H), 3.72-3.67 (dt, 1.08H), 3.39 (s, 2.7H, OCH_3), 3.23 (s, 0.6H), 1.14 (s, 0.4H, 2'-CH_3), 1.03 (s, 2.84H, 2'-CH_3).

[0431] To a solution of dried 2',3'-O-(methoxymethylene)-2'-C-methyladenosine (720 mg, 2.22 mmol) and imidazole (348 mg, 5.12 mmol) in anhydrous DMF (5 mL) was added tert-butyldimethylsilylchloride (579 mg, 3.84 mmol), and stirred at room temperature under nitrogen atmosphere overnight. The reaction mixture was then concentrated into a crude residue, and co-evaporated with toluene. The above crude residue was applied to a short column of silica gel eluted with dichloromethane-methanol (10:1) to give a pure 5'-O-(t-butyldimethylsilyl)-2',3'-O-(methoxymethylene)-2'-C-methyladenosine as amorphous solid (1.09 g, 100%).

Step 2. Preparation of 2',3'-O-(methoxymethylene)-N^6-(4-methoxytrityl)-2'-C-methyladenosine

[0432]



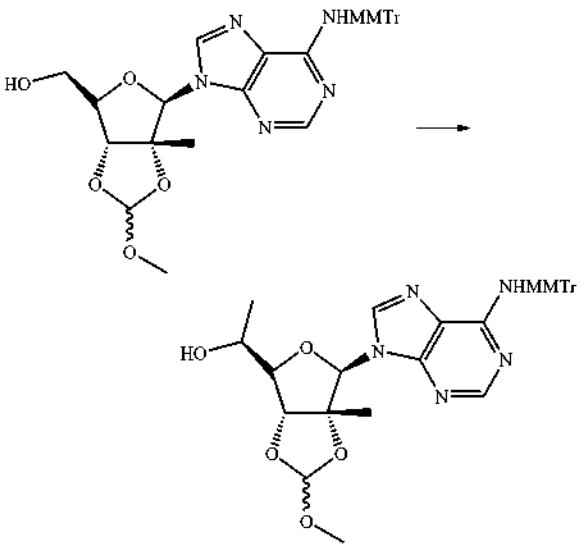
[0433] To a solution of dried 5'-O-(t-butyldimethylsilyl)-2',3'-O-(methoxymethylene)-2'-C-methyladenosine (1.09 g, 2.49 mmol), triethylamine (703 μL), and DMAP (290 mg) in anhydrous dichloromethane (5 mL) was added MMTrCl (1.15 g, 3.74 mmol), and stirred at 45-50° C. under nitrogen

atmosphere overnight. Another portion of MMTrCl (1.15 g) was added after stiffing at 45-50° C. for 16 h, and continued to be stirred at the same temperature for total of 48 h. The reaction mixture was then concentrated into a crude residue, and co-evaporated with toluene. The above crude residue was applied to a short column of silica gel eluted with hexanes-ethyl acetate (4:1) to give a pure 5'-O-(t-butyldimethylsilyl)-2',3'-O-(methoxymethylene)-N^6-(4-methoxytrityl)-2'-C-methyladenosine as amorphous solid (1.10 g, 62%).

[0434] To a solution of dried 5'-O-(t-butyldimethylsilyl)-2',3'-O-(methoxymethylene)-N^6-(4-methoxytrityl)-2'-C-methyladenosine (1.1 g, 1.55 mmol) in tetrahydrofuran (THF) (6 mL) was added tetrabutylammonium fluoride hydrate (349 mg), and stirred at room temperature under nitrogen atmosphere overnight. The reaction mixture was then concentrated into a crude residue, which was applied to a short column of silica gel eluted with dichloromethane-methanol (10:1) to give a pure 2',3'-O-(methoxymethylene)-N^6-(4-methoxytrityl)-2'-C-methyladenosine as an amorphous solid (610 mg, 66%).

Step 3. Preparation of 2',5'-C-dimethyl-2',3'-O-(methoxymethylene)-N^6-(4-methoxytrityl)adenosine

[0435]

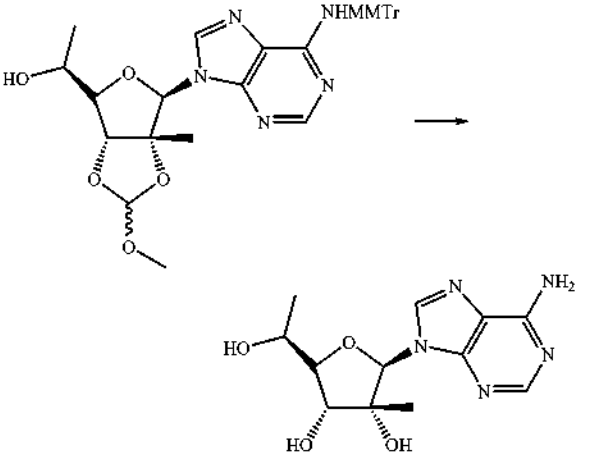


[0436] To a solution of dried 2',3'-O-(methoxymethylene)-N^6-(4-methoxytrityl)-2'-C-methyladenosine (610 mg, 1.02 mmol) in a mixture of anhydrous dichloromethane (15 mL) and anhydrous pyridine (1.02 mL) was added Dess-Martin periodinane (647 mg, 1.53 mmol), and stirred at room temperature under nitrogen atmosphere for 2 h. The reaction mixture was quenched with a mixture of sat. sodium bicarbonate aq. solution and 10% $Na_2S_2O_3$ aq. The organic phase was separated and the aqueous phase was extracted with dichloromethane (3×20 mL). The combined organic phase was dried with anhydrous sodium sulfate and concentrated into a crude residue, which was applied to a short column of silica gel eluted with hexanes-ethyl acetate (1:1 and 1:2), then dichloromethane-methanol (10:1) to give a pure 5'-C,5'-O-didehysro-2',3'-O-(methoxymethylene)-N^6-(4-methoxytrityl)-methyladenosine as an amorphous solid (200 mg, 33%). Two-isomer: ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 9.5 (s, 1H, CH═O), 9.49 (s, 1H, CH═O), 3.79 (s, 20CH_3), 3.39 (s, 2.7H, OCH_3), 1.14 (s, 0.4H, 2'-CH_3), 1.03 (s, 2.84H, 2'-CH_3).

[0437] To a cold solution of dried 5'-C,5'-O-didehysro-2',3'-O-(methoxymethylene)-N^6-(4-methoxytrityl)-2'-C-methyladenosine (350 mg, 0.59 mmol) in anhydrous tetrahydrofuran (3-5 mL) cooled with an ice-sodium chloride bath to −20° C. was added methylmagnesium bromide (0.80 mL, 3.0 M in ether) and stirred at −20 to RT overnight under nitrogen. The reaction mixture was then quenched with sat ammonium chloride and concentrated to removal of tetrahydrofuran, and extracted with ethyl acetate (3×20 mL). The combined organic phase was concentrated and co-evaporated with toluene into a crude residue. The above crude residue was applied to a short column of silica gel eluted with hexanes-ethyl acetate (1:2) to give a pure 2',5'-C-dimethyl-2',3'-O-(methoxymethylene)-N^6-(4-methoxytrityl)adenosine as amorphous solid (170 mg, 47%).

Step 4. Preparation of 2',5'-C-dimethyladenosine

[0438]

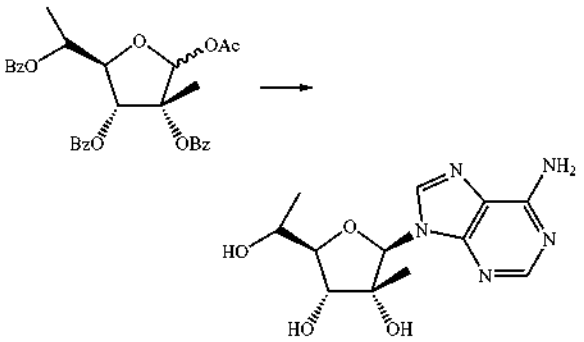


[0439] A solution of 2',5'-C-dimethyl-2',3'-O-(methoxymethylene)-N^6-(4-methoxytrityl)adenosine (110 mg, 0.181 mmol) in a mixture of methanol (6 mL), acetic acid (3 mL), and water (1 mL) was stirred at 50° C. for 16 h, The reaction mixture was then concentrated and co-evaporated with toluene into a crude residue, which was applied to a short column of silica gel eluted with dichloromethane-methanol (10:1 and 6:1) to give a pure 2',5'-C-dimethyladenosine (40 mg, 75%) as amorphous solid. Two-isomer A and B, ratio of A vs B is 1.77: ^{1}H-NMR (CD_3OD, 500 MHz): δ 8.57 (s, 1H, isomer-A), 8.55 (s, 1H, isomer-B), B), 8.20 (s, 1H, isomer-A), 8.19 (s, 1H, isomer-B), 6.09 (s, 1H, H'-1, isomer-A), 6.07 (s, 1H, H'-1, isomer-B), 4.58 (s, 0.8H), 4.30-4.28 (m, 4H), 4.19 (d, 1H), 4.08-4.07 (dq, 1H), 3.97 (dd, 1H), 3.88 (dd, 1H), 1.36 (d, 3H, 5'-CH_3, isomer-A, J=6.6 Hz), 1.33 (d, 3H, 5'-CH_3, isomer-B, J=6.9 Hz), 0.90 (s, 3H, 2'-CH_3, isomer-A), 0.89 (s, 3H, 2'-CH_3, isomer-B). ESI-MS (positive mode): 295 [M], 318 [M+Na].

Example 54

Preparation of 2',5'(S)—Cdimethyladenosine (54)

[0440]



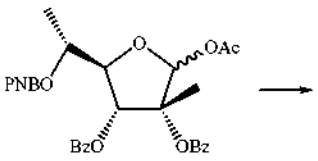
[0441] A mixture of N^6-benzoyladenosine (1.46 g, 6.12 mmol) and N,O-bis(trimethylsilyl)acetamide (2.95 mL, 12.24 mmol) in anhydrous acetonitrile (15 mL) under argon was heated under reflux for 45 min and cooled to rt. A solution of 1-O-acetyl-5(S)—C-methyl-5-O-(4-nitrobenzoyl)-2,3,5-O-tribenzoyl-D-ribofuranose (1.63 g, 3.06 mmol) in anhydrous acetonitrile (15 mL) was added, followed by addition of trimethylsilyl trifluoromethanesulfonate (0.86 mL, 4.59 mmol). The resulting mixture was heated under reflux overnight, cooled with ice, diluted with ethyl acetate, washed with aqueous sodium bicarbonate, dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel with 10-15% ethyl acetate in DCM gave 1.60 g of 5'(S)—C-methyl-2',3',5'-O-tribenzoyladenosine.

[0442] 5'(S)—C-Methyl-2',3',5'-O-tribenzoyladenosine (1.58 g) in methanol (150 mL) and 28% aqueous ammonia (50 mL) was stirred at RT overnight. The solvent was removed and the residue was re-dissolved in 28% aqueous NH_3 (130 mL). The mixture was stirred at rt for 3 days and the solvent was removed. Chromatography on silica gel with 12-14% MeOH in DCM gave 619 mg of 2',5'(S)—C-dimethyladenosine as a white solid; ^1H NMR (CD_3OD): δ 8.55 (s, 1H), 8.19 (s, 1H), 6.07 (s, 1H), 4.29 (d, J=8.4 Hz, 1H), 4.26 (m, 1H), 3.97 (dd, J_1=8.4 Hz, J_2=2.4 Hz,1H), 1.33 (d, J=6.8 Hz, 3H), 0.89 (s, 3H).

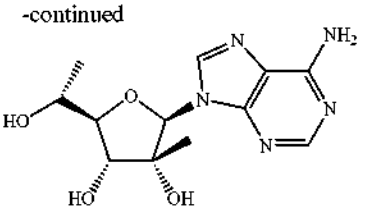
Example 55

Preparation of 2',5'(R)—C-dimethyladenosine (55)

[0443]



-continued



[0444] A mixture of N^6-benzoyladenosine (1.75 g, 7.3 mmol) and N,O-bis(trimethylsilyl)acetamide (3.6 mL, 14.6 mmol) in anhydrous acetonitrile (18 mL) under argon was heated under reflux for 45 min and cooled to rt. A solution of 1-O-acetyl-5(S)—C-methyl-5-O-(4-nitrobenzoyl)-2,3,5-O-tribenzoyl-D-ribofuranose (2.11 g, 3.65 mmol) in anhydrous acetonitrile (18 mL) was added, followed by addition of trimethylsilyl trifluoromethanesulfonate (1.02 mL, 5.48 mmol). The resulting mixture was heated under reflux overnight, cooled with ice, diluted with ethyl acetate, washed with aqueous sodium bicarbonate, dried over anhydrous Na_2SO_4 and concentrated. Chromatography on silica gel with 10-15% ethyl acetate in DCM gave 1.81 g of 5'(R)—C-methyl-5'-O-(4-nitrobenzoyl)-2',3',5'-O-tribenzoyladenosine.

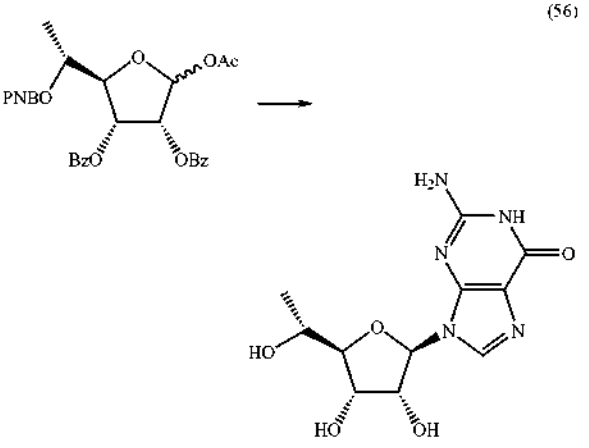
[0445] 5'(R)—C-Methyl-5'-O-(4-nitrobenzoyl)-2',3',5' -O-tribenzoyladenosine (2.04 g) in methanol (200 mL) and 28% aqueous ammonia (65 mL) was stirred at RT overnight. The solvent was removed and the residue was re-dissolved in 28% aqueous NH_3 (240 mL). The mixture was stirred at rt for 2 days and the solvent was removed. Precipitate was washed with 20% MeOH in DCM and then with MeOH to give 402 mg of 2',5'(R)—C-dimethyladenosine as white solid. Chromatography on silica gel with 10-14% MeOH in DCM gave 397 mg of 2',5'(R)—C-dimethyladenosine as a white solid. Total yield was 779 mg. ^1H NMR (CD_3OD): δ 8.59 (s, 1H), 8.19 (s, 1H), 6.09 (s, 1H), 4.19 (d, J=8.8 Hz, 1H), 4.06 (dq, 1H), 3.88 (dd, J_1=8.8 Hz, J_2=2.4 Hz,1H), 1.36 (d, J=6.8 Hz, 3H), 0.90 (s, 3H).

Example 56

Preparation of 5'(R)—C-methylguanosine (56)

[0446]

(56)



[0447] To a solution of 1-O-acetyl-2,3-O-dibenzoyl-5(R)—C-methyl-5-O-(4-nitrobenzoyl)-D-ribofuranose (969 mg, 1.72 mmol), 2-amino-6-chloropurine (0,32 g, 1.87 mmol) and DBU (0.77 mL, 5.10 mmol) in anhydrous acetonitrile (20 mL) was added dropwise trimethylsilyl trifluoromethanesulfonate (1.25 mL, 6.88 mmol). The resulting reaction mixture was stirred at 65° C. overnight, cooled, diluted with ethyl acetate, washed with 10% sodium bicarbonate and dried over sodium sulfate. Chromatography on silica gel with 10-15% ethyl acetate in DCM gave 0.62 g of 1-(2-amino-6-chloropurin-N^9-yl)-2,3-O-dibenzoyl-5(R)—C-methyl-5-O-(4-nitrobenzoyl)-β-D-ribofuranose as a white solid, which was dissolved in 7 M NH_3 in MeOH and stood at RT overnight and concentrated. Chromatography on silica gel with 10-15% MeOH in DCM gave 260 mg of 1-(2-amino-6-chloropurin-N^9-yl)-5(R)—C-methyl-β-D-ribofuranose as a white solid.

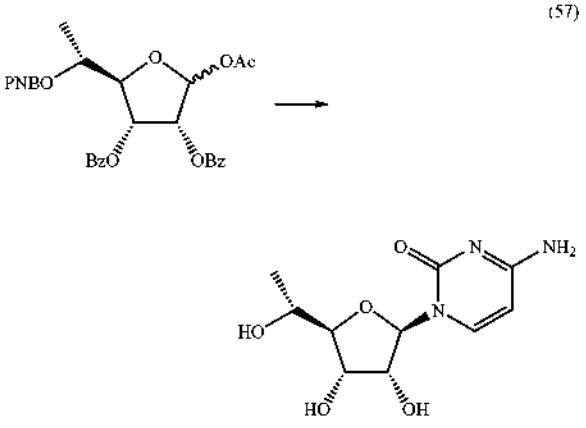
[0448] To a mixture of 1-(2-amino-6-chloropurin-N^9-yl)-5(R)—C-methyl-β-D-ribofuranose (253 mg, 0.8 mmol) and mercaptoethnaol (2.28 mL, 4.0 mmol) in MeOH (5 mL) was added 0.5 M NaOMe in MeOH (8 mL, 4.0 mmol). The resulting mixture was refluxed overnight, cooled, neutralized with AcOH. Reverse phase HPLC with acetonitrile/water gave 5'(R)—C-methylguanosine as a white solid (167 mg); ^{1}H NMR (DMSO-d_6) δ 1.08 (d, J=6.8 Hz, 3H), 3.16 (d, J=4.8 Hz, 1H), 3.7 (t, J=3.2 Hz, 1H), 3.73-3.79 (m, 1H), 4.06-4.10 (m, 2H), 4.30-4.34 (m, 1H), 5.34 (d, J=5.2 Hz, 1H), 5.68 (d, J=5.6 Hz, 1H), 6.47 (br s, 2H), 7.95 (s, 1H), 10.72 (br s, 1H).

Example 57

Preparation of 5'(R)—C-methylcytidine (57)

[0449]

(57)



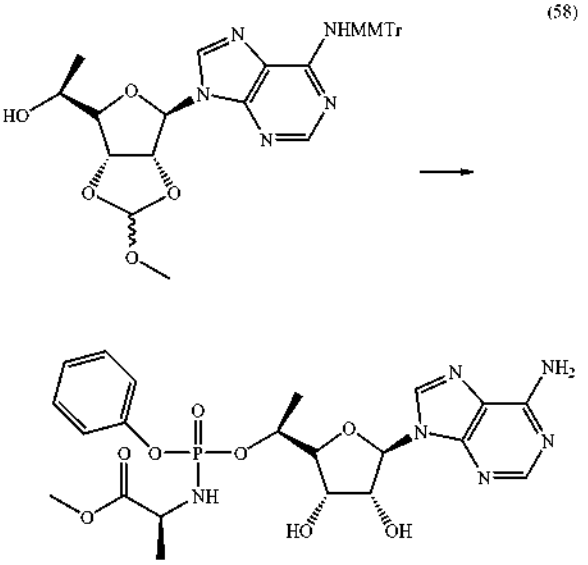
[0450] N^4-Benzoylcytosine (215 mg, 1.0 mmol) and N,O-bis(trimethylsilyl)acetonitrile (0.49 mL, 2.0 mmol) in anhydrous acetonitrile (2 mL) was refluxed for 30 min and cooled. A solution of 1-O-acetyl-2,3-O-dibenzoyl-5(R)—C-methyl-5-O-(4-nitrobenzoyl)-D-ribofuranose (289 mg, 0.5 mmol) in acetonitrile (2 mL) was added, followed by addition of tin tetrachloride (0.24 mL, 2.0 mmol). The resulting reaction mixture was refluxed overnight, cooled, diluted with ethyl acetate, washed with 10% sodium bicarbonate and dried over sodium sulfate. Chromatography on silica gel with 10-20% ethyl acetate in DCM gave 2',3'-O,N^4-tribenzoyl-5'(R)—C-methyl-5'-O-(4-nitrobenzoyl)cytidine, which was dissolved in 7.0 M NH_3/MeOH and stood at RT for 3 h. The solution was concentrated and the residue dissolved in 29% aqueous ammonia and stood at RT for 3 days. Volatile was evaporated and the residue was subjected to reverse-phase HPLC purification to give 122 mg of 5'(R)—C-methylcytidine.

Example 58

Preparation of 5'(S)—C-methyladenosine 5'-[phenyhmethoxy-L-alaninyl)]phosphate (58)

[0451]

(58)

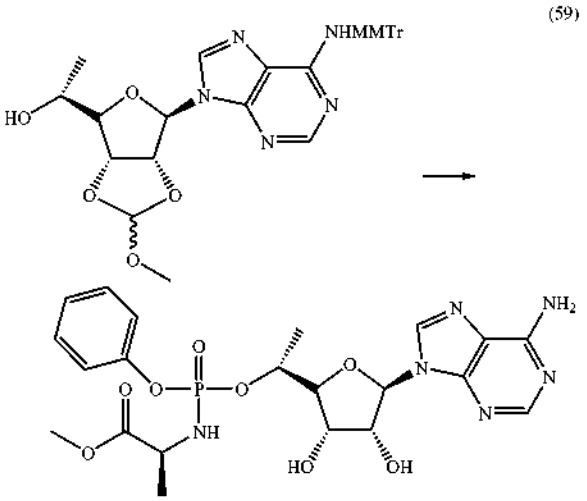


[0452] To a solution of 2',3'-O-methoxymethylene-N^6-(4-methoxytrityl)-5'(S)-methyladenosine (60 mg, 0.1 mmol) in THF (1 mL) under argon was added 1.0 M t-BuMgBr in THF (0.25 mL, 0.25 mmol). The resulting solution was stirred at RT for 30 min and phenyl(methoxy-L-alaninyl)phosphorochloridate (85 mg, 0.3 mmol). The reaction mixture was stirred at RT for 3 days, cooled with ice, quenched with water, diluted with ethyl acetate, washed with brine three times. Chromatography on silica gel with ethyl acetate/hexanes (1:1 to 2:1) gave a mixture of four isomers as a white solid. The product was dissolved in 80% formic acid (5 mL) and stood at RT overnight. Solvent was evaporated at RT and co-evaporated with MeOH/toluene three times. Chromatography on silica gel with 10-15% MeOH in DCM, followed by repurification by reverse-phase HPLC with acetonitrile/water, gave 9.5 mg of 5'(S)—C-methyladenosine 5'-[phenyl(methoxy-L-alaninyl)]phosphate as white solid; ^{1}H NMR (CD_3OD) δ 1.28 (d, J=6.8 Hz, 3H), 1.44 (d, J=6.4 Hz, 3H), 3.65 (s, 3H), 3.89-3.93 (m, 1H), 4.01-4.04 (m, 1H), 4.45-4.47 (m, 1H), 4.70 (t, J=6.0 Hz, 1H), 4.58-5.98 (d, J=6.8 Hz, 1H), 7.12-7.33 (m, 6H), 8.19 (s, 1H), 8.31 (s, 1H); ^{31}P NMR (CD_3OD) δ 3.39

Example 59

Preparation of 5'(R)—C-methyladenosine 5'-[phenylmethoxy-L-alaninyl)]phosphate (59)

[0453]

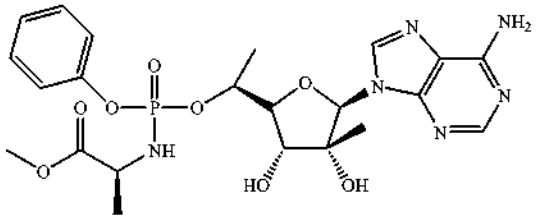


[0454] To a solution of 2',3'-O-methoxymethylene-N^6-(4-methoxytrityl)-5'(R)-methyladenosine (60 mg, 0.1 mmol) in THF (1 mL) under argon was added 1.0 M t-BuMgBr in THF (0.25 mL, 0.25 mmol). The resulting solution was stirred at RT for 30 min and phenyl(methoxy-L-alaninyl)phosphorochloridate (85 mg, 0.3 mmol) was added. The reaction mixture was stirred at RT for 3 days, cooled with ice, quenched with water, diluted with ethyl acetate, washed with brine three times. Chromatography on silica gel with ethyl acetate/hexanes (1:1 to 2:1) gave a mixture of four isomers as a white solid. The product was dissolved in 80% formic acid (5 mL) and stood at RT overnight. Solvent was evaporated at RT and co-evaporated with MeOH/toluene three times. Chromatography on silica gel with 10-15% MeOH in DCM, followed by re-purification by reverse-phase HPLC with acetonitrile/water, gave 12 mg of 5'(R)—C-methyladenosine 5'-[phenyl (methoxy-L-alaninyl)]phosphate as white solid; ^{1}H NMR (CD_3OD) δ 1.24 (d, J=6.8 Hz, 3H), 1.52 (d, J=6.4 Hz, 3H), 3.66 (s, 3H), 3.91-3.97 (m, 1H), 4.05-4.08 (m, 1H), 4.35 (t, J=4.4 Hz, 1H), 4.52 (t, J=4.8 Hz, 1H), 4.82-4.85 (m, 1H), 6.04 (d, J=5.6 Hz, 1H), 7.10-7.31 (m, 6H), 8.2 (s, 1H), 8.29 (s, 1H); ^{31}P NMR (CD_3OD) δ 3.72.

Example 60

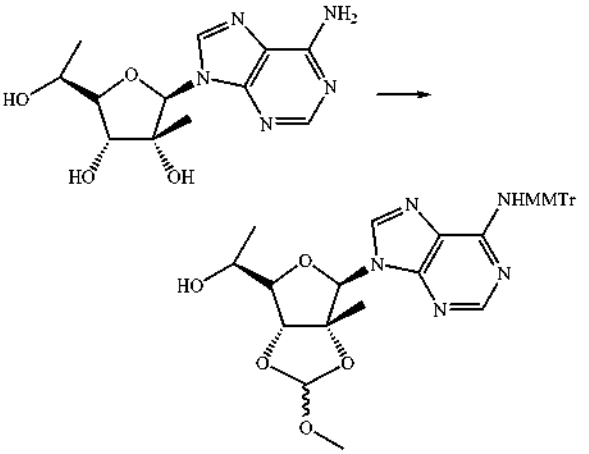
Preparation of 2',5'(S)—C-dimethyladenosine 5'-[phenyl(methoxy-L-alaninyl)]phosphate (60)

[0455]



Step 1. Preparation of 2',3'-O-methoxymethylidene-N^6-(4'-methoxytrityl)-5'(S)-methyladenosine

[0456]

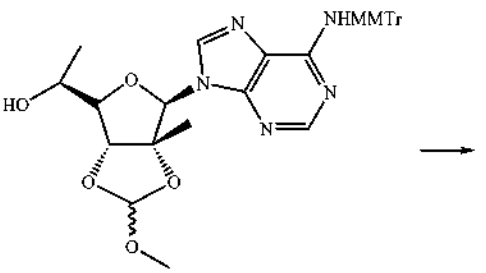


[0457] A mixture of 2,5'(S)—C-dimethyladenosine (585 mg, 1.98 mmol), trimethyl orthoformate (5.6 mL) and p-toluenesulfonic acid monohydrate (565 mg, 2.97 mmol) in 1,4-dioxane (7 mL) was stirred at 30° C. for 24 h, cooled with ice and quenched by adding triethylamine (1 mL) and concentrated. Chromatography on silica gel with 5-7% MeOH in DCM gave 716 mg of 2',3'-O-methoxymethylidene-2,5'(S)—C-dimethyladenosine.

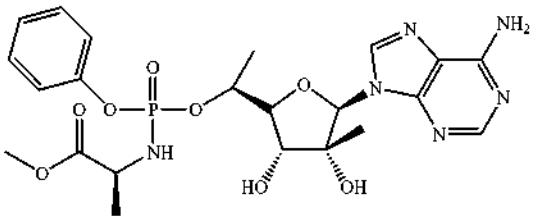
[0458] A solution of 2',3'-O-methoxymethylidene-2,5'(S)—C-dimethyladenosine (575 mg, 1.71 mmol) and 4-methoxytrityl chloride (714 mg, 2.32 mmol) in pyridine (16 mL) was stirred at rt for 3 days. Additional 4-methoxytrityl chloride (72 mg) was added and the mixture was heated at 40° C. for 24 h. Additional 144 mg of 4-methoxytrityl was added the mixture was heated at 50° C. for 24 h, diluted with ethyl acetate, washed with brine three times. Solvent was evaporated and the residue was chromatographed on silica gel with 25-60% ethyl acetate in hexanes to give 151 mg of 5'-O,N^6-di(4'-methoxytrityl)-2',3'-O-methoxymethylidene-5'(S)-methyladenosine and 489 mg of 2',3'-O-methoxymethylidene-N^6-(4'-methoxytrityl)-5'(S)-methyladenosine as amophous solid.

Step 2. Preparation of 2',5'(S)—C-dimethyladenosine 5'-[phenyl(methoxy-L-alaninyl)]phosphate

[0459]



-continued

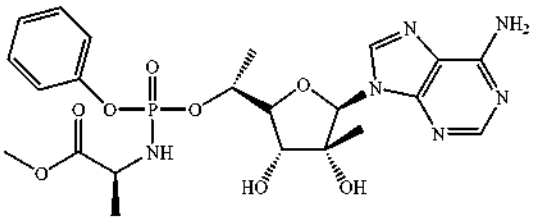


[0460] To a solution of 2',5'(S)—C-dimethyl-2',3'-O-methyomethylene-N^6-(4-methoxytrityl)adenosine (60 mmg, 0.1 mmol) in THF (1 mL) under argon was added 1.0 M t-BuMgBr in THF (0.25 mL, 0.25 mmol). The resulting solution was stirred at RT for 30 min and phenyl(methoxy-L-alaninyl)phosphorochloridate (85 mg, 0.3 mmol). The reaction mixture was stirred at RT for 3 days, cooled with ice, quenched with water, diluted with ethyl acetate, washed with brine three times. Chromatography on silica gel with ethyl acetate/hexanes (1:1 to 2:1) gave a mixture of four isomers as a white solid. The product was dissolved in 80% formic acid (5 mL) and stood at RT overnight. Solvent was evaporated at RT and co-evaportaed with MeOH/toluene three times. Chromatography on silica gel with 10-15% MeOH in DCM, followed by re-purification by reverse-phase HPLC with acetonitrile/water, gave 6.8 mg of 2',5'(S)—C-dimethyladenosine 5'-[phenyl(methoxy-L-alaninyl)]phosphate as white solid; ^{1}H NMR (CD_3OD) δ 0.95 (d, J=4.4 Hz, 3H), 1.21 (dd, J=1.2, 7.2 Hz, 1H), 1.30 (dd, J=0.8, 7.2 Hz, 1H), 1.55 (dd, J=1.6, 6.8 Hz, 1H), 2.32 (s, 1H), 3.58 (s, 1H), 3.64 (s, 2H), 3.82-3.99 (m, 1H), 4.07-4.11 (m, 1H), 4.27 & 4.36 (each d, J=8.8, 8.4 Hz, 1H), 4.99-5.05 (m, 1H), 6.10 & 6.13 (2×s, 1H), 7.1-7.39 (m, 7H), 8.18 & 8.19 (2×s, 1H), 8.29 & 8.31 (2×s, 1H); ^{31}P NMR (CD_3OD) δ 3.59, 3.74.

Example 61

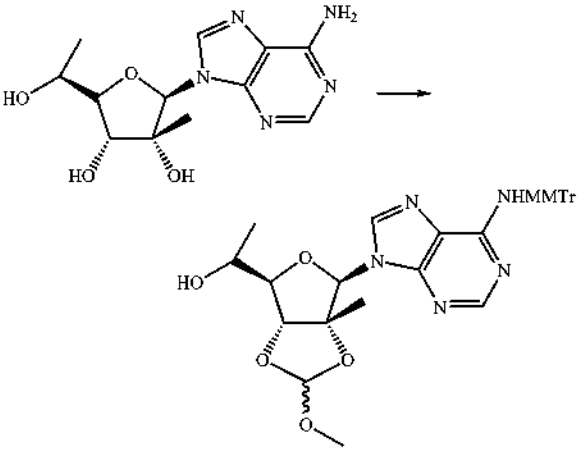
Preparation of 2',5'(R)—C-dimethyladenosine 5'-[phenyhmethoxy-L-alaninyl)]phosphate (61)

[0461]



Step 1. Preparation of 2',3'-O-methoxymethylidene-N^6-(4'-methoxytrityl)-5'(R)-methyladenosine

[0462]

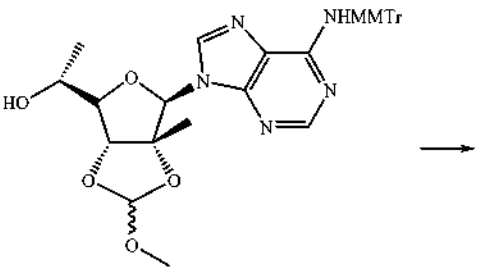


[0463] A mixture of 2,5'(R)—C-dimethyladenosine (395 mg, 1.34 mmol), trimethyl orthoformate (3.8 mL) and p-toluenesulfonic acid monohydrate (382 mg, 2.01 mmol) in 1,4-dioxane (4.8 mL) was stirred at 30° C. for 24 h, cooled with ice and quenched by adding triethylamine (1 mL) and concentrated. Chromatography on silica gel with 5-7% MeOH in DCM gave 360 mg of 2',3'-O-methoxymethylidene-2,5'(R)—C-dimethyladenosine.

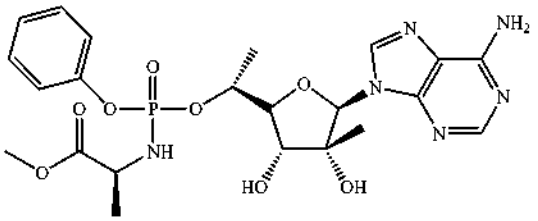
[0464] A solution of 2',3'-O-methoxymethylidene-2,5'(R)—C-dimethyladenosine (357 mg, 1.06 mmol) and 4-methoxytrityl chloride (444 mg, 1.44 mmol) in pyridine (10 mL) was stirred at rt for 3 days. Additional 222 mg of 4-methoxytrityl was added the mixture was heated at 50° C. for 24 h, diluted with ethyl acetate, washed with brine three times. Solvent was evaporated and the residue was chromatographed on silica gel with 25-60% ethyl acetate in hexanes to give 142 mg of 5'-O,N^6-di(4'-methoxytrityl)-2',3'-O-methoxymethylidene-5'(R)-methyladenosine and 301 mg of 2',3'-O-methoxymethylidene-N^6-(4'-methoxytrityl)-5'(R)-methyladenosine as amophous solid.

Step 2. Preparation of 2',5'(R)—C-dimethyladenosine 5'-[phenyl(methoxy-L-alaninyl)]phosphate

[0465]



-continued

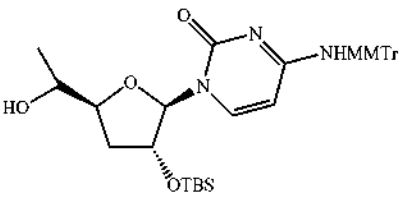


[0466] To a solution of 2',5'(R)—C-dimethyl-2',3'-O-methyomethylene-N^6-(4-methoxytrityl)adenosine (61 mg, 0.1 mmol) in THF (1 mL) under argon was added 1.0 M t-BuMgBr in THF (0.25 mL, 0.25 mmol). The resulting solution was stirred at RT for 30 min and phenyl(methoxy-L-alaninyl)phosphorochloridate (85 mg, 0.3 mmol). The reaction mixture was stirred at RT for 3 days, cooled with ice, quenched with water, diluted with ethyl acetate, washed with brine three times. Chromatography on silica gel with ethyl acetate/hexanes (1:1 to 2:1) gave a mixture of four isomers as a white solid. The product was dissolved in 80% formic acid (5 mL) and stood at RT overnight. Solvent was evaporated at RT and co-evaportaed with MeOH/toluene three times. Reverse-phase HPLC with acetonitrile/water gave 16.1 mg of 2',5'(R)—C-dimethyladenosine 5'-[phenyl(methoxy-L-alaninyl)]phosphate as white solid. Isomer A: ^{1}H NMR (CD_3OD) δ 0.89 (s, 3H), 1.27 (dd, J=1.2, 7.2 Hz, 3H), 1.61 (d, J=6.4, 3H), 2.32 (s, 2H), 3.96-4.02 (m, 2H), 4.09 (d, J=9.2 Hz, 1H), 4.96-5.00 (m, 1H), 3.65 (s, 3H), 3.94-4.02 (m, 2H), 4.09 (d, J=9.2 Hz, 1H), 4.96 (m, 1H), 7.09-7.32 (m, 7H), 8.20 (s, 1H), 8.25 (s, 1H); ^{31}P NMR (CD_3OD) δ 3.73; Isomer B: ^{1}H NMR (CD_3OD) δ 0.94 & 0.98 (each s, 3H), 1.25 (d, J=10.4 Hz, 3H), 1.50 (d, J=6.8, 3H), 2.32 (s, 1H), 3.55 (s, 3H), 3.92-4.01 (m, 2H), 4.26 (d, J=9.2 Hz, 1H), 6.09 (s, 1H), 7.08-7.36 (m, 7H), 8.21 (s, 1H), 8.2 (s, 1H); ^{31}P NMR (CD_3OD) δ 3.61, 3.70.

Example 62

Preparation of 2'-O-(t-butyldimethysilyl)-3'-deoxy-5'(R,S)-O-methyl-N^4-(4-methoxytrityl)cytidine (62)

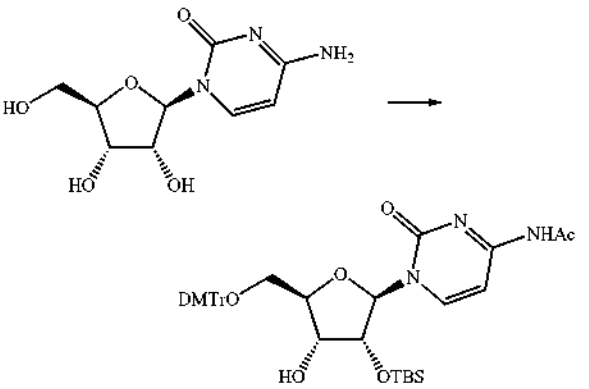
[0467]



(62)

Step 1. Preparation of N^4-acetyl-2'-O-(t-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)cytidine

[0468]

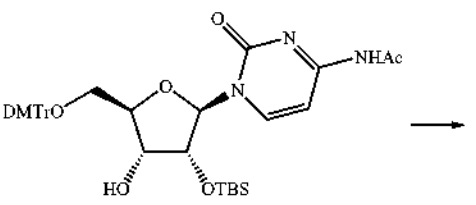


[0469] Cytidine (100.0 g, 0.41 mol) was dissolved in DMF (500 ml), acetic anhydride (42.5 ml, 45.9 g, 0.45 mol) was added and the whole was left for 24 h. Solvent was evaporated, the residue boiled with methanol (40 ml) and cooled. Crystals were filtered and dried to furnish N^4-acetylcytidine (102 g, 87.0%).

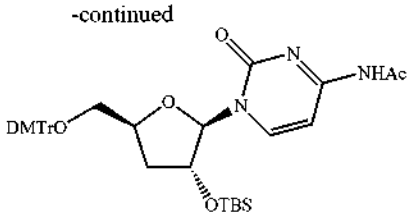
[0470] To a solution of N^4-acetylcytidine (65.0 g, 0.228 mol) in anhydrous pyridine (600 mL) cooled in an ice bath, DMTrCl (84.7 g, 0.251 mol) was added. The reaction mixture was stirred at room temperature overnight. To the reaction mixture cooled with an ice bath, THF (600 ml) and $AgNO_3$ (58.1 g, 0.342 mmol) were added. Then TBSCl (51.5 g, 0.342 mmol) was added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was filtered, solvent was removed under vacuum to give a residue which was diluted with EtOAc (500 mL) and washed with water (200 ml) and brine (200 ml). The organic layer was separated and dried over anhydrous Na_2SO_4 and the filtrate was concentrated to a syrup which was purified by chromatography on silica gel (eluted with PE:EA=5:1 to 3:1) to give N^4-acetyl-2'-O-(t-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)cytidine solid (80 g, 50%). ^{1}H NMR ($CDCl_3$) δ 8.39 (d, J=7.6 Hz, 1H), 7.34 (dd, J1=1.6 Hz, J2=8.4 Hz, 2H), 7.21-7.27 (m, 6H), 7.02 (d, J=7.2 Hz, 1H), 6.80 (dd, J1=2.0 Hz, J2=6.8 Hz, 4H), 5.82 (d, J=1.2 Hz, 1H), 4.26-4.32 (m, 1H), 4.20 (dd, J1=1.2 Hz, J2=4.4 Hz, 1H), 4.00-4.02 (m, 1H), 3.74 (d, J=1.6 Hz, 6H), 3.43-3.53 (m, 2H), 2.32 (d, J=9.6 Hz, 1H), 2.18 (s, 3H), 0.86 (s, 9H), 0.22 (s, 3H), 0.11 (s, 3H).

Step 2. Preparation of N^4-acetyl-2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)cytidine

[0471]



-continued

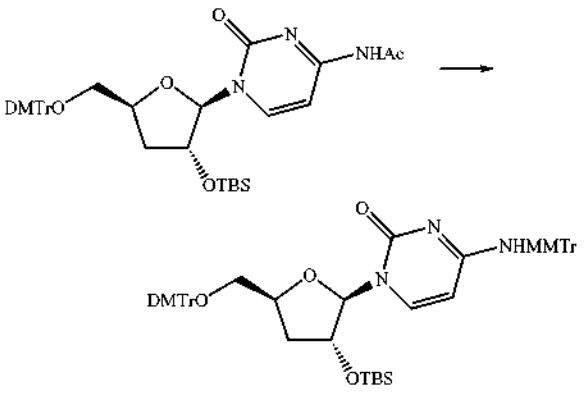


[0472] N^4-Acetyl-2'-O-(t-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)cytidine (50.0 g, 71.3 mmol) and DMAP (26.1 g, 213.9 mmol) was dissolved in ACN (2000 ml), and PTCCl (18.5 g, 106.9 mmol) was added dropwise under nitrogen atmosphere at room temperature, then the reaction mixture was stirred at room temperature overnight. Then solvent was removed under vacuum to give a residue which was diluted with EtOAc (500 mL) and washed with water (200 ml) and brine (200 ml). The organic layer was separated and dried over anhydrous Na_2SO_4 and the filtrate was concentrated to a syrup which was purified by chromatography on silica gel (eluted with PE:EA=5:1 to 3:1) to give N^4-Acetyl-2'-O-(t-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-3'-O-(phenoxythiono)cytidine as yellow solid (27.0 g, 45.2%).

[0473] To a solution of N^4-acetyl-2'-O-(t-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-3'-O-(phenoxythiono)cytidine (24.0 g, 28.7 mmol) and AIBN (5.1 g, 31.6 mmol) in anhydrous toluene (1000 ml), $(Bu)_3SnH$ (16.7 g, 57.3 mmol) was added dropwise under nitrogen atmosphere at room temperature, then the reaction mixture was refluxed at 120° C. for 10 h. The solvent was removed under vacuum to give a residue which was diluted with EtOAc (500 mL) and washed with water (200 ml) and brine (200 ml). The organic layer was separated and dried over anhydrous Na_2SO_4 and the filtrate was concentrated to a syrup which was purified by silica gel chromatography (eluted with PE:EA=8:1 to 5:1) to give N^4-acetyl-2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)cytidine as yellow solid (16.0 g, 81.6%).

Step 3. Preparation of 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-N^4-(4-methoxytrityl)cytidine

[0474]

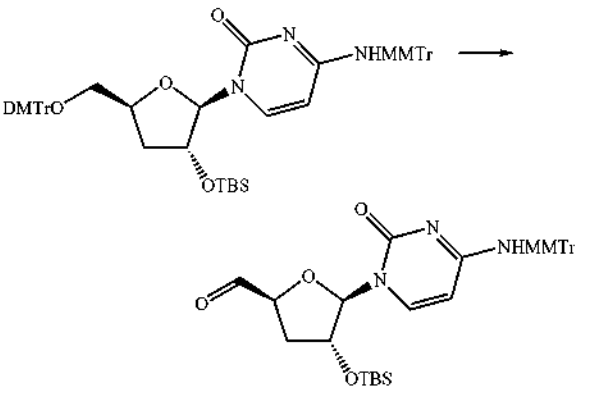


[0475] A solution of N^4-acetyl-2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)cytidine (11.0 g, 16.0 mmol) in NH_3/MeOH (300 ml) was stirred at room temperature overnight. The solvent was removed under vacuum to give a residue which was purified by silica gel chromatography (eluted with PE:EA=1:1 to 1:3) to give 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'-O-(4,4'-dimethoxytrityl) cytidine as yellow solid (6.0 g, 58.2%). 1HNMR (400 MHz) ($CDCl_3$) δ 8.09 (d, J=7.2 Hz, 1H), 7.35 (d, J=8.8 Hz, 2H), 7.21-7.26 (m, 7H), 6.77 (dd, J_1=1.2 Hz, J_2=8.8 Hz, 4H), 5.70 (s, 1H), 5.18 (d, J=7.2 Hz, 1H), 4.50-4.51 (m, 1H), 4.33 (d, J=3.6 Hz, 1H), 3.72 (s, 6H), 3.55 (dd, J1=2.0 Hz, J2=11.2 Hz, 1H), 3.26 (dd, J1 =3.6 Hz, J2 =10.8 Hz, 1H), 1.97 (s, 1H), 1.94 (s, 1H), 1.63 (dd, J1=4.4 Hz, J2=12.4 Hz, 1H), 0.81 (s, 9H), 0.14 (s, 3H), 0.04 (s, 3H).

[0476] To a solution of 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)cytidine (6.0 g, 9.3 mmol), $AgNO_3$ (4.7 g, 28.0 mmol) and MMTrCl (8.6 g, 28.0 mmol) in anhydrous DCM (150 ml), collidine (16.9 g, 139.5 mmol) was added dropwise under nitrogen atmosphere at room temperature. Then the reaction mixture was refluxed at 50° C. for 12 h. The reaction mixture was filtered, solvent was removed under vacuum to give a residue which was purified by silica gel chromatography (eluted with PE:EA=5:1 to 3:1) to give 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-N^4-(4-methoxytrityl)cytidine as yellow solid (8.0 g, 93.7%). 1HNMR (400 MHz) ($CDCl_3$):δ7.84 (dd, J=2.8 Hz, 7.6 Hz, 1H), 6.62-7.20 (m, 27H), 5.20 (d, J=4.8 Hz, 1H), 4.57 (dd, J_1=7.6 Hz, J_2=12.0 Hz, 1H), 4.40 (d, J=8.8 Hz, 1H), 4.28 (d, J=2.8 Hz, 1H), 3.64-3.68 (m, 9H), 3.41-3.45 (m, 1H), 3.23 (ddd, J=3.2 Hz, 11.2 Hz, 1H), 1.88-1.91 (m, 1H), 1.55-1.61 (m, 1H), 1.19 (s, 9H), 0.14 (s, 3H), 0.05 (s, 3H).

Step 4. Preparation of 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'C,5'-O-didehydro-N^4-(4-methoxytrityl)cytidine

[0477]



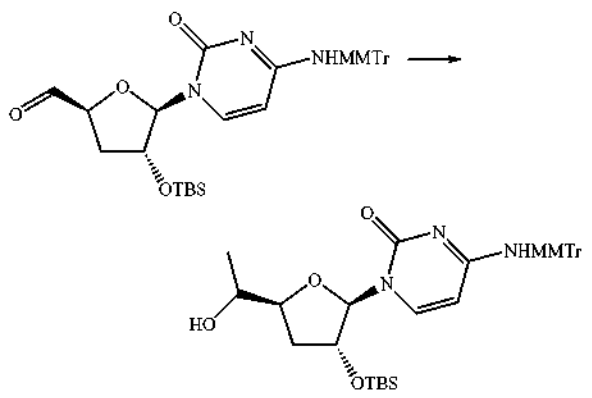
[0478] A solution of 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-N^4-(4-methoxytritylcytidine (6.0 g, 6.6 mmol) in 80% AcOH (200 mL) was stirred at room temperature for 7 h. The reaction mixture was neutralized with $NaHCO_3$ to pH=7, then was diluted with EtOAc (100 mL) and washed with water (100 ml) and brine (100 ml). The organic layer was separated and dried over anhydrous Na_2SO_4 and the filtrate was concentrated to a syrup which was purified by silica gel chromatography (eluted with PE:EA=3:1 to 2:1) to give 2'-O-(t-butyldimethylsilyl)-3'-deoxy-N^4-(4-methoxytrityl)cytidine (2.9 g, 72.5%); ^{1}HNMR

(400 MHz) ($CDCl_3$) δ 7.29 (d, J=7.6 Hz, 1H),7.15-7.25 (m, 10H), 7.12 (d, J=24 Hz, 2H), 6.75 (d, J=8.8 Hz, 2H), 5.28 (d, J=2.4 Hz, 1H), 4.99 (d, J=7.6 Hz, 1H), 4.59-4.62 (m, 1H), 4.36-4.40 (m, 1H), 3.88 (dd, J=2.0 Hz, 12.0 Hz, 1H), 3.73 (s, 3H), 3.54 (dd, J1=3.2 Hz, 12.0 Hz, 1H), 2.00-2.03 (m, 1H), 1.70-1.76 (m, 1H), 0.78 (s, 9H), 0.02 (s, 3H), 0.01 (s, 3H).

[0479] A mixture of 2'-O-(t-butyldimethylsilyl)-3'-deoxy-N^4-(4-methoxytrityl)cytidine (2.9 g, 4.7 mmol), pyridine (1.9 g, 23.8 mmol), anhydrous DCM (20 ml), and a solution of Dess-Martin reagents (3.0 g, 7.2 mmol) in anhydrous DCM (20 ml) was added dropwise under nitrogen atmosphere in an ice bath. Then the reaction mixture was stirred at room temperature overnight. The reaction mixture was filtered, and filtrate was washed with saturated $Na_2S_2O_3$ solution (20 ml). The organic layer washed with water brine (20 ml), dried over anhydrous Na_2SO_4 and the filtrate was concentrated to a syrup which was purified by silica gel chromatography (eluted with PE:EA=3:1 then PE:EA=1:1) to give 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'C,5'-O-didehydro-N^4-(4-methoxytrityl)cytidine as yellow solid (1.5 g, 51.9%); ^{1}HNMR (400 MHz) ($CDCl_3$) δ 9.68 (s, 1H), 7.35 (d, J=7.6 Hz, 1H), 7.06-7.23 (m, 11H), 6.75 (d, J=8.8 Hz, 4H), 5.57 (s, 1H), 4.99 (d, J=7.6 Hz, 1H), 4.81 (dd, J=6.4 Hz, 10.4 Hz, 1H), 4.53 (d, J=2.0 Hz, 1H), 3.73 (s, 3H), 2.05 (dd, J=2.0 Hz, 5.2 Hz, 1H), 1.71-1.78 (m, 1H), 0.82 (s, 9H), 0.11 (s, 3H), 0.05 (s, 3H).

Step 5. Preparation of 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'-C-methyl-N^4-(4-methoxytrityl)cytidine

[0480]

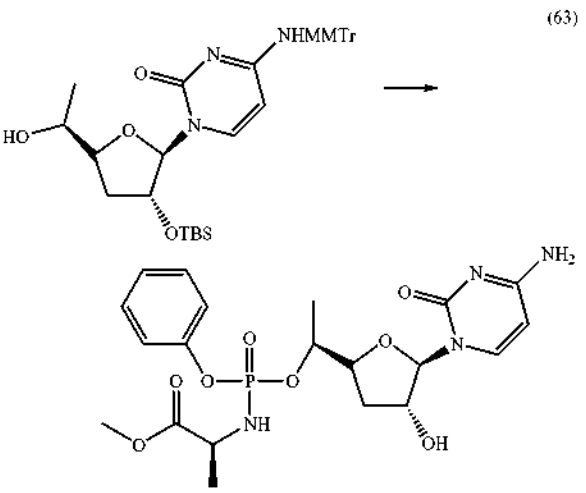


[0481] To a solution of 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'C,5'-O-didehydro-N^4-(4-methoxytrityl)cytidine (0.85 g, 4.66 mmol) in anhydrous THF (10 ml), MeMgBr (2.8 ml, 8.50 mmol) was added dropwise under nitrogen atmosphere at −20° C., then it was warmed up to room temperature and stirred overnight. The reaction mixture was slowly quenched with saturated NH_4Cl solution, and then extracted with EA (20 mL×3). The combined organic phase was dried with anhydrous Na_2SO_4 and the filtrate was concentrated to a syrup which was purified by silica gel chromatography (eluted with PE:EA=5:1 then PE:EA=3:1) to give 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'-C-methyl-N^4-(4-methoxytrityl)cytidine yellow solid (0.31 g, 35.6%), which was subjected to SFC separation to afford 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'(S)—C-methyl-N^4-(4-methoxytrityl)cytidine and 2'-O-(t-butyldimethylsilyl)-3'-deoxy-5'(R)—C-methyl-N^4-(4-methoxytrityl)cytidine. The isomer with shorter retention time in SFC was designated as 5'(S)-isomer; ^{1}H NMR (400 MHz) ($CDCl_3$) δ 7.18-7.28 (m, 10H), 7.11 (d, J_1=8.8 Hz, 2H), 6.80, (d, J=8.8 Hz, 3H), 5.15 (d, J=3.6 Hz, 1H), 4.99 (d, J=7.6 Hz, 1H), 4.75-4.79 (m, 1H), 4.17-4.20 (m, 1H), 4.05-4.07 (m, 1H), 3.88 (br, 1H), 3.78 (s, 3H), 2.18-2.25 (m, 1H), 1.72-1.78 (m, 1H), 1.09 (d, J=6.4 Hz, 1H), 0.82 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H). The isomers with longer retention time in SFC was designated as 5'(R)-isomer; ^{1}H NMR (400 MHz) ($CDCl_3$) δ 7.15-7.26 (m, 10H), 7.07 (d, J=8.8 Hz, 2H), 6.25 (d, J=8.8 Hz, 3H), 5.31 (d, J=2.4 Hz, 1H), 4.93 (d, J=6.8 Hz, 1H), 4.57-4.59 (m, 1H), 4.11-4.16 (m, 1H), 3.72 (s, 3H), 3.63-3.66 (m, 1H), 1.78-1.85 (m, 1H), 1.70-1.75 (m, 1H), 1.15 (d, J=6.4 Hz, 3H), 0.79 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H).

Example 63

Preparation of 3'-deoxy-5'(R)—C-methylcytidine 5'-[phenyhmethoxy-L-alaninyl)]phosphate (63)

[0482]

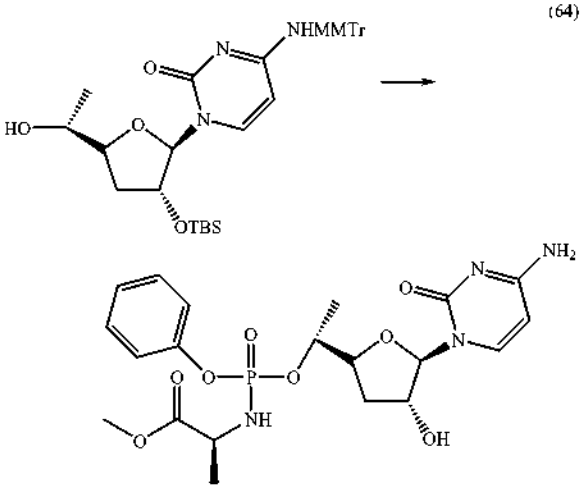


[0483] To a solution of 2'-(t-butyldimethysilyl)-3'-deoxy-5'(R)-methyl-N^4-(4-methoxytrityl)cytidine (63 mmg, 0.1 mmol) in THF (1 mL) under argon was added 1.0 M t-BuMgBr in THF (0.25 mL). The resulting solution was stirred at RT for 30 min and phenyl(methoxy-L-alaninyl) phosphorochloridate (0.34 g, 1.2 mmol) was added. The reaction mixture was stirred at RT overnight, cooled with ice, quenched with water, diluted with ethyl acetate, washed with brine three times. Chromatography on silica gel with ethyl acetate/hexanes (1:1 to 2:1) gave a mixture of four isomers as a white solid. The product was dissolved in 80% formic acid (5 mL) and stood at RT overnight. Solvent was evaporated at RT and co-evaporated with MeOH/toluene three times. Reverse-phase HPLC purification with 1% formic acid in acetonitrile/water, followed by chromatography on silica gel with 10-15% MeOH in DCM, gave 13 mg of 3'-deoxy-5'(R)—C-methylcytidine 5'-[phenyl(methoxy-L-alaninyl)] phosphate as white solid; ^{1}H NMR (DMSO-d_6) δ 1.17-1.39 (m, 7H), 1.72-1.94 (m, 3H), 2.29 (s, 1H), 3.54, 3.59 (each s, 3H), 3.82-3.91 (m, 1H), 4.12 (br s, 1H), 4.22-4.32 (m, 2H), 4.54-4.59 (m, 1H), 5.57-5.76 (m, s, 1H4H), 5.97-6.04(m, 1H), 7.14-7.46 (m, 10H), 7.72 (d, J=7.6 Hz, 1H), 8.13 (s, 1H), 12.84 (br s, 1H); ^{31}P NMR (DMSO-d_6) δ 3.9, 4.08

Example 64

Preparation of 3'-deoxy-5'(S)—C-methylcytidine 5'-[phenyhmethoxy-L-alaninyl)]phosphate (64)

[0484]



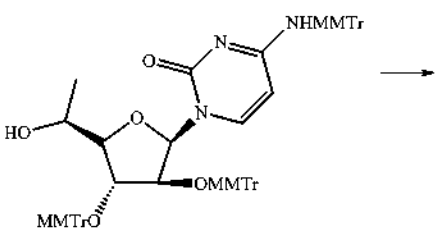
(64)

[0485] To a solution of 2'-(t-butyldimethylsilyl)-3'-deoxy-5'(S)-methyl-N^4-(4-methoxytrityl)cytidine (94 mmg, 0.1 mmol) in THF (1 mL) under argon was added 1.0 M t-BuMgBr in THF (0.38 mL). The resulting solution was stirred at RT for 30 min and phenyl(methoxy-L-alaninyl) phosphorochloridate (0.51 g, 1.8 mmol). The reaction mixture was stirred at RT overnight, cooled with ice, quenched with water, diluted with ethyl acetate, washed with brine three times. Chromatography on silica gel with ethyl acetate/hexanes (1:1 to 2:1) gave a mixture of four isomers as a white solid. The product was dissolved in 80% formic acid (5 mL) and stood at RT overnight. Solvent was evaporated at RT and co-evaportaed with MeOH/toluene three times. Reverse-phase HPLC purification with 1% formic acid in acetonitrile/water, followed by chromatography on silica gel with 10-15% MeOH in DCM, gave 12 mg of 3'-deoxy-5'(S)—C-methyl-cytidine 5'-[phenyl(methoxy-L-alaninyl)]phosphate as white solid; ^{1}H NMR (DMSO-d_6) δ 1.20 (d, J=6.0 Hz, 3H), 1.29 (d, J=7.2 Hz, 3H), 1.77-1.93 (m, 2H), 2.29 (s, 2H), 3.05 (s, 3H), 3.79-3.86 (m, 1H), 4.12-4.15 (m, 2H), 4.64-4.67 (m, 1H), 5.55 (br s, 1H), 5.62-5.76 (m, 3H), 6.06 (dd, J=10.4 Hz, 1H), 7.12-7.39 (m, 12H), 7.66 (d, J=7.2 Hz, 1H); ^{31}P NMR (DMSO-d_6) δ 3.32, 3.65.

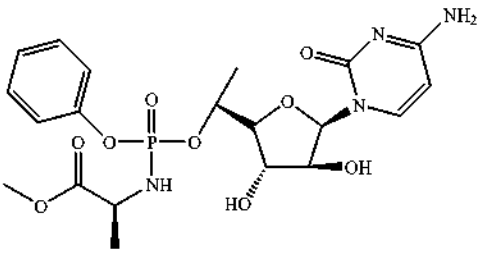
Example 65

Preparation of 5'(S)—C-methylarabinocytidine 5'-[phenyhmethoxy-L-alaninyl)]phosphate (65)

[0486]



-continued

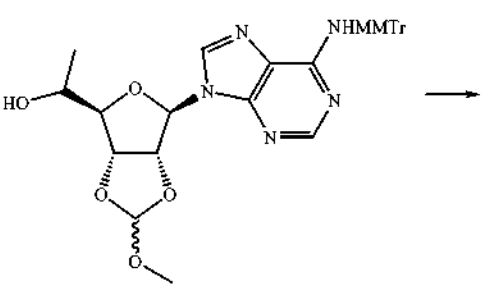


[0487] To a solution of 5'(S)-methyl-2',3'-O,N^4-tris(4-methoxytrityl)arabinocytidine (212 mg, 0.2 mmol) in THF (2 mL) under argon was added 1.0 M t-BuMgBr in THF (0.45 mL). The resulting solution was stirred at RT for 15 min and phenyl(methoxy-L-alaninyl) phosphorochloridate (0.17 g, 0.6 mmol) was added. The reaction mixture was stirred at RT for 4 days, cooled with ice, quenched with water, diluted with ethyl acetate, washed with brine three times. Chromatography on silica gel with ethyl acetate/hexanes (1:1 to 3:1) gave a mixture of four isomers as a white solid (132 mg). The product was dissolved in 80% formic acid (5 mL) and stood at 40-50° C. for 4 h. Solvent was evaporated at RT and co-evaporated with MeOH/toluene three times. Reverse-phase HPLC purification with acetonitrile/water, followed by chromatography on silica gel with 15-30% MeOH in DCM, gave 31 mg of 5'(S)—C-methylarabinocytidine 5'-[phenyl(methoxy-L-alaninyl)]phosphate as white solid. Isomer A: ^{1}H NMR (CD_3OD) δ 1.29-1.33 (m, 5H), 1.51 (d, J=6.4 Hz, 3H), 3.22 (m, 1H), 3.66 (s, 3H), 3.8-3.82 (m, 1H), 3.98-4.06 (m, 2H), 4.16-4.18 (m, 1H), 5.66 (d, J=7.6 Hz, 1H), 6.18 (d, J=3.6 Hz, 1H), 7.14-7.34 (m, 6H), 7.93 (d, J=7.2 Hz, 1H), 8.32 (br s, 1H), ^{31}P NMR (CD_3OD) δ 3.3; Isomer B: ^{1}H NMR (CD_3OD) δ 1.32-1.33 (m, 6H), 3.63 (s, 3H), 3.73-3.79 (m, 1H), 3.97-4.03 (m, 2H), 4.16-4.17 (m, 1H), 4.77-4.83 (m, 1H), 5.85 (d, J=7.6 Hz, 1H), 6.21 (d, J=3.6 Hz, 1H), 7.14-7.39 (m, 6H), 7.99 (d, J=7.2 Hz, 1H), 8.24 (br s, 1H); ^{31}P NMR (CD_3OD) δ 4.07.

Example 66

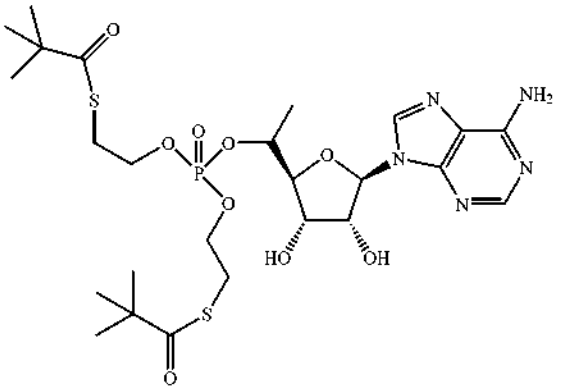
Preparation of 5'(S)—C-methyladenosin-5'-yl bis(S-pivaloyl-2-thioethyl)phosphate (66)

[0488]

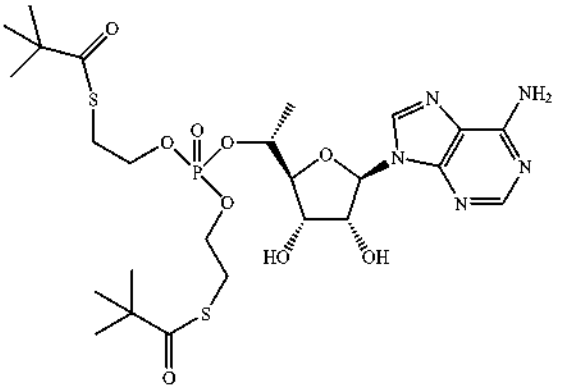


(66)

-continued



-continued



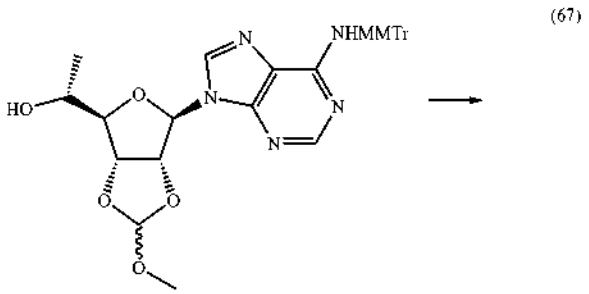
[0489] To a solution of 2',3'-O-methyomethylene-5'(S)-methyl-N^6-(4-methoxytrityl)adenosine (120 mg, 0.2 mmol) in acetonitrile (0.4 mL) under argon was added bis(S-pivaloyl-2-thioethyl)N,N-diisopropylphosphoramidite (136 mg, 0.3 mmol), 0.25 mmol), followed by addition of 0.45 M tetrazole in acetonitrile (1.5 mL, 0.66 mmol). The resulting solution was stirred at RT for 1.5 h, cooled to −40° C. and a solution of mCPBA (69 mg, 0.4 mmol) in DCM (0.75 mL) was added. The mixture was warmed up to RT and stirred for 10 min, diluted with ethyl acetate, washed with 10% $Na_2S_2O_3$ two times and washed with brine. Chromatography on silica gel with 15-25% ethyl acetate in DCM gave 140 mg of purified product, which was dissolved in 80% AcOH (8 mL) and the solution was heated at 50° C. for 24 h. Solvent was evaporated and the residue was chromatographed on silica gel with 7-10% MeOH in DCM to give 5'(S)—C-methyladenosin-5'-yl bis(S-pivaloyl-2-thioethyl)phosphate (61 mg) as white solid; ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 18H), 1.45 (d, J=6.4 Hz, 3H), 3.02-3.11 (m, 4H), 3.94-4.07 (m, 4H), 4.16-4.18 (m, 1H), 4.58-4.75 (m, 3H), 5.91 (br s, 2H), 5.94 (d, J=6.0 Hz, 1H), 6.05-6.15 (br s, 1H), 8.07 (s, 1H), 8.26 (s, 1H).

[0491] To a solution of 2',3'-O-methyomethylene-5'(R)-methyl-N^6-(4-methoxytrityl)adenosine (238 mg, 0.4 mmol) in acetonitrile (0.8 mL) under argon was added bis(S-pivaloyl-2-thioethyl)N,N-diisopropylphosphoramidite (272 mg, 0.6 mmol), followed by addition of 0.45 M tetrazole in acetonitrile (3.0 mL, 1.32 mmol). The resulting solution was stirred at RT for 3 h, cooled to -40° C. and a solution of mCPBA (172 mg, 1.0 mmol) in DCM (2 mL) was added. The mixture was warmed up to RT and stirred for 10 min, diluted with ethyl acetate, washed with 10% $Na_2S_2O_3$ two times and washed with brine. Chromatography on silica gel with 25-35% ethyl acetate in DCM gave 326 mg of purified product, 207 mg of which was dissolved in 80% AcOH (12 mL) and the solution was heated at 50° C. for 24 h. Solvent was evaporated and the residue was chromatographed on silica gel with 5-7% MeOH in DCM to give 5'(R)—C-methyladenosin-5'-yl bis(S-pivaloyl-2-thioethyl)phosphate (105 mg) as a white solid; ^{1}H NMR ($CDCl_3$) δ 1.17 (s, 9H), 1.23 (s, 9H), 1.51 (d, J=6.4 Hz, 3H), 3.04-3.13 (m, 4H), 4.02-4.10 (m, 4H), 4.21-4.22 (m, 1H), 4.46 (t, J=3.6 Hz, 1H), 4.62 (t, J=5.2 Hz, 1H), 4.74-4.78 (m, 1H), 6.00 (br s, 2H), 6.06 (d, J=4.8 Hz, 1H), 8.17 (s, 1H), 8.26 (s, 1H).

Example 67

Preparation of 5'(R)—C-methyladenosin-5'-yl bis(S-pivaloyl-2-thioethyl)phosphate (67)

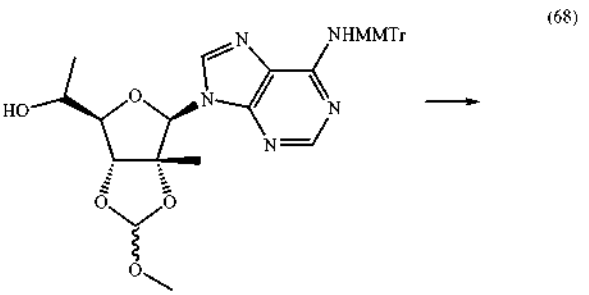
[0490]



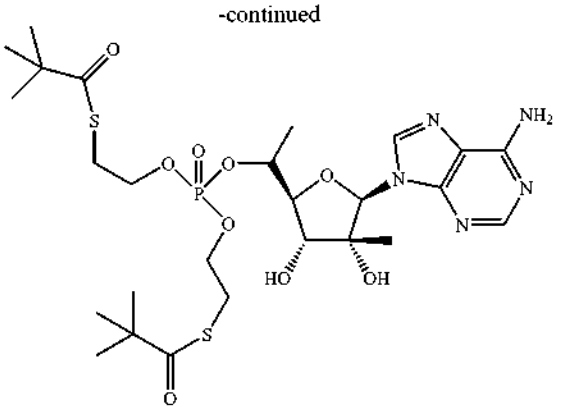
Example 68

Preparation of 2',5'(S)—C-dimethyladenosin-5'-yl bis(S-pivaloyl-2-thioethyl)phosphate (68)

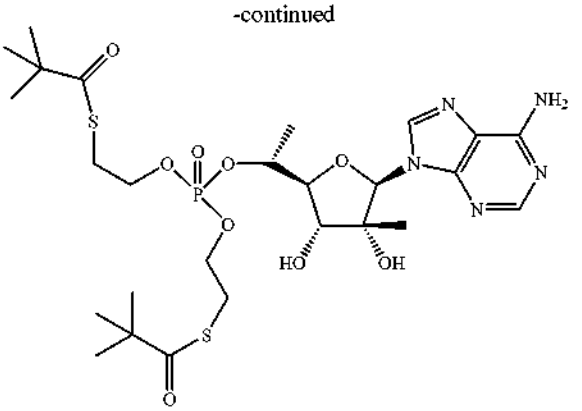
[0492]



-continued



-continued

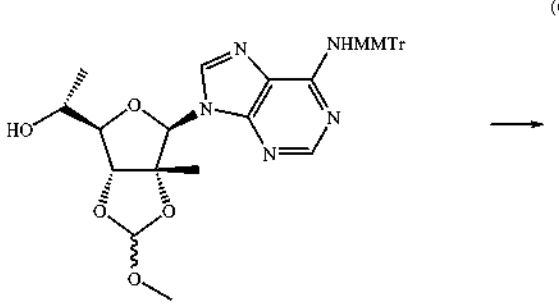


[0493] To a solution of 2',3'-O-methyomethylene-2',5'(S)-dimethyl-N^6-(4-methoxytrityl)adenosine (122 mg, 0.2 mmol) in acetonitrile (0.4 mL) under argon was added bis(S-pivaloyl-2-thioethyl)N,N-diisopropylphosphoramidite (136 mg, 0.3 mmol), followed by addition of 0.45 M tetrazole in acetonitrile (1.5 mL, 0.66 mmol). The resulting solution was stirred at RT for 3 h, cooled to −40° C. and a solution of mCPBA (86 mg, 0.5 mmol) in DCM (1 mL) was added. The mixture was warmed up to RT and stirred for 10 min, diluted with ethyl acetate, washed with 10% $Na_2S_2O_3$ two times and washed with brine. Chromatography on silica gel with 25-35% ethyl acetate in DCM gave a purified product, which was dissolved in 80% AcOH (10 mL) and the solution was heated at 50° C. for 24 h. Solvent was evaporated and the residue was chromatographed on silica gel with 4-7% MeOH in DCM to give 2',5'(S)—C-dimethyladenosin-5'-yl bis(S-pivaloyl-2-thioethyl)phosphate (68 mg) as a white solid; ^{1}H NMR ($CDCl_3$) δ 1.02 (s, 3H), 1.22, 1.24 (2×s, each 9H), 1.49 (d, J=6.8 Hz, 3H), 3.14-3.20 (m, 4H), 4.01 (t, J=5.6 Hz, 1H), 4.12-4.20 (m, 5H), 4.43-4.46 (m, 1H), 4.73 (br s, 1H), 4.83-4.88 (m, 1H), 5.64 (br s, 2H), 5.97 (s, 1H), 7.95 (s, 1H), 8.33 (s, 1H).

Example 69

Preparation of 2',5'(R)—C-dimethyladenosin-5'-yl bis(S-pivaloyl-2-thioethyl)phosphate (69)

[0494]



(69)

[0495] To a solution of 2',3'-O-methyomethylene-2',5'(R)-dimethyl-N^6-(4-methoxytrityl)adenosine (183 mg, 0.3 mmol) in acetonitrile (0.6 mL) under argon was added bis(S-pivaloyl-2-thioethyl)N,N-diisopropylphosphoramidite (204 mg, 0.45 mmol), followed by addition of 0.45 M tetrazole in acetonitrile (2.2 mL, 0.99 mmol). The resulting solution was stirred at RT for 3 h, cooled to −40° C. and a solution of mCPBA (129 mg, 0.75 mmol) in DCM (1.5 mL) was added. The mixture was warmed up to RT and stirred for 10 min, diluted with ethyl acetate, washed with 10% Na_2S2O_3 two times and washed with brine. Chromatography on silica gel with 25-35% ethyl acetate in DCM gave a purified product, which was dissolved in 80% AcOH (10 mL) and the solution was heated at 50° C. for 24 h. Solvent was evaporated and the residue was chromatographed on silica gel with 4-7% MeOH in DCM to give 2',5'(R)—C-dimethyladenosin-5'-yl bis(S-pivaloyl-2-thioethyl) phosphate (85 mg) as a white solid; ^{1}H NMR ($CDCl_3$) δ 1.01 (s, 3H), 1.20, 1.24 (2×s, each 9H), 1.56 (d,=6.8 Hz, 3H), 3.14-3.19 (m, 4H), 4.01-4.19 (m, 7H), 4.36 (s, 1H), 4.79-4.83 (m, 1H), 5.7 (br s, 2H), 6.15 (s, 1H), 8.13 (s, 1H), 8.35 (s, 1H).

Example 70

General Procedure for Synthesis of 5'-Alkylated Nucleoside 5'-Triphosphates

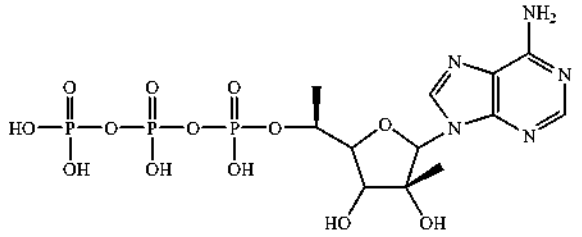
[0496] 1,2,3-Triazol (41 mg, 0.6 mmol) was dissolved in the mixture of 1 ml of dry CH_3CN and 88 ul of dry triethylamine in 1.5 ml centrifuge tube. The solution was cooled down to 0° C. and $POCl_3$ (19 ul, 0.2 mmol) was added. The mixture was vortexed and left at 5° C. for 20 min. The white precipitate was centrifugated and supernatant was added to 0.1 mmol of dry nucleoside in 10 ml flask. Reaction mixture was kept at +5° C. for 2 hours, then tris(tetrabutylammonium) pyrophosphate was added (360 mg, 0.4 mmol). The reaction was left for 2 hours more at room temperature and solvents were evaporated. The residue was dissolved in 80% HCOOH and left for 2 hours more at ambient temperature. Formic acid was evaporated, the residue distributed between 6 ml of water and 3 ml of DCM. Organic fraction was separated and the aqueous fraction was extracted with DCM (2×3 ml). Aqueous fraction containing target NTP was loaded on ion-exchange column HiLoad 16/10 Q Sepharose High Performance. Target NTP was eluted by gradient of NaCl from 0 to 1 M in 50 mmol TRIS-buffer (pH 8). Corresponding fractions were collected and desalted by RP Chromatography on Synergi 4u

Hydro-RP 80A 100×21 in linear gradient of methanol in TEAB-buffer (pH 8.5) from 0 to 40%. Fraction containing target NTP was lyophilized from water (3×5 ml).

[0497] The general procedure was used for synthesis of following nucleoside 5'-triphosphates.

2',5'(S)—C-Dimethyladenosine 5'-triphosphate

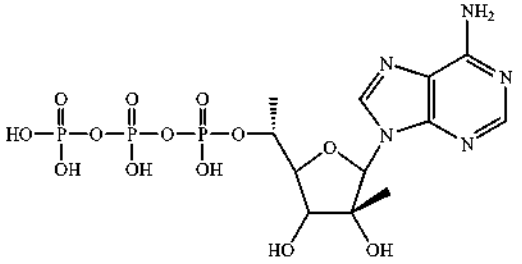
[0498]



[0499] MS: 534.1 (M-1). H^1 NMR (D_2O): 0.83 (s, 3H, methyl); 1.13 (t, 34H, Et_3N-salt); 1.36-1.37 (d, 3H, methyl); 3.02-3.08 (dd, 22H, Et_3N-salt); 3.96-3.99 (m, 1H, 4'-H), 4.20-4.22 (d, 1H, 5'-H); 4.60-4.63 (m, 1H, H-3'); 6.10 (s, 1H, H-1'); 8.10 and 8.31 (s, 1H, adenine). ^{31}P NMR (D_2O): −8.75 (d, 1P); −11.45 (d, 1P), −22.48(t, 1P).

2',5'(R)—C-Dimethyladenosine 5'-triphosphate

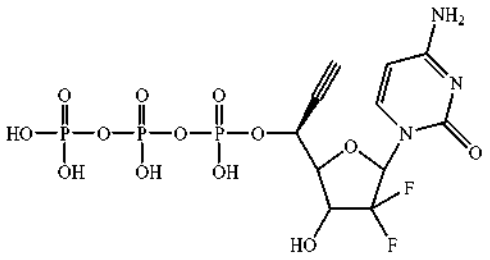
[0500]



[0501] MS: 534.4 (M-1). H^1 NMR (D_2O): 0.85 (s, 3H, methyl); 1.15 (t, 29H, Et_3N-salt); (s, 3H, methyl); 3.04-3.10 (dd, 18H, Et_3N-salt); 3.92-3.94 (m, 1H, 4'-H), 4.29-4.27 (d, 1H, 5'-H, J=9.2 Hz); 6.04 (s, 1H, H-1'); 8.12 and 8.46 (s, 1H, adenine). ^{31}P NMR (D_2O): −8.58 (bs, 1P); −11.09 (d, 1P), −22.15 (t, 1P).

2'-Deoxy-2'2'-difluoro-5'(S)-ethynylcytidine 5'-triphosphate

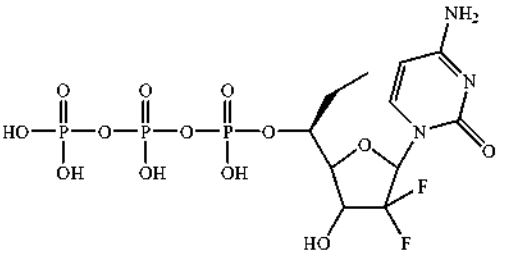
[0502]



[0503] MS: 526.2 (M-1). H^1 NMR (D_2O): 1.15 (t, 16H, Et_3N-salt); 2.89-3.05 (dd, 10H, Et_3N-salt); 4.10-4.12 (d, 1H, 4'-H), 4.68-4.90 (m, 1H, 5'-H, J=9.2 Hz); 5.14-5.16 (d, 1H, J-3'); 6.03-6.05 (d, 1H, H-5); 6.16-6.20 (t, 1H, H-1'); 7.74-7.76 (d, 1H, H-6) ^{31}P NMR (D_2O): −9.58 (bs, 1P); −11.65 (d, 1P), −21.92 (bs, 1P)

2'-Deoxy-2'2'-difluoro-5'(S)-ethylcytidine 5'-triphosphate

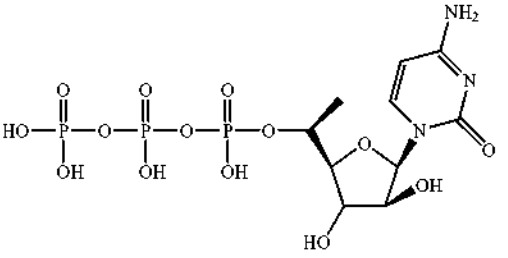
[0504]



[0505] MS: 529.9 (M-1). H^1 NMR (D_2O): 0.84-0.88 (t, 3H, CH_2CH_3); 1.14 (t, 16H, Et_3N-salt); 1.71-1.84 (m, 2H, CH_2CH$_3$); 3.03-3.09 (dd, 12H, Et_3N-salt); 3.97-4.00 (d, 1H, 4'-H), 4.30-4.36 (m, 1H, 5'-H); 4.45-4.50 (m, 1H, H-3'); 6.05-6.07 (d, 1H, H-5); 6.10-6.14 (m, 1H, H-1'); 7.81-7.83 (d, 1H, H-6). ^{31}P NMR (D_2O): −10.15 (d, 1P); −11.20 (d, 1P), −22.45 (t, 1P).

5'(S)-methylarabinocytidine 5'-triphosphate

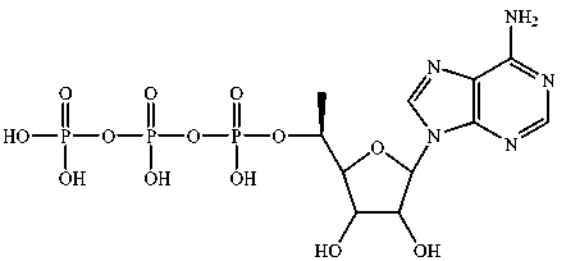
[0506]



[0507] MS: 496.0 (M-1). H^1 NMR (D_2O): 1.14 (t, 15H, Et_3N-salt); 1.33 (d, 1H, methyl), 3.04-3.10 (dd, 12H, Et_3N-salt); 3.67-3.70 (m, 1H, 4'-H), 4.12-4.16 (m, 1H, 5'-H); 4.29-4.32 (m, 1H, H-3'); 4.50-4.60 (m, 1H, H-2'); 6.03-6.05 (d, 1H, H-5); 6.10-6.11(d, 1H, H-1'); 7.90-7.92 (d, 1H, H-6). ^{31}P NMR (D_2O): −10.15 (d, 1P); −11.30 (d, 1P), −22.54 (t, 1P).

5'(S)-methyladenosine 5'-triphosphate

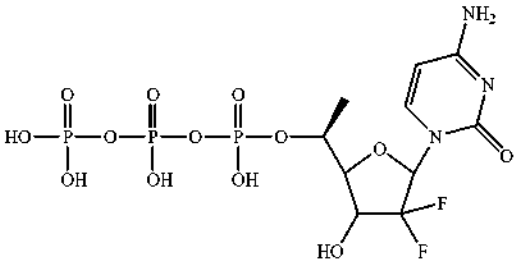
[0508]



[0509] MS: 520.1 (M-1). H^1 NMR (D_2O): 1.23-1.24 (s, 3H, methyl); 1.11 (t, 30H, Et_3N-salt); 3.01-3.07 (dd, 18H, Et_3N-salt); 4.06 (bs, 1H, 4'-H), 4.45-4.53 (m, 2H); 6.00 (d, 1H, H-1'): 8.09 and 8.45 (s, 1H, adenine). ^{31}P NMR (D_2O): −9.75 (d, 1P); −11.41 (d, 1P), −22.51 (t, 1P).

2'-Deoxy-2'2'-difluoro-5'(S)-methylcytidine 5'-triphosphate

[0510]



[0511] MS: 516.0 (M-1). H^1 NMR (D_2O): 1.15 (t, 25H, Et_3N-salt); 1.36-1.37 (d, 3H, CH_3); 3.04-3.10 (dd, 16H, Et_3N-salt); 3.83-3.85 (d, 1H, 4'-H), 4.35-4.58 (m, 2H), 6.02-6.04 (d, 1H, H-5); 6.11-6.14 (m, 1H, H-1'); 7.81-7.83 (d, 1H, H-6). ^{31}P NMR (D_2O): −9.50 (bs, 1P); −11.30 (d, 1P), −22.33 (t, 1P).

Additional Exemplary Compounds

[0512] Compounds prepared by similar protocols and procedures to the preceding examples include, for example, the compounds shown in Table 1. The compounds show in Table 1 are illustrative only and are not intended, or are they to be construed, to limit the scope of the claims in any manner whatsoever.

TABLE 1

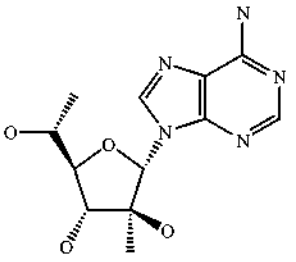
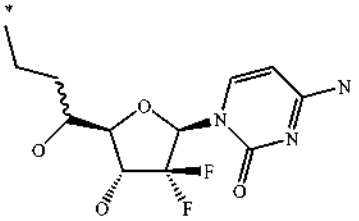
Exemplary Compounds
Structure
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TABLE 1-continued

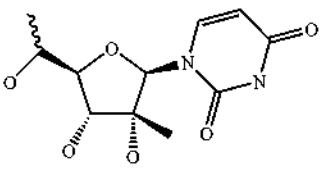
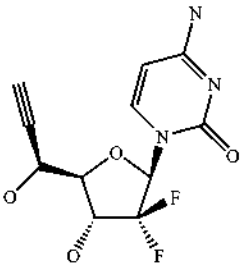
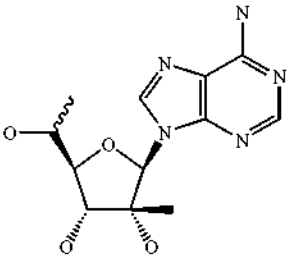
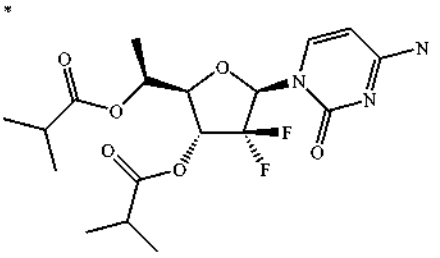
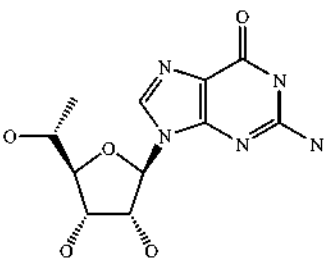
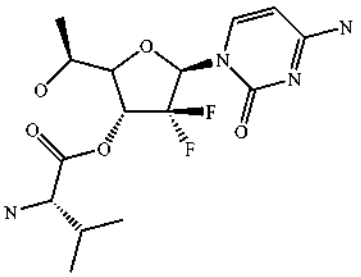
Exemplary Compounds
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TABLE 1-continued

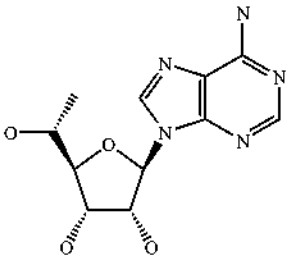
Exemplary Compounds

Structure

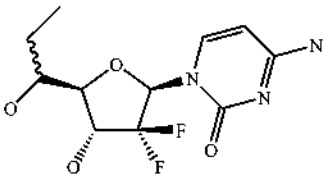
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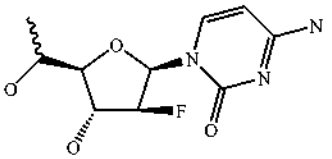
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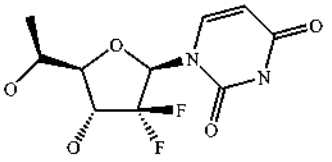
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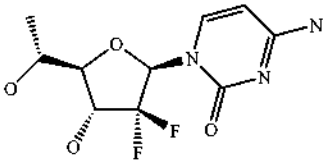
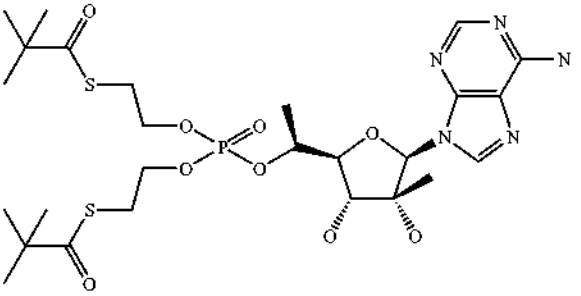


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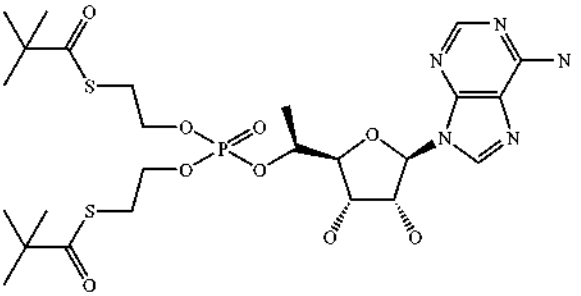
Exemplary Compounds

Structure

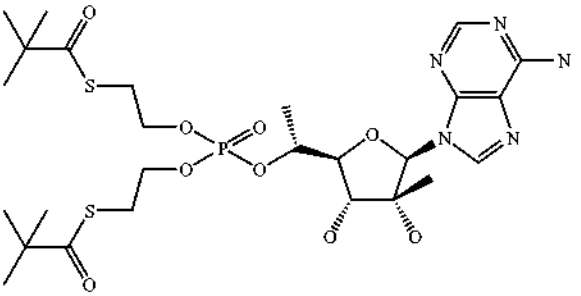
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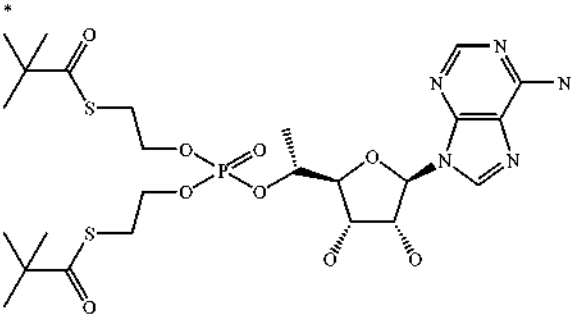
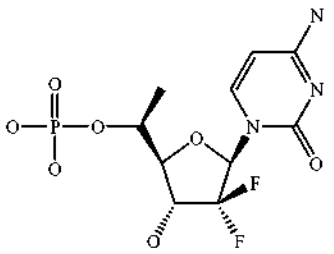


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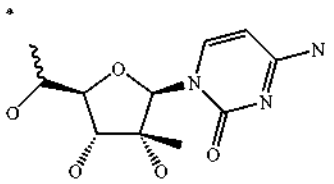
Exemplary Compounds

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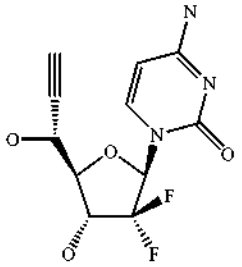
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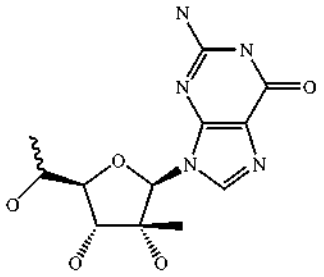
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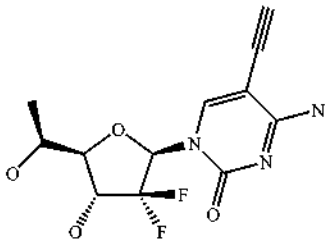
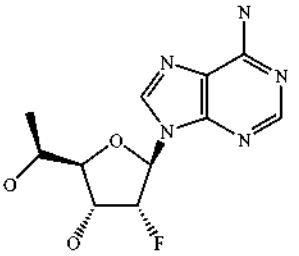


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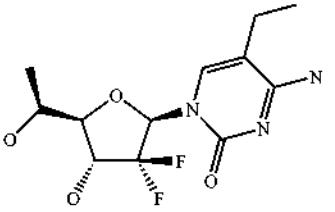
Exemplary Compounds

Structure

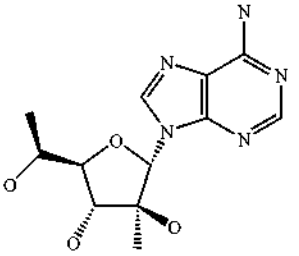
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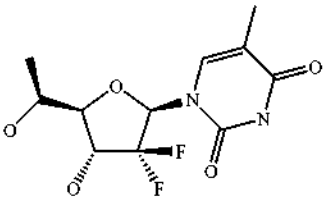
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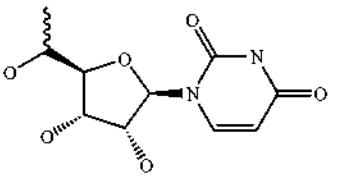
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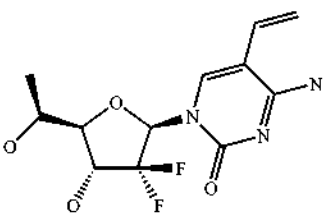
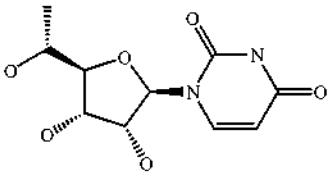


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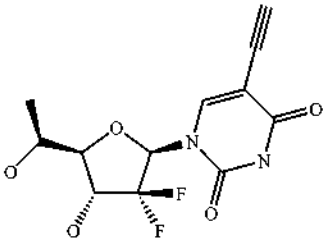
Exemplary Compounds

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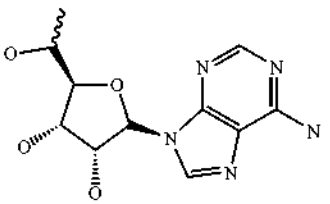
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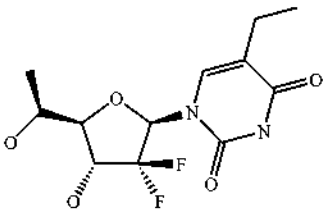
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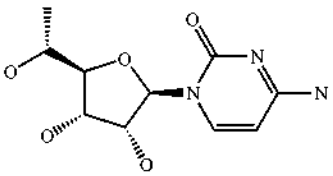
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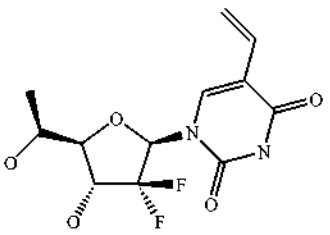
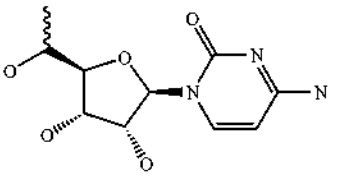


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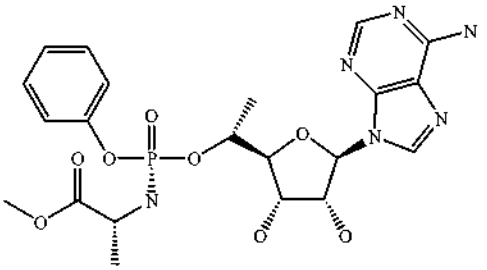
Exemplary Compounds

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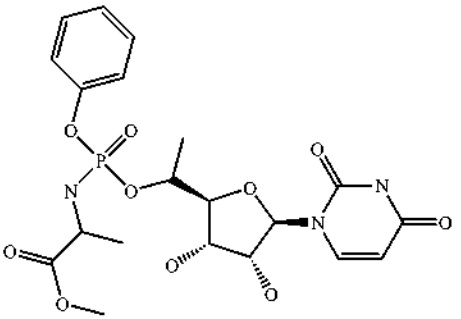
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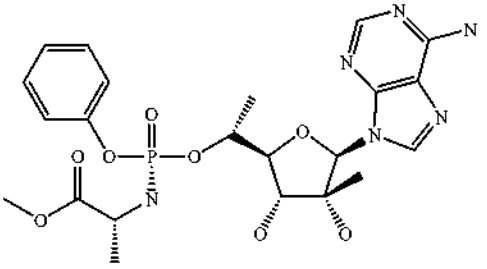
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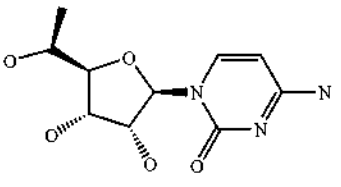
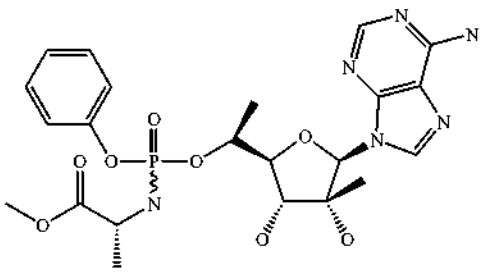


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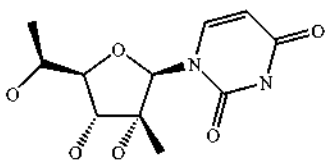
Exemplary Compounds

Structure

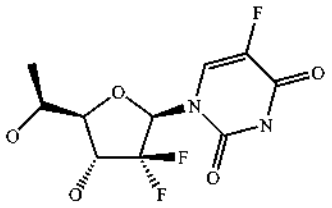
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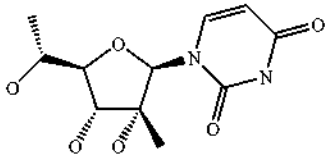
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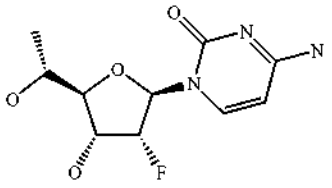
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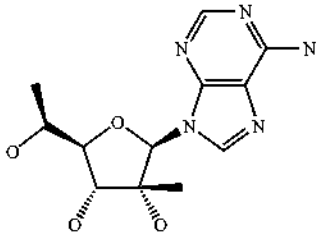
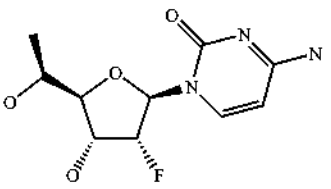


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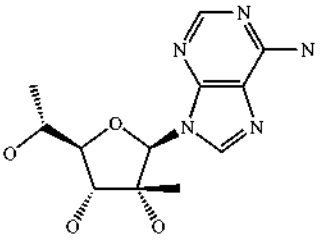
Exemplary Compounds

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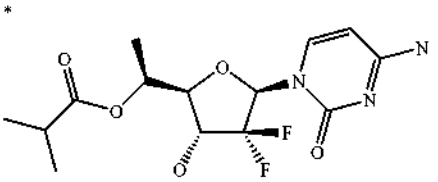
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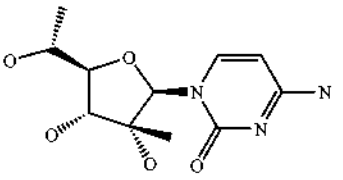
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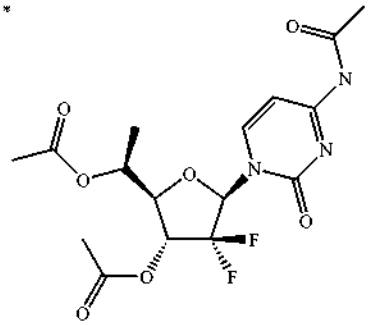
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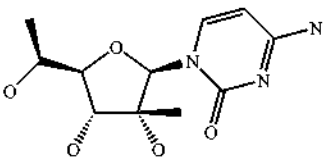
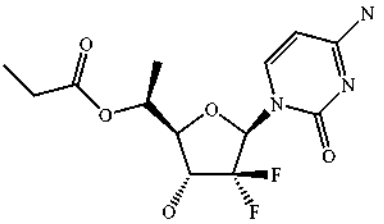


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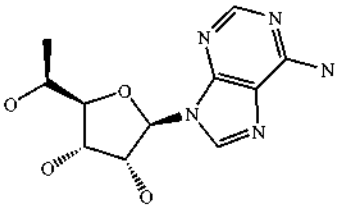
Exemplary Compounds

Structure

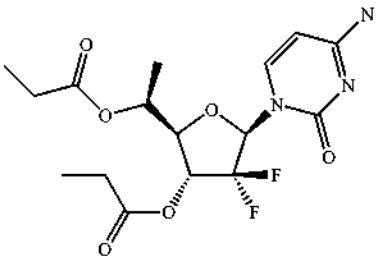
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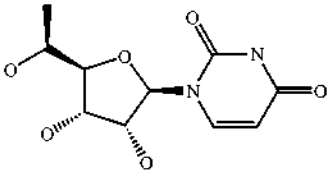
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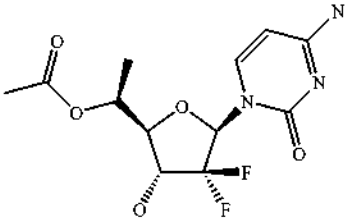
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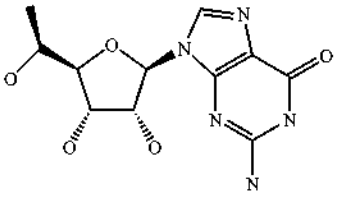
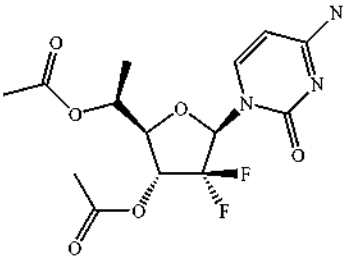


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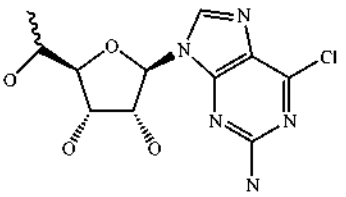
Exemplary Compounds

Structure

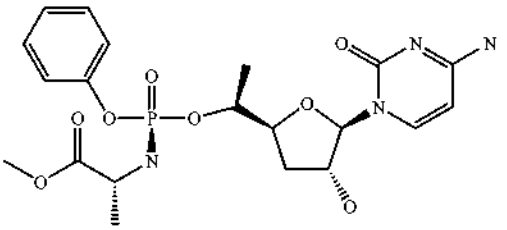
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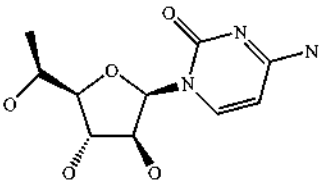
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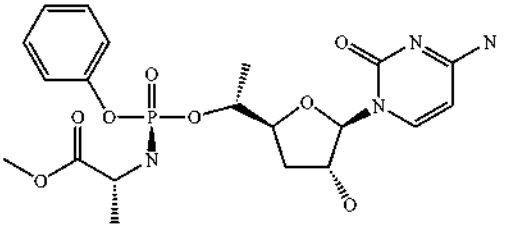
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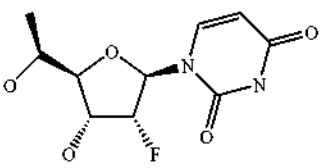
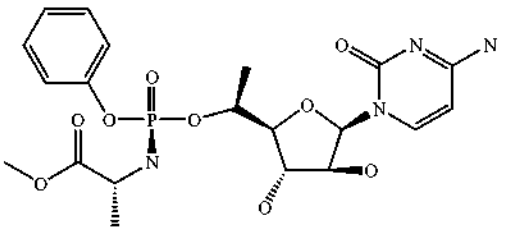


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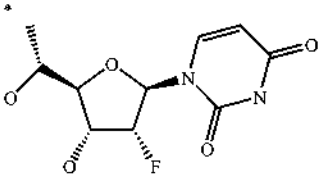
Exemplary Compounds

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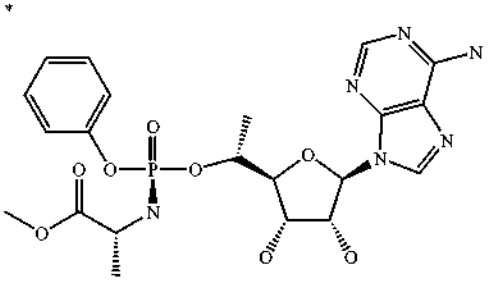
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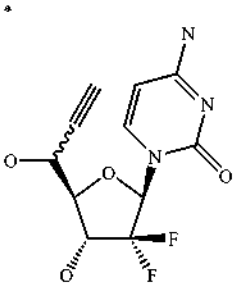
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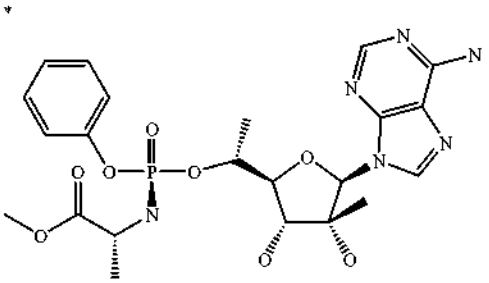
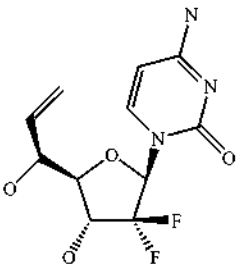


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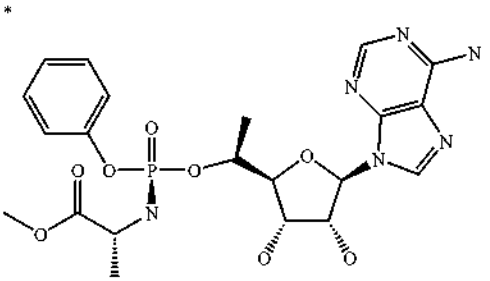
Exemplary Compounds

Structure

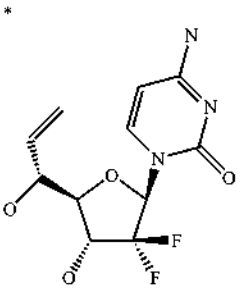
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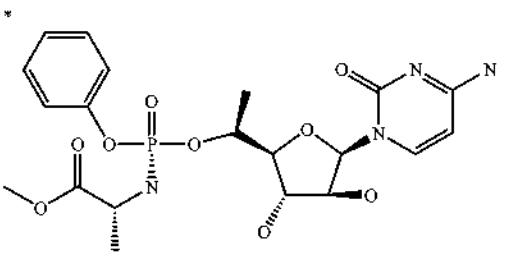
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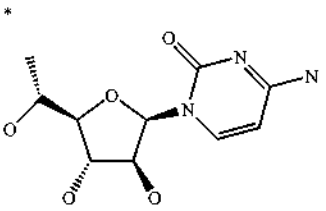
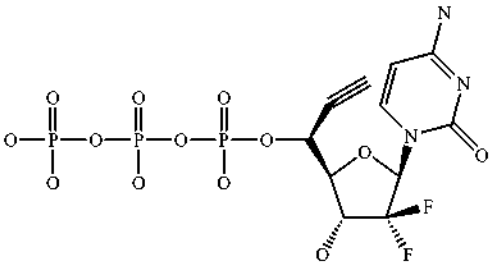


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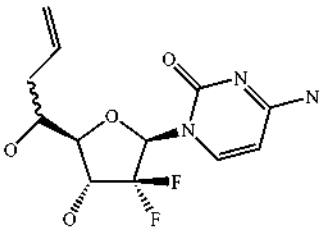
Exemplary Compounds

Structure

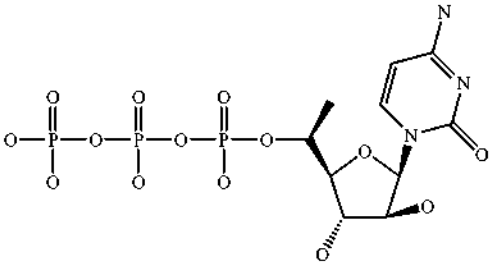
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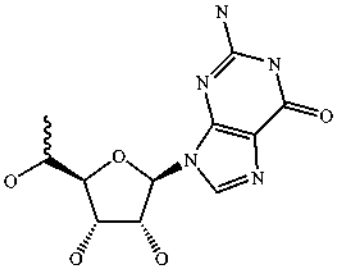
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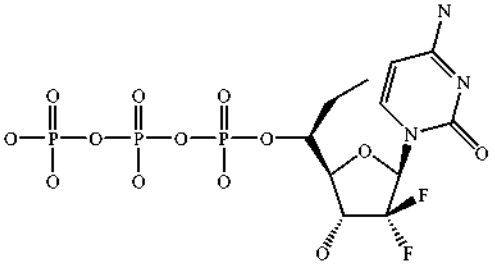
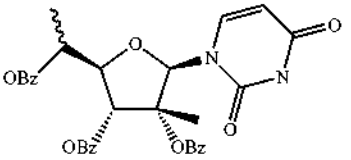


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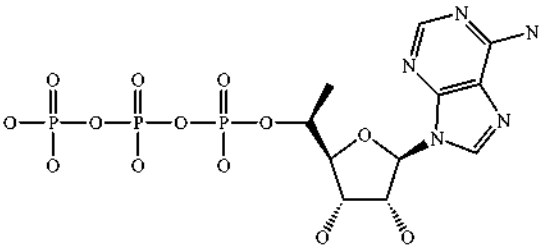
Exemplary Compounds

Structure

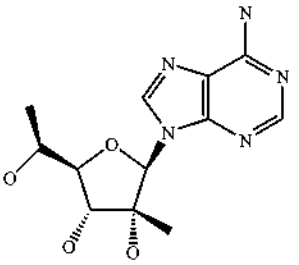
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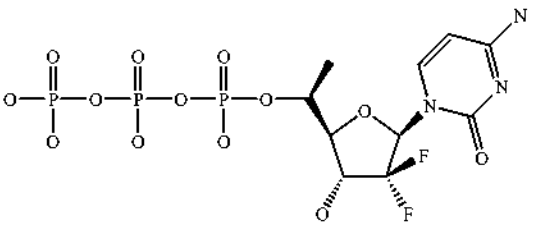
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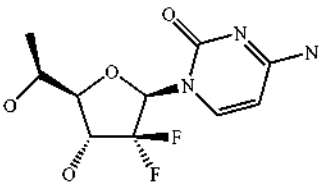
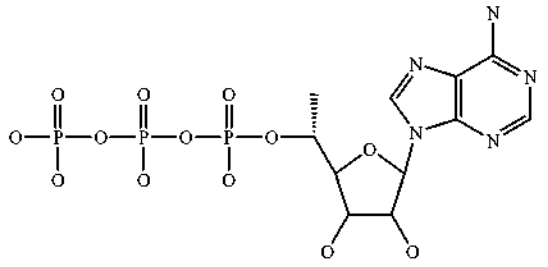


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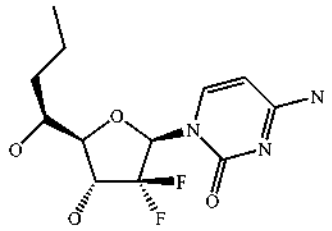
Exemplary Compounds

Structure

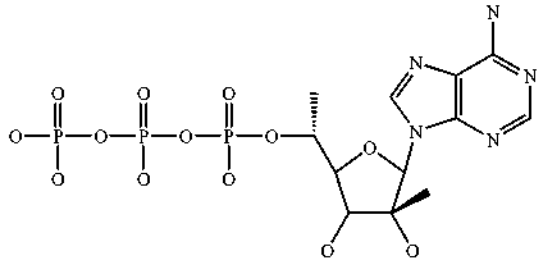
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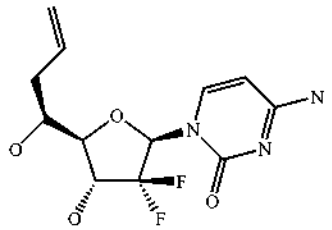
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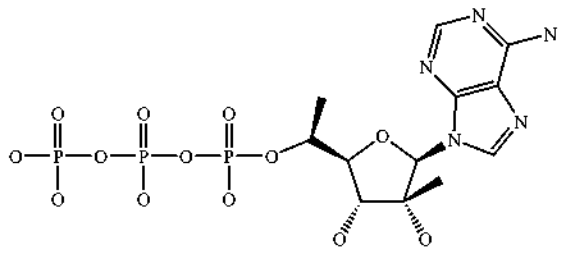
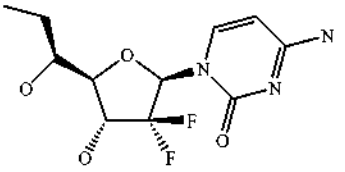


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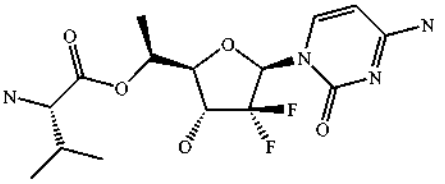
Exemplary Compounds

Structure

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Example 71

HCV Replicon Assay

[0513] Antiviral activity of test compounds was assessed (Okuse, et al., *Antivir. Res.* 2005 65:23) in the stably HCV RNA-replicating cell line, AVA5 (genotype 1b, subgenomic replicon, Blight, et al., *Sci.* 2000 290:1972). Compounds were added to dividing cultures daily for three days. Cultures generally start the assay at 30-50% confluence and reach confluence during the last day of treatment. Intracellular HCV RNA levels and cytotoxicity were assessed 72 hours after treatment.

[0514] Quadruplicate cultures for HCV RNA levels and cytoxicity (on 96-well plates) were used. A total of 12 untreated control cultures, and triplicate cultures treated with α-interferon (concentrations of: 10 IU/mL, 3.3 IU/mL, 1.1 IU/mL and 0.37 IU/mL) and 2'C-Me-C (concentrations of: 30 μM, 10 μM, 3.3 μM and 1.1 μM) served as assay controls.

[0515] Intracellular HCV RNA levels were measured using a conventional blot hybridization method, in which HCV RNA levels are normalized to the levels of β-actin RNA in each individual culture (Okuse, et al., *Antivir. Res.* 2005 65:23). Cytotoxicity was measured using an established neutral red dye uptake assay (Korba and Gerin, *Antivir. Res.* 1992 19:55; Okuse, et al., *Antivir. Res.* 2005 65:23). HCV RNA levels in the treated cultures are expressed as a percentage of the mean levels of RNA detected in untreated cultures. The absorbance of the internalized dye at 510 nM (A_{510}) was used for quantitative analysis.

[0516] Compounds were dissolved in 100% tissue culture grade DMSO (Sigma, Inc.) at 10 mM. Aliquots of test compounds sufficient for one daily treatment were made in individual tubes and all material was stored at −20° C. For the test, the compounds were suspended into culture medium at room temperature, and immediately added to the cell cultures. Compounds were analyzed separately in two groups with separate assay controls. The concentrations of the test compounds were run at concentrations of 10 μM, 3.3 μM, 1.1 μM and 0.37 μM. CC_{50}, EC_{50} and EC90 were determined using the concentration response curve.

[0517] The results demonstrate that compounds 8a and 9 are active and have an EC_{50} (μM) between 1.0 and 10. The antiviral activity of additional exemplary compounds is shown in Table 2, wherein 'A' represents an EC_{50} of less than 5 μM, 'B' represents an EC_{50} of less than 30 μM, and 'C' represents an EC_{50} of less than 200 μM.

TABLE 2

Activity of Exemplary Compounds	
Structure	Activity
*	B
*	B
*	A
*	B
*	C

TABLE 2-continued

Activity of Exemplary Compounds	
Structure	Activity
*	C
*	C
*	B
*	A
*	A
*	A

TABLE 2-continued

Activity of Exemplary Compounds

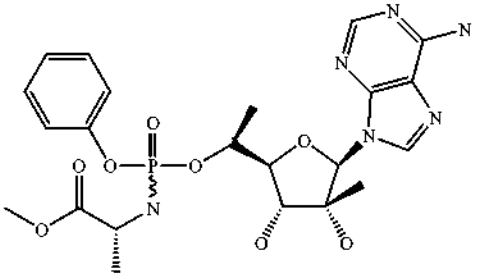
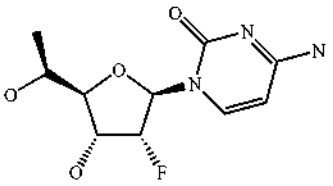
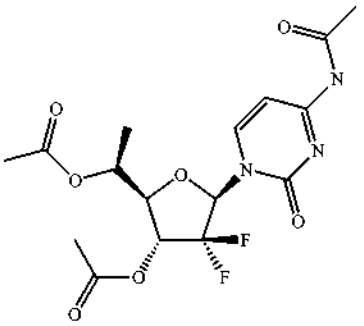
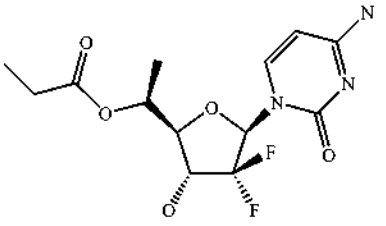
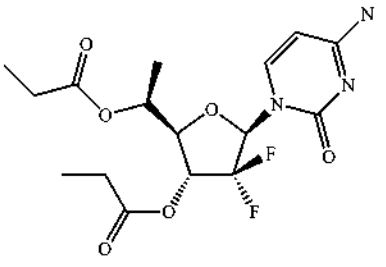
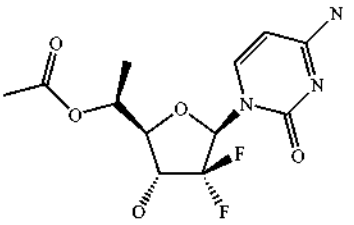
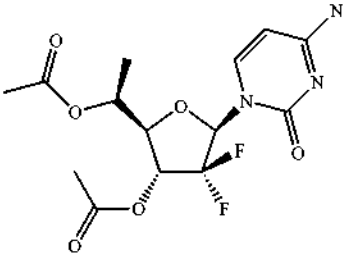
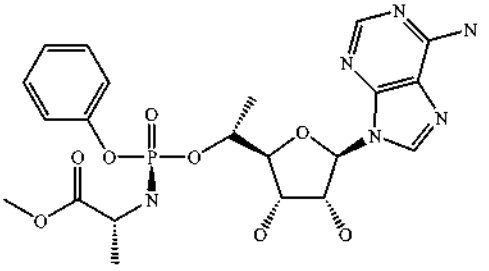
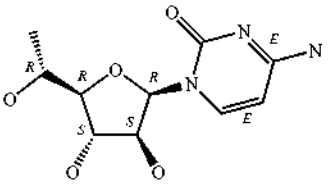
Structure	Activity
* 	C
* 	B
* 	A
* 	A
* 	A

TABLE 2-continued

Activity of Exemplary Compounds

Structure	Activity
* 	A
* 	A
* 	A
	A

(C < 200 μM, B < 30 μM, A < 5 μM)

Example 72

Stability Studies

[0518] Preparation of the cell extract. 10×10^6 of human prostate carcinoma cells (PC3) are treated with 10 mL of RIPA-buffer [15 mM Tris-HCl pH 7.5, 120 mM NaCl, 25 mM KCl, 2 mM EDTA, 2 mM EGTA, 0.1% Deoxycholic acid, 0.5% Triton X-100, 0.5% PMSF supplemented with Complete Protease Inhibitor Cocktail (Roche Diagnostics GmBH, Germany)] at 0° C. for 10 min. Most of the cells are disrupted by this hypotonic treatment and the remaining ones are disrupted mechanically. The cell extract obtained is centrifuged (900 rpm, 10 min) and the pellet is discarded. The extract is stored at −20° C.

[0519] Stability of nucleotides and nucleotides analogs in the cell extract. The cell extract is prepared as described above

(1 mL), and is diluted with a 9-fold volume of HEPES buffer (0.02 mol L^{-1}, pH 7.5, I=0.1 mol L^{-1} with NaCl). A nucleoside analog or a nucleotide analog (0.1 mg) is added into 3 mL of this HEPES buffered cell extract and the mixture is kept at 22±1° C. Aliquots of 150 μL are withdrawn at appropriate intervals, filtered with SPARTAN 13A (0.2 pm) and cooled in an ice bath. The aliquots are analyzed immediately by HPLC-ESI mass spectroscopy (Hypersil RP 18, 4.6×20 cm, 5 μm). For the first 10 min, 0.1% aq. formic acid containing 4% MeCN is used for elution and then the MeCN content is increased to 50% by a linear gradient during 40 min.

[0520] Stability of nucleoside and nucleotide analogs towards Porcine Liver Esterase. A nucleoside analog or a nucleotide analog (1 mg) and 3 mg (48 units) of Sigma Porcine Liver Esterase (66H7075) are dissolved in 3 mL of HEPES buffer (0.02 mol L^{-1}, pH 7.5, I=0.1 mol L^{-1} with NaCl). The stability test is carried out as described above for the cell extract

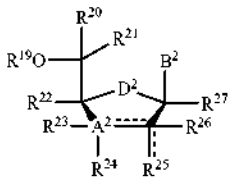
[0521] Stability tests in human serum. Stability tests in human serum are carried out as described for the whole cell extract. The measurements are carried out in serum diluted 1:1 with HEPES buffer (0.02 mol L^{-1}, pH 7.5, I=0.1 mol L^{-1} with NaCl).

[0522] It will be understood by those of skill in the art that numerous and various modifications can be made without departing from the spirit of the present disclosure. Therefore, it should be clearly understood that the forms disclosed herein are illustrative only and are not intended to limit the scope of the present disclosure.

What is claimed is:

1. A compound of Formula (II) or a pharmaceutically acceptable salt or a prodrug thereof:

(II)



wherein:

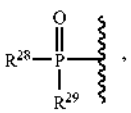
each ------ is independently a double or single bond;

A^2 is selected from the group consisting of C (carbon), O (oxygen) and S (sulfur);

B^2 is an optionally substituted heterocyclic base or a derivative thereof;

D^2 is selected from the group consisting of C=CH_2, CH_2, O (oxygen), S (sulfur), CHF, and CF_2;

R^{19} is selected from the group consisting of hydrogen, an optionally substituted alkyl, an optionally substituted cycloalkyl, an optionally substituted aralkyl, dialkylaminoalkylene, alkyl-C(=O)—, aryl-C(=O)—, alkoxyalkyl-C(=O)—, aryloxyalkyl-C(=O)—, alkylsulfonyl, arylsulfonyl, aralkylsulfonyl,



an —O-linked amino acid, diphosphate, triphosphate or derivatives thereof;

R^{20} and R^{21} are independently selected from the group consisting of hydrogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl and an optionally substituted C_{1-6} haloalkyl, provided that at least one of R^{20} and R^{21} is not hydrogen; or R^{20} and R^{21} are taken together to form a group selected from among C_{3-6} cycloalkyl, C_{3-6} cycloalkenyl, C_{3-6} aryl, and a C_{3-6} heteroaryl;

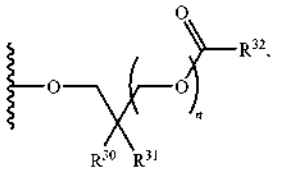
R^{22} and R^{27} is independently selected from the group consisting of hydrogen, halogen, —NH_2, —NHR^{a2}, $NR^{a2}R^{b2}$, —OR^{a2}, —SR^{a2}, —CN, —NC, —N_3, —NO_2, —$N(R^{c2})$—$NR^{a2}R^{b2}$, —$N(R^{c2})$—OR^{a2}, —S—SR^{a2}, —C(=O)R^{a2}, —C(=O)OR^{a2}, —C(=O)$NR^{a2}R^{b2}$, —O—C(=O)OR^{a2}, —O—C(=O)$NR^{a2}R^{b2}$, —$N(R^{c2})$—C(=O)$NR^{a2}R^{b2}$, —S(=O)R^{a2}, S(=O)$_2R^{a2}$, —O—S(=O)$_2NR^{a2}R^{b2}$, —$N(R^{c2})$—S(=O)$_2NR^{a2}R^{b2}$, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl and an —O-linked amino acid;

R^{23}, R^{24} and R^{25} are independently absent or selected from the group consisting of hydrogen, halogen, —NH_2, —NHR^{a2}, $NR^{a2}R^{b2}$, —OR^{a2}, —SR^{a2}, —CN, —NC, —N_3, —NO_2, —$N(R^{c2})$—$NR^{a2}R^{b2}$, —$N(R^{c2})$—OR^{a2}, —S—SR^{a2}, —C(=O)R^{a2}, —C(=O)OR^{a2}, —C(=O)$NR^{a2}R^{b2}$, —O—(C=O)R^{a2}, —O—C(=O)OR^{a2}, —O—C(=O)$NR^{a2}R^{b2}$, —$N(R^{c2})$—C(=O)$NR^{a2}R^{b2}$, —S(=O)R^{a2}, S(=O)$_2R^{a2}$, —O—S(=O)$_{2I\ NR}{}^{a2}R^{b2}$, —$N(R^{c2})$—S(=O)$_2NR^{a2}R^{b2}$, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl, an optionally substituted aralkyl and an —O-linked amino acid; or R^{24} and R^{25} taken together form —O—C(=O)—O—;

R^{26} is absent or selected from the group consisting of hydrogen, halogen, —NH_2, —NHR^{a2}, $NR^{a2}R^{b2}$, —OR^{a2}, —SR^{a2}, —CN, —NC, —N_3, —NO_2, —$N(R^{c2})$—$NR^{a2}R^{b2}$, —$N(R^{c2})$—OR^{a2}, —S—SR^{a2}, —C(=O)R^{a2}, —C(=O)OR^{a2}, —C(=O)$NR^{a2}R^{b2}$, —O—C(=O)OR^{a2}, —O—C(=O)$NR^{a2}R^{b2}$, —$N(R^{c2})$—C(=O)$NR^{a2}R^{b2}$, —S(=O)R^{a2}, S(=O)$_2R^{a2}$, —O—S(=O)$_2NR^{a2}$, R^{b2}, —$N(R^{c2})$—S(=O)$_2NR^{a2}R^{b2}$, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl, an optionally substituted haloalkyl, an optionally substituted hydroxyalkyl and an —O-linked amino acid, or when the bond to R^{25} indicated by ------ is a double bond, then R^{25} is a C_{2-6} alkylidene and R^{26} is absent;

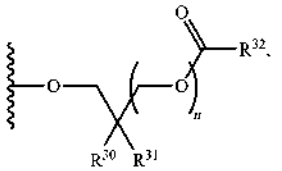
R^{a2}, R^{b2} and R^{c2} are each independently selected from the group consisting of hydrogen, an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted alkynyl, an optionally substituted aryl, an optionally substituted heteroaryl, an optionally substituted aralkyl and an optionally substituted heteroaryl (C_{1-6} alkyl);

R^{28} is selected from the group consisting of O^-, —OH, an optionally substituted aryloxy or aryl-O—,



alkyl-C(=O)—O—CH_2—O—, alkyl-C(=O)—S—CH_2CH_2—O— and an —N-linked amino acid;

R^{29} is selected from the group consisting of O^-, –OH, aryloxy or aryl-O—,



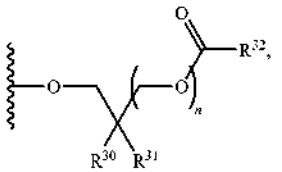
alkyl-C(=O)—O—CH_2—O—, alkyl-C(=O)—S—CH_2CH_2—O— and an —N-linked amino acid;

each R^{30} and each R^{31} are independently —C≡N or an optionally substituted substituent selected from the group consisting of C_{1-8} organylcarbonyl, C_{1-8} alkoxycarbonyl and C_{1-8} organylaminocarbonyl;

each R^{32} is hydrogen or an optionally substituted C_{1-6}-alkyl;

each n is independently 1 or 2; and

if both R^{28} and R^{29} are



each R^{30}, each R^{31}, each R^{32} and each n can be the same or different.

2. The compound of claim **1**, wherein A^2 is C (carbon), D^2 is O (oxygen), and both bonds indicated by ====== are single bonds.

3. The compound of claim **1**, wherein R^{22} is selected from the group consisting of hydrogen, halogen, —OR^{a2}, —CN, —N_3, and an optionally substituted C_{1-6} alkyl;

R^{23} is absent or selected from the group consisting of hydrogen, halogen, —OR^{a2} and an optionally substituted C_{1-6} alkyl;

R^{24} is absent or selected from the group consisting of hydrogen, halogen, —NH_2, —OR^{a2}, —N_3, an optionally substituted C_{1-6} alkyl and an —O-linked amino acid;

R^{25} is selected from the group consisting of hydrogen, halogen, —OR^{a2}, —CN, —NC, an optionally substituted C_{1-6} alkyl and an —O-linked amino acid; and

R^{26} is selected from the group consisting of hydrogen, halogen, an optionally substituted C_{1-6} alkyl, an optionally substituted haloalkyl, an optionally substituted hydroxyalkyl.

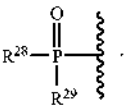
4. The compound of claim **1**, wherein at least one of R^{25} and R^{26} is halogen.

5. The compound of claim **1**, wherein both R^{25} and R^{26} are halogen.

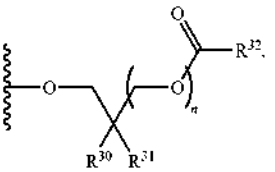
6. The compound of claim **1**, wherein R^{27} is selected from the group consisting of hydrogen, halogen, and an optionally substituted C_{1-6} alkyl.

7. The compound of claim **1**, wherein R^{19} is selected from the group consisting of hydrogen, a monophosphate, a diphosphate, and a triphosphate.

8. The compound of claim **1**, wherein R^{19} is



9. The compound of claim **8**, wherein at least one of R^{28} and R^{29} is

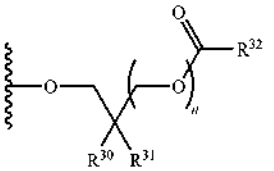


10. The compound of claim **9**, wherein R^{30} is —C≡N and R^{31} is an optionally substituted C_{1-8} alkoxycarbonyl or an optionally substituted C_{1-8} organylaminocarbonyl.

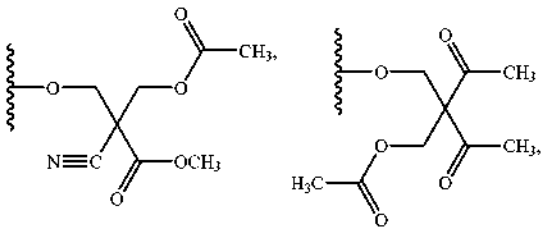
11. The compound of claim **9**, wherein both R^{30} and R^{31} are an optionally substituted C_{1-8} organylcarbonyl or an optionally substituted C_{1-8} alkoxycarbonyl.

12. The compound of claim **9**, wherein n is 2, both R^{30} and R^{31} are an optionally substituted C_{1-8} alkoxycarbonyl, and R^{32} is an optionally substituted C_{1-6}-alkyl.

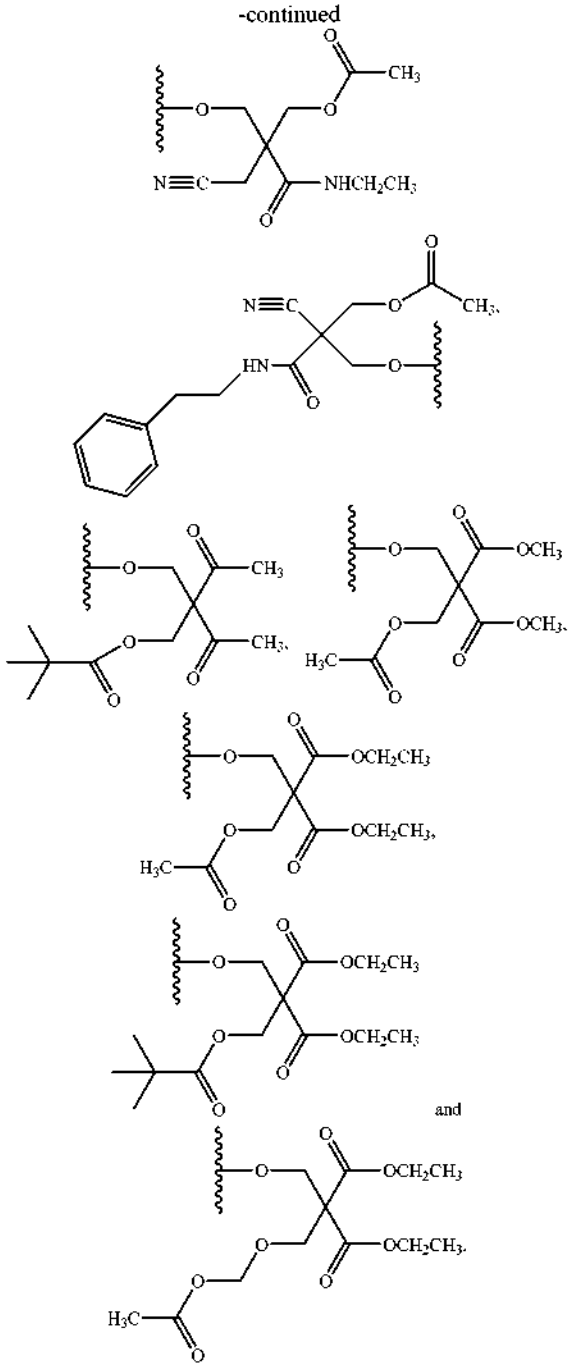
13. The compound of claim **9**, wherein



is selected from the group consisting of:

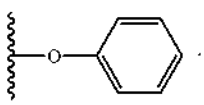


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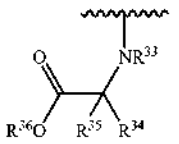
and

14. The compound of claim **8**, wherein at least one of R^{28} and R^{29} is



15. The compound of claim **8**, wherein at least one of R^{28} and R^{29} is an —N-linked amino acid.

16. The compound of claim **15**, wherein the —N-linked amino acid has the structure:



R^{33} is hydrogen or an optionally substituted C_{1-4}-alkyl;

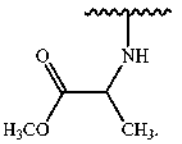
R^{34} is selected from the group consisting of hydrogen, an optionally substituted C_{1-6}-alkyl, an optionally substituted aryl, an optionally substituted aryl(C_{1-6} alkyl) and an optionally substituted haloalkyl;

R^{35} is hydrogen or an optionally substituted C_{1-6}-alkyl; and

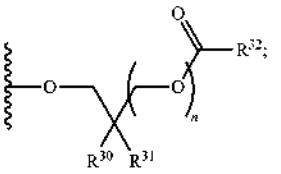
R^{36} is selected from the group consisting of an optionally substituted C_{1-6} alkyl, an optionally substituted C_6 aryl, an optionally substituted C_{10} aryl, and an optionally substituted C_{3-6} cycloalkyl.

17. The compound of claim **16**, wherein R^{33} is hydrogen, and R^{36} is an optionally substituted C_{1-6} alkyl.

18. The compound claim **16**, wherein at least one of R^{28} and R^{29} is:



19. The compound of claim **8**, wherein both R^{28} and R^{29} are both



and wherein each R^{30}, each R^{31}, each R^{32} and each n can be the same or different.

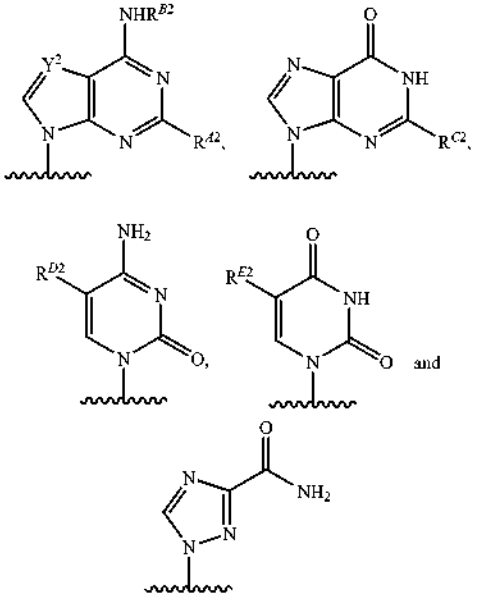
20. The compound of claim **8**, wherein when R^{28} and R^{29} are both O^-.

21. The compound of claim **1**, wherein at least one of R^{24} and R^{25} is —OR^{a2} or an —O-linked amino acid, and wherein R^{a2} is hydrogen.

22. The compound claim **21**, wherein the —O-linked amino acid is selected from the group consisting of alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, tyrosine, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

23. The compound claim **21**, wherein the —O-linked amino acid is selected from the group consisting of —O-linked α-amino acid, —O-linked β-amino acid, —O-linked γ-amino acid and —O-linked δ-amino acid.

24. The compound of claim **1**, wherein B^2 is selected from the group consisting of:



and

wherein:

R^{A2} is hydrogen or halogen;

R^{B2} is hydrogen, an optionally substituted C_{1-6} alkyl, or an optionally substituted C_{3-8} cycloalkyl;

R^{C2} is hydrogen or amino;

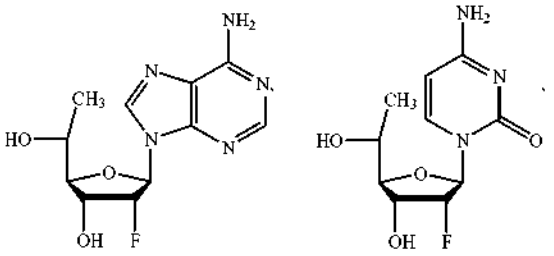
R^{D2} is selected from the group consisting of hydrogen, halogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl and an optionally substituted C_{2-6} alkynyl;

R^{E2} is selected from the group consisting of hydrogen, halogen, an optionally substituted C_{1-6}alkyl, an optionally substituted C_{2-6} alkenyl and an optionally substituted C_{2-6} alkynyl; and

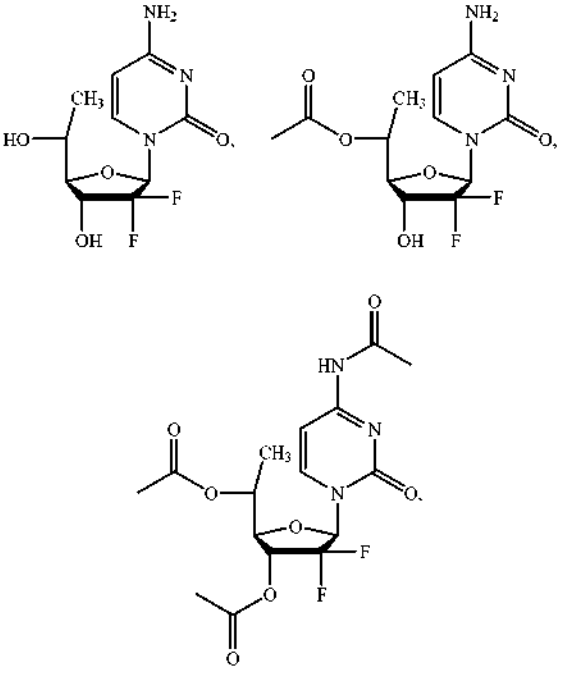
Y^2 is N or CR^{F2}, wherein R^{F2} can be selected from the group consisting of hydrogen, halogen, an optionally substituted C_{1-6}-alkyl, an optionally substituted C_{2-6}-alkenyl and an optionally substituted C_{2-6}-alkynyl.

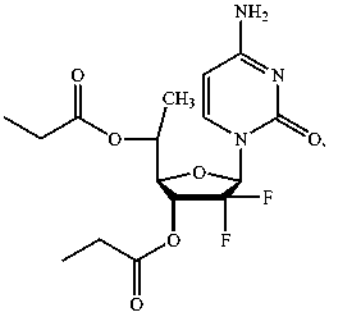
25. The compound of claim **1**, wherein R^{20} is methyl or CF_3; and R^{21} is hydrogen.

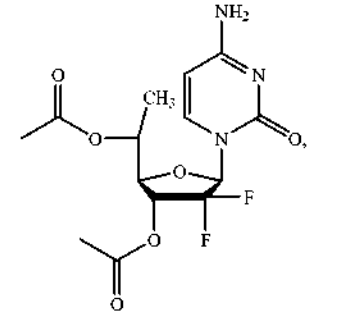
26. The compound of claim **1**, wherein the compound of Formula (II) is selected from the group consisting of:

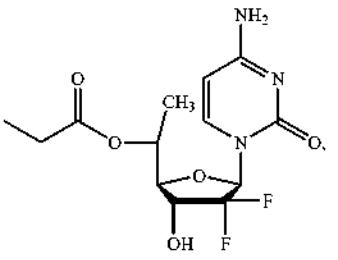


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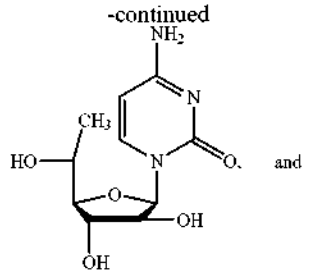




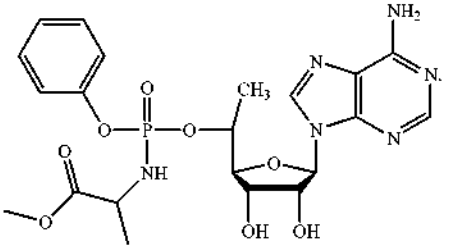




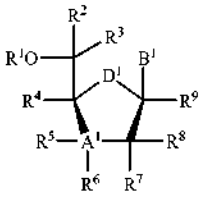
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and



27. A compound of Formula (I) or a pharmaceutically acceptable salt or a prodrug thereof:



(I)

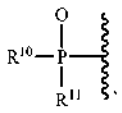
wherein:

A^1 is selected from the group consisting of C (carbon), O (oxygen) and S (sulfur);

B^1 is an optionally substituted heterocyclic base or a derivative thereof;

D^1 is selected from the group consisting of $C{=}CH_2$, CH_2, O (oxygen), S (sulfur), CHF, and CF_2;

R^1 is selected from the group consisting of hydrogen, an optionally substituted alkyl, an optionally substituted cycloalkyl, an optionally substituted aralkyl, dialkylaminoalkylene, alkyl-C(=O)—, aryl-C(=O)—, alkoxyalkyl-C(=O)—, aryloxyalkyl-C(=O)—, alkylsulfonyl, arylsulfonyl, aralkylsulfonyl,



an —O-linked amino acid, diphosphate, triphosphate or derivatives thereof;

R^2 and R^3 are each independently selected from the group consisting of hydrogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl and an optionally substituted C_{1-6} haloalkyl, provided that at least one of R^2 and R^3 is not hydrogen; or R^2 and R^3 are taken together to form a group selected from among C_{3-6} cycloalkyl, C_{3-6} cycloalkenyl, C_{3-6} aryl, and a C_{3-6}heteroaryl;

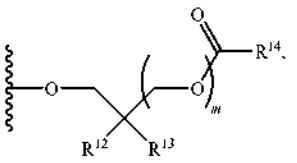
R^4, R^7 and R^9 is independently selected from the group consisting of hydrogen, halogen, $—NH_2$, $—NHR^{a1}$, $NR^{a1}R^{b1}$, $—OR^{a1}$, $—SR^{a1}$, —CN, —NC, $—N_3$, $—NO_2$, $—N(R^{c1})—NR^{a1}R^{b1}$, $—N(R^{c1})—OR^{a1}$, $—S—SR^{a1}$, $—C(=O)R^{a1}$, $—C(=O)OR^{a1}$, $—C(=O)NR^{a1}R^{b1}$, $—O—(C=O)R^{a1}$, $—O—C(=O)OR^{a1}$, $—O—C(=O)NR^{a1}R^{b1}$, $—N(R^{c1})—C(=O)NR^{a1}R^{b1}$, $—S(=O)R^{a1}$, $S(=O)_2R^{a1}$, $—O—S(=O)_2NR^{a1}R^{b1}$, $—N(R^{c1})—S(=O)_2NR^{a1}R^{b1}$, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl, an optionally substituted aralkyl and an —O-linked amino acid;

R^5 and R^6 is independently absent or selected from the group consisting of hydrogen, halogen, $—NH_2$, $—NHR^{a1}$, $NR^{a1}R^{b1}$, $—OR^{a1}$, $—SR^{a1}$, —CN, —NC, $—N_3$, $—NO_2$, $—N(R^{c1})—NR^{a1}R^{b1}$, $—N(R^{c1})—OR^{a1}$, $—S—SR^{a1}$, $—C(=O)R^{a1}$, $—C(=O)OR^{a1}$, $—C(=O)NR^{a1}R^{b1}$, $—O—C(=O)OR^{a1}$, $—O—C(=O)NR^{a1}R^{b1}$, $—N(R^{c1})—C(=O)NR^{a1}R^{b1}$, $—S(=O)R^{a1}$, $S(=O)_2R^{a1}$, $—O—S(=O)_2NR^{a1}R^{b1}$, $—N(R^{c1})—S(=O)_2NR^{a1}R^{b1}$, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl and an —O-linked amino acid; or R^6 and R^7 taken together form —O—C(=O)—O—;

R^8 is halogen, $—OR^{a1}$, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{2-6} alkynyl and an optionally substituted C_{1-6} haloalkyl;

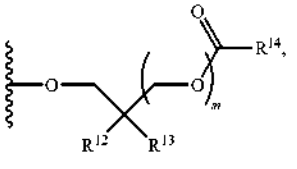
R^{a1}, R^{b1} and R^{c1} are each independently selected from the group consisting of hydrogen, an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted alkynyl, an optionally substituted aryl, an optionally substituted heteroaryl, an optionally substituted aralkyl and an optionally substituted heteroaryl (C_{1-6} alkyl);

R^{10} is selected from the group consisting of O^-, –OH, an optionally substituted aryloxy or aryl-O—,



alkyl-C(=O)—O—CH_2—O—, alkyl-C(=O)—S—CH_2CH_2—O— and an —N-linked amino acid;

R^{11} is selected from the group consisting of O^-, –OH, aryloxy or aryl-O—,



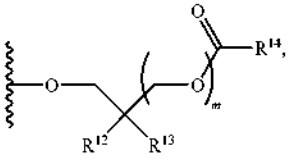
alkyl-C(=O)—O—CH_2—O—, alkyl-C(=O)—S—CH_2CH_2—O— and an —N-linked amino acid;

each R^{12} and each R^{13} are independently —C≡N or an optionally substituted substituent selected from the group consisting of C_{1-8} organylcarbonyl, C_{1-8} alkoxycarbonyl and C_{1-8} organylaminocarbonyl;

each R^{14} is hydrogen or an optionally substituted C_{1-6}-alkyl; and

each m is independently 1 or 2;

and if both R^{10} and R^{11} are



each R^{12}, each R^{13}, each R^{14} and each m can be the same or different.

28. The compound of claim **27**, wherein A^1 is C (carbon), and D^1 is O (oxygen).

29. The compound of claim **27**, wherein R^4 is selected from the group consisting of hydrogen, halogen, —OR^{a1}, —CN, —N_3, and an optionally substituted C_{1-6} alkyl;

R^5 is absent or selected from the group consisting of hydrogen, halogen, —OR^{a1} and an optionally substituted C_{1-6} alkyl;

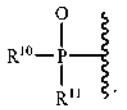
R^6 is absent or selected from the group consisting of hydrogen, halogen, —NH_2, —OR^{a1}, —N_3, an optionally substituted C_{1-6} alkyl and an —O-linked amino acid;

R^7 is selected from the group consisting of hydrogen, halogen, —OR^{a1}, —CN, —NC, an optionally substituted C_{1-6} alkyl and an —O-linked amino acid; and

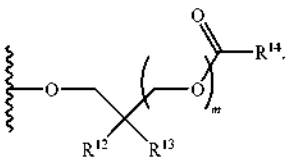
R^9 is selected from the group consisting of hydrogen, halogen, and an optionally substituted C_{1-6} alkyl.

30. The compound of claim **27**, wherein R^1 is selected from the group consisting of hydrogen, a monophosphate, a diphosphate, and a triphosphate.

31. The compound of claim **27**, wherein R^1 is



32. The compound of claim **31**, wherein at least one of R^{10} and R^{11} is

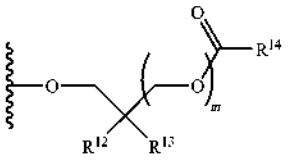


33. The compound of claim **32**, wherein R^{12} is —C≡N, and R^{13} is an optionally substituted C_{1-8} alkoxycarbonyl or an optionally substituted C_{1-8}organylaminocarbonyl.

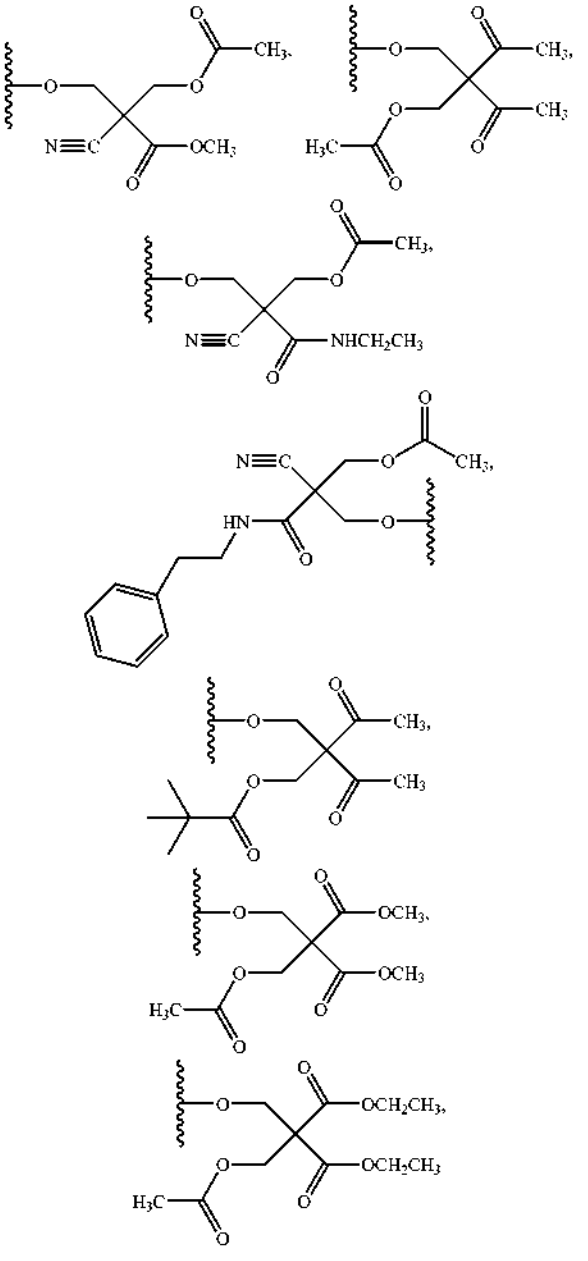
34. The compound of claim **32**, wherein both R^{12} and R^{13} are an optionally substituted C_{1-8} organylcarbonyl or an optionally substituted C_{1-8} alkoxycarbonyl.

35. The compound of claim **32**, wherein m is 2, both R^{12} and R^{13} are an optionally substituted C_{1-8} alkoxycarbonyl, and R^{14} is an optionally substituted C_{1-6}-alkyl.

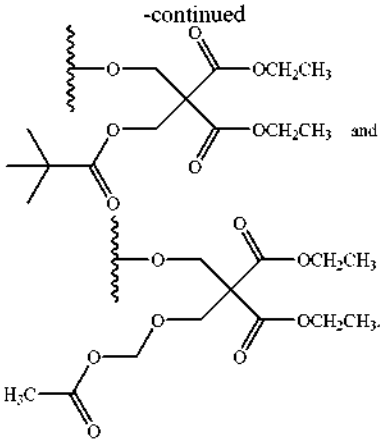
36. The compound of claim **32**, wherein



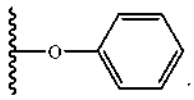
is selected from the group consisting of:



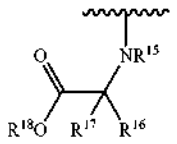
-continued



37. The compound of claim **31**, wherein at least one of R^{10} and R^{11} is



38. The compound of claim **27**, wherein the —N-linked amino acid has the structure:



R^{15} is hydrogen or an optionally substituted C_{1-4}-alkyl;

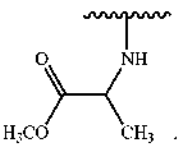
R^{16} is selected from the group consisting of hydrogen, an optionally substituted C_{1-6}-alkyl, an optionally substituted aryl, an optionally substituted aryl(C_{1-6} alkyl) and an optionally substituted haloalkyl;

R^{17} is hydrogen or an optionally substituted C_{1-6}-alkyl; and

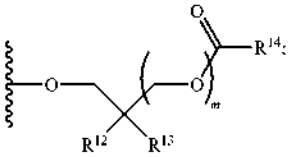
R^{18} is selected from the group consisting of an optionally substituted C_{1-6} alkyl, an optionally substituted C_6 aryl, an optionally substituted C_{10} aryl, and an optionally substituted C_{3-6} cycloalkyl.

39. The compound of claim **38**, wherein R^{15} is hydrogen, and R^{18} is an optionally substituted C_{1-6} alkyl.

40. The compound of claim **38**, at least one of R^{10} and R^{11} is:



41. The compound of claim **31**, wherein when R^{10} and R^{11} are both



and wherein each R^{12}, each R^{13}, each R^{14} and each m can be the same or different.

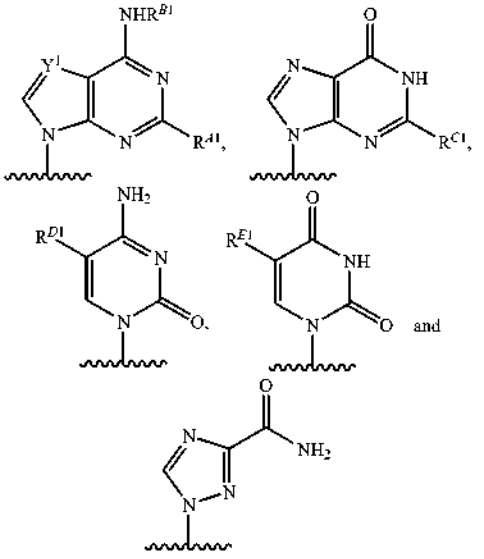
42. The compound of claim **31**, R^{10} and R^{11} are both O^-.

43. The compound of claim **27**, wherein at least one of R^6 and R^7 is —OR^{a1} or an —O-linked amino acid, and wherein R^{a1} is hydrogen.

44. The compound claim **43**, wherein the —O-linked amino acid is selected from the group consisting of alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, tyrosine, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

45. The compound claim **43**, wherein the —O-linked amino acid is selected from the group consisting of —O-linked α-amino acid, —O-linked β-amino acid, —O-linked γ-amino acid and —O-linked δ-amino acid.

46. The compound of claim **27**, wherein B^1 is selected from the group consisting of:



wherein:

R^{A1} is hydrogen or halogen;

R_{B1} is hydrogen, an optionally substituted C_{1-6} alkyl, or an optionally substituted C_{3-8} cycloalkyl;

R^{C1} is hydrogen or amino;

R^{D1} is selected from the group consisting of hydrogen, halogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl and an optionally substituted C_{2-6} alkynyl;

R^{E1} is selected from the group consisting of hydrogen, halogen, an optionally substituted C_{1-6}alkyl, an optionally substituted C_{2-6} alkenyl and an optionally substituted C_{2-6} alkynyl; and

Y^1 is N or CR^{F1}, wherein R^{F1} can be selected from the group consisting of hydrogen, halogen, an optionally substituted C_{1-6}-alkyl, an optionally substituted C_{2-6}-alkenyl and an optionally substituted C_{2-6}-alkynyl.

47. The compound of claim **27**, wherein R^2 is methyl or CF_3; and R^3 is hydrogen.

48. The compound of claim **27**, wherein R^8 is methyl.

49. A pharmaceutical composition comprising a compound of claim **1**, and a pharmaceutically acceptable carrier, diluent, excipient or combination thereof.

50. A method of ameliorating or treating a neoplastic disease comprising administering to a subject suffering from the neoplastic disease a therapeutically effective amount of a compound of claim **1**.

51. The method of claim **50**, wherein the neoplastic disease is cancer.

52. The method of claim **50**, wherein the neoplastic disease is leukemia.

53. A method of ameliorating or treating a viral infection comprising administering to a subject suffering from the viral infection a therapeutically effective amount of a compound of claim **1**.

54. The method of claim **53**, wherein the viral infection is caused by a virus selected from the group consisting of an adenovirus, an Alphaviridae, an Arbovirus, an Astrovirus, a Bunyaviridae, a Coronaviridae, a Filoviridae, a Flaviviridae, a Hepadnaviridae, a Herpesviridae, an Alphaherpesvirinae, a Betaherpesvirinae, a Gammaherpesvirinae, a Norwalk Virus, an Astroviridae, a Caliciviridae, an Orthomyxoviridae, a Paramyxoviridae, a Paramyxoviruses, a Rubulavirus, a Morbillivirus, a Papovaviridae, a Parvoviridae, a Picornaviridae, an Aphthoviridae, a Cardioviridae, an Enteroviridae, a Coxsackie virus, a Polio Virus, a Rhinoviridae, a Phycodnaviridae, a Poxviridae, a Reoviridae, a Rotavirus, a Retroviridae, an A-Type Retrovirus, an Immunodeficiency Virus, a Leukemia Viruses, an Avian Sarcoma Viruses, a Rhabdoviruses, a Rubiviridae and a Togaviridae.

55. The method of claim **53**, wherein the viral infection is a hepatitis C viral infection or a HIV viral infection.

56. A method of ameliorating or treating a parasitic disease comprising administering to a subject suffering from the parasitic disease a therapeutically effective amount of a compound of claim **1**.

57. The method of claim **56**, wherein the parasitic disease is Chagas' disease.

* * * * *

THE CHEMICAL RECORD

2′-Deoxy-4′-*C*-Ethynyl-2-Fluoroadenosine, a Nucleoside Reverse Transcriptase Inhibitor, Is Highly Potent Against All Human Immunodeficiency Viruses Type 1 and Has Low Toxicity

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ABSTRACT: An idea to use 4′-*C*-substituted-2′-deoxynucleoside derivatives was proposed based on a working hypothesis to solve the problems of existing acquired immune deficiency syndrome chemotherapy (highly active antiretroviral therapy). Subsequent studies have successfully proved the validity of the idea and resulted in the development of 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, which is highly potent to all human immunodeficiency viruses type 1 (HIV-1s) including multidrug-resistant HIV-1 and has a low toxicity. © 2006 The Japan Chemical Journal Forum and Wiley Periodicals, Inc. Chem Rec 6: 133–143; 2006: Published online in Wiley InterScience (www.interscience.wiley.com) DOI 10.1002/tcr.20078

Key words: AIDS; anti-HIV agent; reverse transcriptase; nucleoside reverse transcriptase inhibitor; highly active antiretroviral therapy; drug-resistant HIV; 4′-*C*-substituted-2′-deoxynucleoside

Introduction

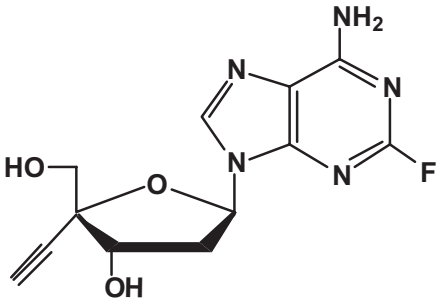
A highly active antiretroviral therapy (HAART) using two or more nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors has dramatically improved the quality of life and prognosis of patients infected by human immunodeficiency virus type 1 (HIV-1).[1,2] However, the existing HAART has several critical problems that remain to be solved. These problems include: (i) the emergence of new drug-resistant HIV-1 mutants, (ii) the need to take large dosages, and (iii) drug side effects. Therefore, the development of new, highly potent anti-human immunodeficiency virus (HIV) drugs that prevent the emergence of drug-resistant mutants and have few side effects is urgently needed.

These problems prompted me to speculate on the reason for the emergence of HIV-1 mutants resistant to clinical NRTIs, which belong to the family of 2′,3′-dideoxynucleoside (ddN). I proposed a working hypothesis for the chemical structure of a new, highly potent NRTI that might prevent the emergence of drug-resistant mutants and which has a low toxicity. The design of 4′-*C*-substituted-2′-deoxynucleoside (4′SdN) was based on the working hypothesis. Studies on the synthesis and biological evaluation of 4′SdNs have proved the validity of the working hypothesis and resulted in the development of 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine (4′Ed2FA) (Fig. 1). This substance is highly potent against all HIV-1s and is expected to prevent the emergence of drug-resistant mutants, has a low toxicity, and is stable to enzymatic catabolism. Hence, 4′Ed2FA is an important key for its further development of potential therapeutics to solve the problems being encountered by the present HAART.

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4′Ed2FA

Fig. 1. Structure of 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine (4′Ed2FA).

Working Hypothesis of the Study

Design of the Nucleoside Derivatives That Might Be Effective for Drug-Resistant HIV-Mutants and Possibly Prevent the Emergence of New Drug-Resistant HIV-1 Mutants

The structures of the clinical NRTIs are shown in Figure 2. All belong to the ddN family. The ddN structure is considered essential for the nucleoside derivatives as the chain terminator of the reverse transcriptase (RT)-catalyzed biosynthesis of proviral DNA. However, HIV-1 mutants resistant to all NRTIs have emerged.

The emergence of HIV-1 mutants resistant to ddNs indicates that HIV-1 can acquire the ability to discriminate ddNs from physiological 2′-deoxynucleosides (dNs) and does not accept ddNs into the active center of RT and/or cuts off the incorporated ddNs from the proviral DNA terminus.

Therefore, the anti-HIV nucleoside that might prevent the emergence of drug-resistant HIV-1 mutants must satisfy the following two conditions:

1. To prevent discrimination by HIV-1, the nucleoside must have a structure very much like those of dNs. Because the striking difference of the ddN and dN structures is whether they have 3′-OH, the nucleoside must have 3′-OH.
2. In spite of having 3′-OH, the nucleoside must be the chain terminator of RT-catalyzed biosynthesis of proviral DNA.

Based on the following hypothesis, 4′SdN (Fig. 2) was designed as a nucleoside that could satisfy the mentioned conditions:

1. It would be difficult for HIV-1 to discriminate 4′SdN from dN because 4′SdN has all the functional groups of dN.
2. The introduction of a substituent at the 4′-position makes the 3′-OH into a very unreactive neopentyl-type secondary alcohol. Thus, the 3′-OH of 4′SdN will be used for HIV to mistake 4′SdN for dN, but is too unreactive to be used for the elongation of proviral DNA by RT. Therefore, 4′SdN could be the chain terminators of proviral DNA biosynthesis.
3. Steric hindrance between 3′-OH and 4′-substituent changes the conformation of the furanose ring of 4′SdN preferably to the 3′-endo conformation (*N*-type). This results in 4′SdN being less susceptible to both acidic and enzymatic degradation than that of dN and ddN.
4. Further, the electron-withdrawing 3′-OH makes 4′SdN more acid stable than does ddN even with purines. Thus, various purine derivatives can be made in this way.



Hiroshi Ohrui was born in 1942 in Tokyo, Japan. He received his B.S. (1965) and Ph.D. degrees (1971) from The University of Tokyo under the direction of Professor Masanao Matsui. He joined the Institute for Physical and Chemical Research (RIKEN) in 1966 as a researcher. In 1981, he moved to Tohoku University as Associate Professor in the Department of Food Chemistry, Faculty of Agriculture. In 1997, he was promoted to Full Professor, Graduate School of Life Sciences. In 2006, he moved to Yokohama College of Pharmacy as Professor. He worked for Dr. J. J. Fox at Sloan-Kettering Institute for Cancer Research (1972–1973) as Research Associate, and for Dr. J. G. Moffatt at Syntex Research (1973–1974) as a postdoctoral fellow. He has received several awards including The Agricultural Chemical Society of Japan Award for Young Scientists (1974), Inoue Prize for Science (2001), Japan Prize of Agricultural Science (2004), and The Japan Society for Analytical Chemistry Award (2004). His research interests cover organic synthesis, chiral discrimination, and medicinal chemistry. ■

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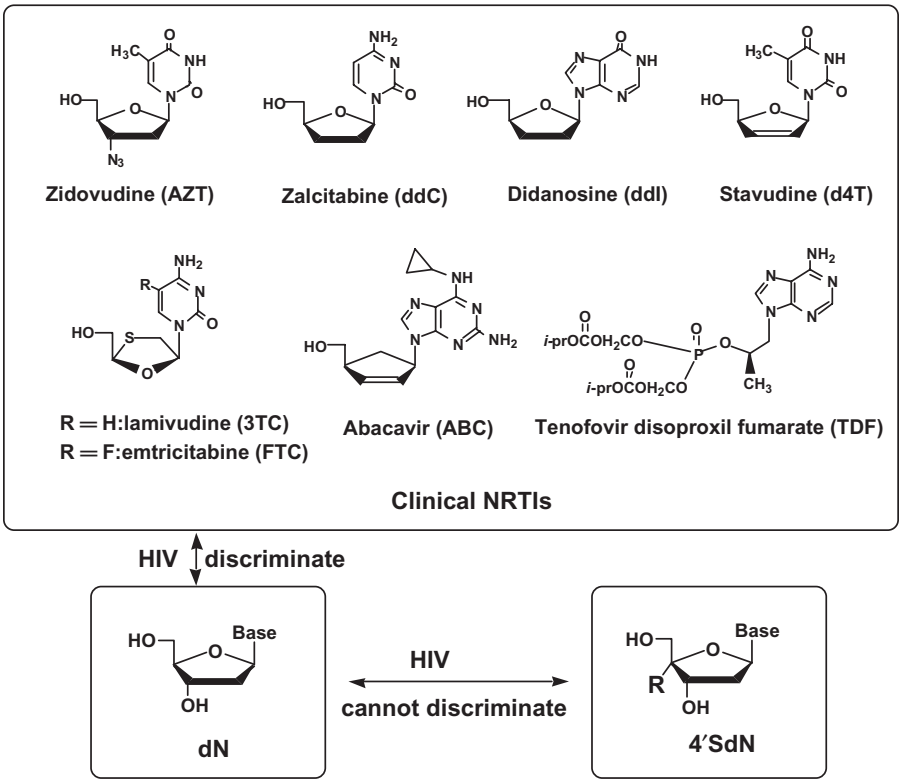


Fig. 2. Structures of clinical nucleoside reverse transcriptase inhibitors (NRTIs), physiologic 2′-deoxynucleoside (dN), and 4′-*C*-substituted-2′-deoxynucleoside (4′SdN). HIV = human immunodeficiency virus; dN = 2′-deoxynucleoside; 4′SdN = 4′-*C*-substituted-2′-deoxynucleoside.

5. The lipophilic substituent at the 4′-position imparts more lipophilicity to 4′SdNs, thus enabling them to penetrate the cell membrane efficiently. This may possibly enhance their bioavailability.

Method of Decreasing the Toxicity of Nucleoside Drugs Based on My Past Findings of the Relationship Between the Biological Activity and Structure of Nucleoside Derivatives

If DNA polymerases also mistake 4′SdN for dN, 4′SdN should be highly toxic. However, ddNs have been used as anti-HIV-1 drugs, meaning that DNA polymerases hardly accept ddNs as their substrates. Thus, the ability of DNA polymerases to distinguish substrates is superior to that of RT.

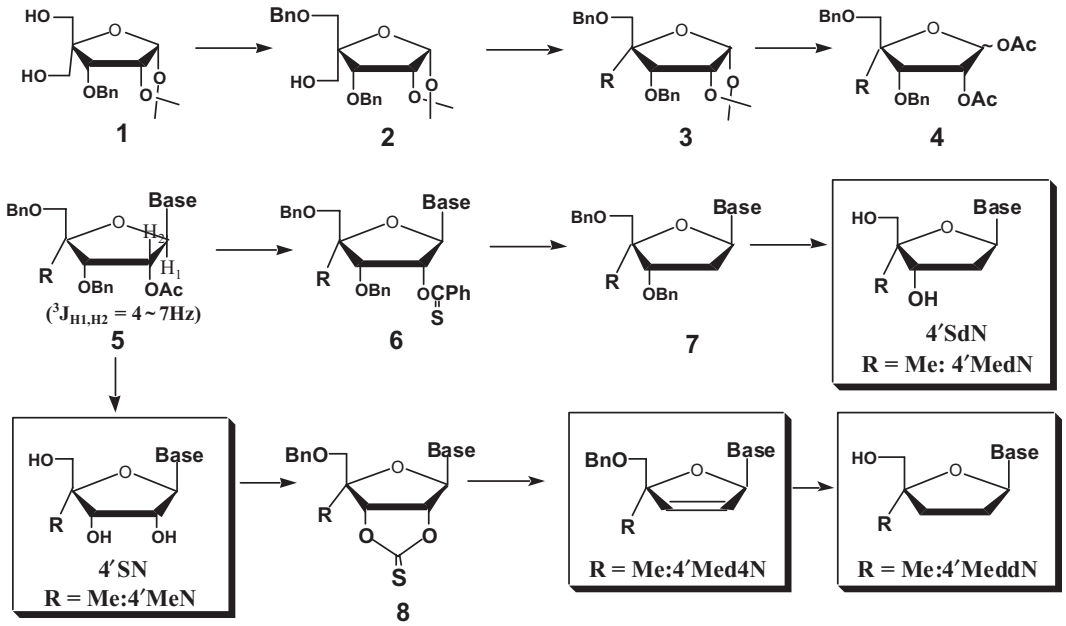
In the 1960s and 1970s, many organic chemists synthesized nucleoside derivatives modified at two or more positions of physiologic nucleosides expecting to get nucleoside derivatives having new and/or higher biological activity. However, none of these modified nucleosides exhibited antimicrobial activity. These results indicate that intracellular enzymes do not recognize the nucleoside derivatives modified at two or more positions of physiologic nucleosides as substrates. Thus, there is a chance of decreasing the toxicity of 4′SdNs further by additional modification.

Results and Discussion

Examination of the Validity of the Working Hypothesis With 4′-*C*-Methyl Nucleosides

At first, to examine the validity of the working hypothesis, 4′-*C*-methylnucleosides (4′MeNs), 4′-*C*-methyl-2′-deoxynucleosides (4′MedNs), 4′-*C*-methyl-2′,3′-dideoxynucleosides (4′MeddNs), and 4′-*C*-methyl-2′,3′-didehydrodideoxynucleosides (4′Med4Ns) were synthesized and evaluated for their biological activity.[3,4] They were synthesized by glycosidation of 1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-4-*C*-methyl-D-ribofuranose (**4**), prepared from D-glucose through 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**1**),[5] and nucleobases and then deoxygenation of the hydroxy groups of 4′MeN (Scheme 1). It should be noted that **4** took a longer reaction time to complete the glycosidation reaction than that of normal *ribo*-furanose derivatives, indicating the lower reactivity of **4**. In addition, the $^{3}J_{1',2'}$ value of 4′-*C*-substituted β-*ribo*nucleoside (**5**) was larger (4 ~ 7 Hz) than that of the normal

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Scheme 1. General synthetic scheme of 4′-*C*-substituted-2′-deoxynucleoside (4′SdNs) from D-glucose. 4′SdN = 4′-*C*-substituted-2′-deoxynucleoside; 4′MedN = 4′-*C*-methyl-2′-deoxynucleoside; 4′SN = 4′-*C*-substituted nucleoside; 4′MeN = 4′-*C*-methylnucleoside; 4′Med4N = 4′-*C*-methyl-2′,3′-didehydrodideoxynucleoside; 4′MeddN = 4′-*C*-methyl-2′,3′-dideoxynucleoside.

β-*ribo*nucleoside (0 ~ 1 Hz), indicating the change of the conformation of the furanose ring of **5**.

4′MedN showed remarkable biological activity (both anti-HIV-1 activity and toxicity), but 4′MeddN and 4′Med4N did not show any notable biological activity (Table 1). These results indicate the importance of the 3′-OH for biological activity. Further, we demonstrated that the 5-*O*-triphosphate of 4′-*C*-methyl-2′-deoxycytidine (4′MedCTP) was the chain terminator of the DNA polymerases.[6] The result indicates that 4′SdN was an NRTI, although further study of 4′MedCTP with RT was not performed.

Structure–Activity Relationship (SAR) of 4′SdNs

Next, to study the SAR of 4′SdNs and develop 4′SdNs having more potent anti-HIV activity and less toxicity than 4′MedNs, 4′SdNs having various kinds of 4′-*C*-substituents and nucleobases were synthesized and evaluated for their biological activity.[7–14] While we were working on our project, the anti-HIV-1 activity of several 4′SdNs was reported by the Syntex group[15–19] and others.[20–22] Therefore, the anti-HIV activities of 4′SdNs that we studied together with those reported by other groups are listed in Table 2. The SARs of 4′-*C*-substituted nucleosides against HIV-1 are summarized as follows:

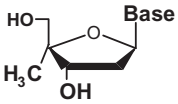
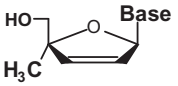
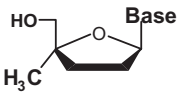
1. The estimated relative order of anti-HIV-1 activity is as follows: CN ≥ C≡CH > N_3 > CH═CH_2 > Me═Et > C≡C—CH_3. Interestingly, the order is the reverse of the $-\Delta G^0$ values between equatorial and axial substituents on a cyclohexane ring: CN < F < C≡CH < CH═CH_2 < Me ≤ Et < ^{t}Bu. Thus, these results indicate that the sterically less demanding substituent at the 4′-position gives more potent anti-HIV activity.
2. Purine analogs are generally less toxic than are pyrimidine analogs; the former generally have greater selectivity indices (SIs). Although 2′-deoxy-4′-*C*-ethynyl-5-fluorocytidine, which is a nucleoside derivative modified at two positions of physiologic 2′-deoxycytidine, gave a very acceptable SI with MT-4 cells, it was toxic with other cells (Kohgo, Yamasa Corporation, private communication).
3. *Arabino* analogs are less active and less toxic compared with their corresponding 2′-deoxy counterparts.
4. Both ddNs and 2′,3′-didehydrodideoxynucleosides (d4Ns), with some exceptions, do not show anti-HIV activity.
5. The *L*-isomers of 4′SdN have no anti-HIV activity,[12,22] although it is known that the *L*-enantiomer of 2′,3′-dideoxy-3′-thia-*L*-cyrtidine (3TC) is as active as the *D*-enantiomer and less toxic than the *D*-enantiomer.[23]

Synthesis of Purine Derivatives of 4′-*C*-Cyano-2′-Deoxynucleoside (4′CNdNs) and 4′-*C*-Ethynyl-2′-Deoxynucleoside (4′EdNs) and Their Anti-HIV Activity

The mentioned results led us to study the biological activity of purine derivatives of 4′CNdN and 4′EdN.[24] Although

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Table 1. Anti-human immunodeficiency virus activity of 4′-*C*-methyl-2′-deoxynucleosides.

Structure	Base	EC_{50} (μM)	CC_{50} (μM)	SI (CC_{50}/EC_{50})
 4′MedN	Ad	2.6	2.6	1.0
	Th	7.2	104	
	Cy	0.072	0.13	1.8
	Pu	1.9	>200	>100
 4′Med4N	Ad	>500	>500	−1
	Th	21	330	16
	Cy	350	350	
 4′MeddN	Ad	30	400	13
	Th	>500	>500	−1
	Cy	27	27	1
AZT		0.001	>20	>2020
ddA		47	>500	>11
d4T		4.1	>500	>120

EC_{50} = 50% effective concentration; CC_{50} = 50% cytotoxic concentration; SI = selectivity index; 4′MedN = 4′-*C*-methyl-2′-deoxynucleoside; 4′Med4N = 4′-*C*-methyl-2′,3′-didehydrodideoxynucleoside; 4′MeddN = 4′-*C*-methyl-2′,3′-dideoxynucleoside; AZT = 3′-azido-3′-deoxythymidine; ddA = 2′,3′-dideoxyadenosine; d4T = 2′,3′-didehydro-3′-didehydro-3′-deoxythymidine.

4′SdNs in our project had been synthesized by the glycosidation of 4-*C*-substituted-D-*ribo*furanose derivatives and nucleobases (Scheme 1), this synthetic route incurred some problems, as follows:

1. Preparation of 4-*C*-substituted-D-*ribo*furanoses and their conversion to the desired 4′SdNs require multistep reactions, and their total yields are low.
2. 4-*C*-Substituted-D-*ribo*furanose derivatives have low reactivity in glycosidation reactions, especially when the substituent is an electron-withdrawing group such as a cyano group.

(In contrast, the low reactivity of the anomeric position of 4-*C*-substituted furanose derivatives indicates that 4′SdN will be more stable to acidic hydrolysis than dN, ddN, and d4N will.)

These problems prompted us to develop another method of preparing purine derivatives of 4′SdN, which starts from dNs (Scheme 2).[24]

The biological activities of the purine derivatives of 4′CNdN and 4′EdN are summarized as follows (Table 3):

1. Some of the purine derivatives of 4′CNdN have high anti-HIV activity, but none of them gives an acceptable SI.
2. All the purine derivatives of 4′EdNs have both high anti-HIV activity and an acceptable SI.

Anti-HIV Activity of 4′SdNs Against Drug-Resistant HIV-1 Mutants[13,14,24]

Many 4′SdNs showed very high anti-HIV activity against wild-type HIV-1. However, the most important point of our study is whether they are active against drug-resistant HIV-1 mutants. The anti-HIV activity of selected 4′SdNs against HIV-1 mutants resistant to various NRTIs is listed in Table 4.

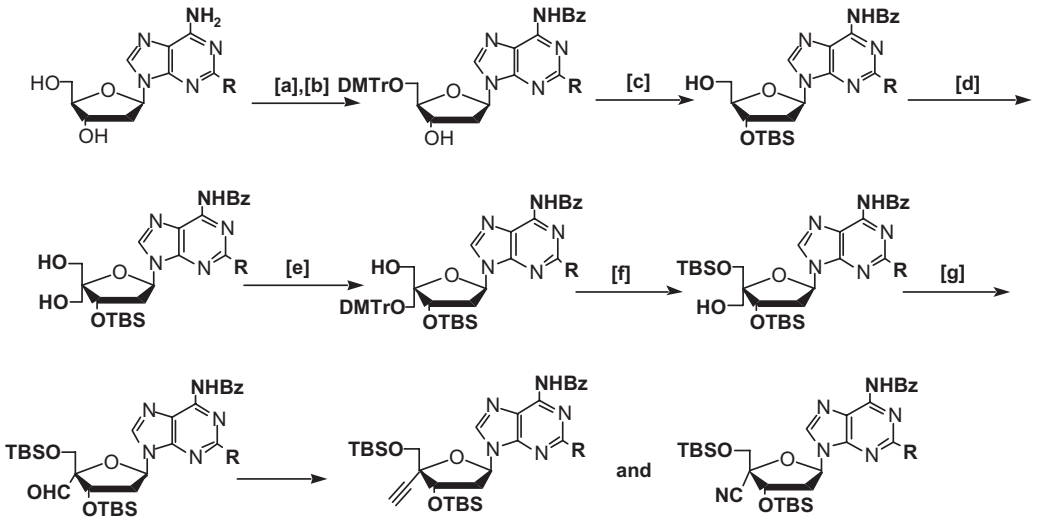
It is noteworthy that the three cytidine derivatives maintained their activity against the drug-resistant HIV-1 mutants, although the activity of 4-*C*-ethynyl-*D*-arabinofuranosylcytosine (4′EaraC) and 4′MedC decreased significantly against M184V, M184I, and 41/69/125/SG. The three purine derivatives, 2′-deoxy-4′-*C*-ethynyladenosine (4′EdA), 2′-deoxy-4′-*C*-ethynyl-2-aminoadenosine (4′Ed2AA), and 2′-deoxy-4′-*C*-ethynylguanosine (4′EdG) except for 2′-deoxy-4′-*C*-ethynylinosine (4′EdI) were highly potent against all drug-resistant HIV-mutants (4′EdI was much less active than the former three derivatives, especially against M184V). Additionally, the three were also active against a non-nucleoside reverse transcriptase inhibitor-resistant Y181C. Further, the three purine derivatives were highly potent against the HIV-1s isolated from seven heavily drug-experienced patients with acquired immune deficiency syndrome (AIDS) as efficiently as against wild-type HIV-1.[13,14,24] Thus, 4′EdA, 4′Ed2AA, and 4′EdG were highly potent against all the existing HIV-1s.

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Table 2. Anti-human immunodeficiency virus activity of 4′-*C*-substituted-2′-deoxynucleosides.

Compound	EC_{50} (μM)[a]	CC_{50} (μM)	SI
4′-*C*-Cyanothymidine	0.002	1	500
4′-*C*-azidothymidine	0.01	8	300
4′-*C*-ethynylthymidine	0.83	>400	>482
4′-*C*-ethynylarabinofuranosylthymine	119	>400	>3.4
4′-*C*-azidomethylthymidine	2.1	333	159
4′-*C*-methylthymidine	7.2	104	14
4′-*C*-ethylthymidine	>400	400	ND
4′-*C*-methoxythymidine	8.49	200	24
4′-*C*-vinylthymidine	>400	>400	ND
4′-*C*-hydroxymethylthymidine	7.0	>400	>57
4′-*C*-propylthymidine	>100	>100	ND
4′-*C*-Cyano-2′-deoxycytidine	0.0012	0.17	142
4′-*C*-azido-2′-deoxycytidine	0.004	0.21	52
4′-*C*-ethyny-2′-deoxycytidine	0.0048	2.2	482
L-4′-*C*-ethynyl-2′-deoxycytidine	>400	>400	ND
4′-*C*-ethynyl-2′-deoxy-5-fluorocytidine	0.030	>100	>3333
4′-*C*-ethynylarabinofuranosylcytidine	0.043	2.0	46.5
4′-*C*-methyl-2′-deoxycytidine	0.015	1.0	66.7
4′-*C*-fluoromethyl-2′-deoxycytidine	0.0068	0.12	18
4′-*C*-methyl-2′-deoxyadenosine	2.6	2.6	1
4′-*C*-azido-2′-deoxyadenosine	0.13	50	385
4′-*C*-ethyny-2′-deoxyadenosine	0.098	16	1630
2′,3′-dideoxy-3′-thia-*L*-cyrtidine (3TC)	0.10	>100	>1000
3′-azido-3′-deoxythymidine (AZT)	0.0032	29.4	9190

[a]Anti-HIV activity was determined by MTT assay. MT-4 cells and HIV-1 LAI were employed. EC_{50} = 50% effective concentration; CC_{50} = 50% cytotoxic concentration; SI = selectivity index; ND = not determined; MTT = 3-(4′5-dimethylthazol-2-yl)-2-5-diphenyl tetrazolium bromide.



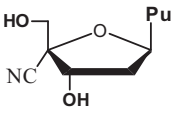
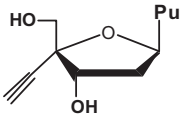
[a] 1.TMSCl, py, 0°C; 2. BzCl, py, 0°C; 3. NH_4OH, H_2O, 0°C. [b] DMtrCl, py, rt. [c] 1.TBSCl, imidazole, DMF, rt; 2.TsOH, H_2O, MeOH, $CHCl_3$, 0°C. [d] 1.EDC·HCl, py, TFA, toluene, DMSO, rt; 2. aq. CH_2O, 1N NaOH, dioxane, rt; 3. $NaBH_4$, EtOH, 0°C, [e]: DMTrCl, Et_3N, CH_2Cl_2, 0°C. [f] 1.TBSCl, imidazole, DMF, rt; 2. TsOH· H_2O, MeOH, $CHCl_3$, rt, 0°C. [g] EDC· HCl, py, TFA, toluene, DMSO, rt.

Scheme 2. Synthetic scheme of purine derivatives of 4′-*C*-cyano-2′-deoxynucleoside and 4′-*C*-ethynyl-2′-deoxynucleoside from 2′-deoxynucleosides. TFA = trifluoroacetic acid; DMF = dimethylformamide; rt = room temperature.

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These results let us suppose that the three purine 4′EdNs could even prevent the emergence of drug-resistant HIV-1s. It should be noted that 4′EdG showed toxicity to *HeLa* cells at 52 μM; thus, 4′EdG will be very toxic.

Table 3. Anti-human immunodeficiency virus activity of purine derivatives of 4′-*C*-cyano-2′-deoxy (4′CNd) and 4′-*C*-ethynyl-2′-deoxynucleoside.

Structure	Base	EC_{50} (μM)[a]	CC_{50} (μM)	SI
	A	0.051	12	235
	I	0.051	23	451
	2AA	0.00079	0.034	43
	G	0.000188	0.034	181
	A	0.098	16	1630
	I	0.15	216	1440
	2AA	0.0003	0.82	2733
	G	0.0014	1.5	975
AZT		0.0032	29.4	9190

[a]Anti-HIV activity was determined by MTT assay. MT-4 cells and HIV-1 LAI were employed.
EC_{50} = 50% effective concentration; CC_{50} = 50% cytotoxic concentration; SI = selectivity index; 2AA = 2-aminoadenine; AZT = 3′-azido-3′-deoxythymidine.

Mouse Toxicity of Purine 4′EdNs

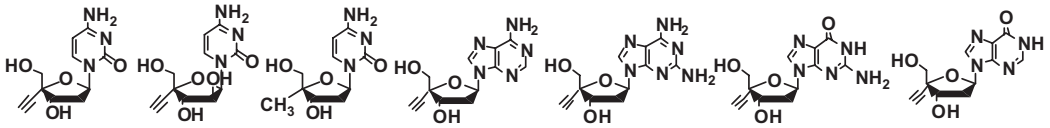
Because the three purine derivatives of 4′EdNs showed high activity against all HIV-1s and acceptable SIs, the mouse toxicity of these 4′EdNs was next examined (Table 5).[22,24]

All eight mice survived after a single dosage of 3 ~ 100 mg kg^{-1} of 4′EdA and 4′EdI by both intravenous and oral administrations, but all mice died after a single dosage of 3 mg kg^{-1} of 4′Ed2AA and 4′EdG irrespective of the administration method (Fig. 3). Thus, it seemed that 4′EdA and 4′EdI were not toxic, but 4′Ed2AA and 4′EdG were highly toxic. However, in mice, it was found that 4′EdA and 4′Ed2AA were easily converted to 4′EdI and 4′EdG, respectively, by adenosine deaminase.[24,25] These results showed that the actual toxicity of 4′EdA and 4′Ed2AA to animals is hard to estimate.

Anti-HIV Activity of 4′EdA Derivatives Stable to Adenosine Deaminase

The fact that both 4′EdA and 4′Ed2AA are deaminated by adenosine deaminase prompted us to prepare 4′EdA derivatives stable to the enzyme. It has been known that the adenine derivatives having a halogen atom at the 2-position of the base are stable to adenosine deaminase.[26,27] Therefore, at first, 4′Ed2FA (Fig. 1) was synthesized and evaluated for anti-HIV

Table 4. Anti-human immunodeficiency virus activity of selected 4′-*C*-substituted-2′-deoxynucleoside.



	EC_{50} (μM)[a]									
Compound	HXB2	KH65R	L74V	41/215	M184V	M184I	41/69/125/SG	MDR	Y181C	CC_{50} (μM)
4′EdC	0.0012	0.0008	0.0013	0.006	0.0024	0.0026	0.015	0.0012	0.0021	>200
4′EaraC	0.0071	0.015	0.026	0.026	0.71	0.48	0.17	0.0079	0.016	>200
4′MedC	0.0058	0.0071	0.0052	ND	0.2	0.74	ND	0.0033	ND	>200
4′EdA	0.008	0.0033	0.004	0.012	0.047	0.022	0.065	0.0062	0.011	>200
4′Ed2AA	0.0014	0.00035	0.0007	0.0017	0.0059	0.0027	0.0041	0.001	0.0008	>200
4′EdG	0.007	0.001	0.0012	0.019	0.008	0.0041	0.0068	0.0048	0.01	52
4′EdI	0.81	0.25	0.61	1.3	1.6	1.5	2.2	0.51	ND	>200
AZT	0.022	0.02	0.02	0.3	0.01	0.017	1.6	15.3	0.014	>100
3TC	0.71	ND	ND	ND	>100	>100	9.9	1.1	ND	>100
ddC	0.2	3.0	1.5	ND	2.2	ND	1.3	5.5	ND	>100
ddI	3.9	12.7	19.5	3.6	10.1	ND	12.2	25	ND	>100

[a]Anti-HIV activity was determined with MAGI assay.
EC_{50} = 50% effective concentration; CC_{50} = 50% cytotoxic concentration; AZT = 3′-azido-3′-deoxythymidine; ND = not determined.

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Table 5. Toxicity of purine derivatives of 4′-*C*-ethynyl-2′-deoxynucleosides to mice. [a]

	Intravenous administration		Oral administration	
	Dose ($mg\,Kg^{-1}$)	Mortality (%)	Dose ($mg\,Kg^{-1}$)	Mortality (%)
4′EdA	100	0	100	0
and	10	0	10	0
4′EdI	3	0	3	0
	1	0	1	0
4′Ed2AA	100	100 (1 day) [b]	100	100 (1 day)
	10	100 (2 days)	10	100 (2 days)
	3	0	3	100 (2 days)
	1	0	1	0
4′EdG	100	100 (1 day)	100	100 (1 day)
	10	100 (2 days)	10	100 (4 days)
	3	100 (4 days)	3	100 (4 days)
	1	0	1	0

[a]Six-week-old ICR male mice were employed.
[b]Numbers in parentheses represent survival days of mice after administration.

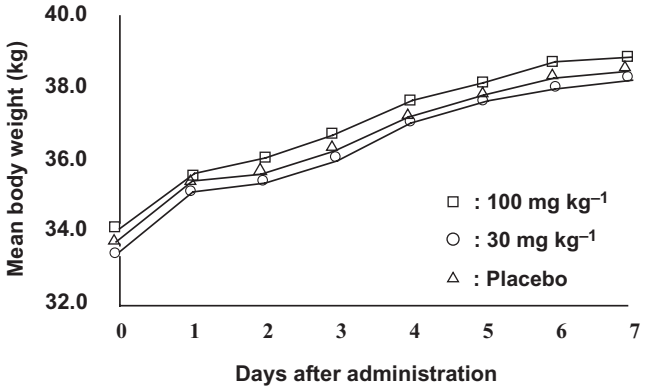


Fig. 3. Body weight change of mice after a single dosage of 2′-deoxy-4′-*C*-ethynyl-2-fluioroadenosine, administrated orally or intravenously to ICR mice.

activity[25,28] and stability to both adenosine deaminase and acidic conditions.

Because 4′Ed2FA is a nucleoside derivative modified at two positions (4′-position and 2-position) of physiologic 2′-deoxyadenosine, the toxicity of 4′Ed2FA is expected to be lower than that of 4′EdA. As shown in Table 6, 4′Ed2FA is highly potent against all HIV-1s including multidrug-resistant and M184V HIV-1 mutants and has an acceptable SI (110,000).[25,28]

Expectedly, 4′Ed2FA was completely stable to adenosine deaminase under the conditions where 4′EdA was completely deaminated in 60 min and, further, fairly stable under acidic conditions. Thus, in 120 min only a small part (3%) of 4′Ed2FA was decomposed under the acidic conditions of gastric juices (pH 1.06) at 24°C, while 2′,3′-dideoxyadenosine (ddA) was completely decomposed in 5 min.

Appearance of Two Papers Claiming that 3′-OH of 4′EdNs Is the Cause of the Toxicity of 4′SdNs

While we were working on our project, two papers on the role of 3′-OH of 4′SdNs appeared in which the authors claimed that 3′-OH was the cause of the toxicity of 4′SdNs. Tanaka and coworkers reported[29] that 4′-*C*-ethynyl-2′,3′-didehydro-3′-deoxythymidine (4′Ed4T) was more potent against wild-type HIV-1 and less toxic than 2′,3′-didehydro-3′-deoxythymidine (d4T). Further, they claimed that 3′-OH of 4′SdNs was the cause of the toxicity. However, they did not note the activity of 4′Ed4T against drug-resistant HIV-1s, the most important point of the chemotherapeutic agent in overcoming the problems of the existing HAART.

In contrast, Marquez and coworkers reported[30] that 3′-OH of 4′SdNs played an important role in the phosphorylation of 5′-OH by cellular kinases, but was the cause of the toxicity of 4′SdNs. This determination was based on their results that 4′-*C*-ethynyl-2′,3′-dideoxycytidine was inactive against HIV-1 in cellular systems, but its 5′-*O*-triphosphate (4′EddCTP) was more active than 3′-azido-3′-deoxythymidine (AZT)-5′-*O*-triphosphate (AZTTP) against the RT of wild-type HIV. They also reported that 4′EddCTP was much less active against the RT of the M184V mutant than against the RT of wild-type HIV-1. In addition, the *L*-isomer of 4′EddCTP was not active against the RT of the M184V mutant.

Anti-HIV Activity of 2′,3′-Dineoxy (dd-) and 2′,3′-Didehydrodideoxy (d4) Analogs of 4′Ed2FA

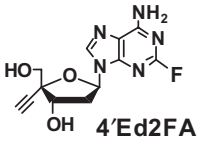
The two papers previously cited caused us to synthesize the dd- and d4-analogs of 4′Ed2FA and evaluate their anti-HIV activity.[25] The anti-HIV activities of 2′,3′-dideoxy-4′-*C*-ethynyl-2-fluoroadenosine (4′Edd2FA) and 2′,3′-didehydro-dideoxy-4′-*C*-ethynyl-2-fluoroadenosine (4′Ed42FA) together with that of 2′-deoxy-4-*C*-ethynyl-2′-chloroadenosine (4′Ed2ClA) are listed in Table 7. Although both 4′Ed42FA and 4′Edd2FA showed some activity against wild-type HIV-1, they significantly lost any activity against drug-resistant HIV-1s. 4′Ed2ClA is active against all HIV-1s, but its activity is lower than that of 4′Ed2FA.

These results indicated that the 3′-OH played important roles not only for the phosphorylation of 5′-OH, but also for the activity against drug-resistant HIV-1s.

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Table 6. Anti-human immunodeficiency virus activity of 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine (4′Ed2FA).

HIV-1	Compound	EC_{50} (nM)	CC_{50} (nM)	SI
Wild type	4′ Ed2AA	0.34	900	2600
	4′ Ed2FA	0.068	7500	110,000
	AZT	3.2	29,400	9190
M184V	4′Ed2FA	3.1		
	AZT	16.0		
MDR	4′Ed2FA	0.15		
	AZT	15,300.00		

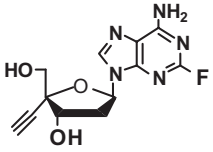


4′Ed2FA

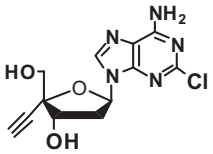
m.p. (decomp.) 224.4–224.6°C [α] D + 4.64 (σ = 0.5, DMSO)

HIV-1 = human immunodeficiency virus type 1; EC_{50} = 50% effective concentration; CC_{50} = 50% cytotoxic concentration; SI = selectivity index.

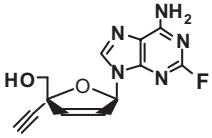
Table 7. Anti-human immunodeficiency virus activity of 4′-*C*-substituted-2′-deoxy-2-haloadenosines.



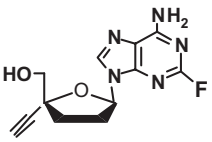
4′Ed2FA



4′Ed2ClA



4′Ed42FA



4′Edd2FA

	Anti-HIV activity (MAGI assay, μg)		
Compound	$HIV\text{-}1_{wild}$	$HIV\text{-}1_{MDR}$	$HIV\text{-}1_{M184V}$
4′Ed2FA	0.00020	0.00014	0.0031
4′Ed2ClA	0.0019	0.0084	0.01
4′Ed42FA	0.80	0.15	1.8
4′Edd2FA	0.94	8.7	97
AZT	0.022	15.3	0.01
3TC	0.71	1.1	>100

MAGI = multinuclear activation of galactosidase indicator; HIV-1 = human immunodeficiency virus type 1; AZT = 3′-azido-3′-deoxythymidine; 3TC = 2′,3′-dideoxy-3′-thia-*L*-cyrtidine.

Toxicity of 4′Ed2FA to Mice and Inhibition of DNA Polymerases

Because 4′Ed2FA is stable to adenosine deaminase, its mouse toxicity was examined.[25,28] 4′Ed2FA did not show any acute toxicity to mice by either oral or intravenous administration up to 100 mg kg^{-1} (Figure 3; Table 8).

It is known that the toxicity of NRTIs to animals is caused by their inhibition of mitochondrial DNA polymerase γ. The 50% effective concentration (EC_{50}) of 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine-5-*O*-triphosphate (4′Ed2FATP) to inhibit the incorporation of 2′-deoxyadenosine-5-*O*-triphosphate (dATP) mediated by human mitochondrion DNA polymerase was 10 μM, which was significantly higher than the 0.2 μM of 2′,3′-dideoxyadenosine-5-*O*-triphosphate (ddATP).[31] The EC_{50} values of 4′Ed2FATP against DNA polymerase α and β were higher than 200 μM. These results indicate that the DNA polymerases scarcely recognize 4′Ed2FATP, a derivative modified at two positions of physiologic dATP, as their substrate but that RT does.

Intracellular Metabolism of 4′Ed2FA[31]

The amounts of all fractions of intracellular 4′Ed2FA metabolites, (4′Ed2FA–monophosphate, 4′Ed2FA–diphosphate, and 4′Ed2FATP) increased proportionately with an increase in the

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Table 8. Toxicity of 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine after a single dosage to ICR mice.

	Survivors/total	
Dose (mg Kg^{-1})	p.o	i.v.
Placebo	8/8	8/8
1	8/8	8/8
3	8/8	8/8
10	8/8	8/8
30	8/8	8/8
100	8/8	8/8

concentration of intracellular 4′Ed2FA, while compared to AZT diphosphate and AZTTP, only AZT monophosphate markedly increased with an increase in intracellular AZT concentration. The intracellular half-life ($T_{1/2}$) of 4′Ed2FATP was ~18 h in complete expansion media (CEM) cells, MT4 cells, and multinuclear activation of galactosidase indicator (MAGI)–CCR5 cells ($T_{1/2}$ of AZTTP was 3 h). About 50% of the cells were protected against the infection of HIV-1 for 24 h after removal of extracellular 4′Ed2FA in both MT4 cells and MAGI cells cultured in the presence of 0.1 μM of 4′Ed2FA.

These results indicate that 4′Ed2FA, 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine-5-*O*-diphosphate (4′Ed2FADP), and 4′Ed2FATP are very stable against intracellular enzymatic catabolism.

Summary

A study of the synthesis and biological evaluation of 4′EdNs was conducted according to a proposed working hypothesis based on the fundamentals of both organic chemistry and biochemistry. Old scientific evidence proved the validity of the working hypothesis and resulted in the development of 4′Ed2FA, which is highly potent against all HIV-1s, is stable to intracellular enzymatic catabolism and acidic degradation, has a very long intracellular $T_{1/2}$, does not greatly inhibit DNA polymerase γ, and does not have acute mouse toxicity.

These results strongly suggest that 4′Ed2FA deserves further study for the development of a highly potent therapeutic agent for HIV-1 infection (AIDS), and which may solve the problems of the existing HAART.

I am looking forward to the results of a further study.

In addition, it should be noted that 4′Ed2FA could be an ideal drug for both HIV and hepatitis B virus (HBV) infections.

HBV is a DNA virus. However, it also belongs to the family of retroviruses because it uses RT when it replicates. It was found that the NRTIs for HIV are also active against HBV, and 3TC and adefovir dipivoxil have been used as anti-HBV drugs.[32,33] However, an HBV resistant to these drugs has emerged.[32,33] Therefore, the development of an anti-HBV drug that prevents the emergence of a drug-resistant HBV is needed. Because 4′Ed2FA is highly active against 3TC-resistant HIV and might prevent the emergence of drug-resistant HIV, it is also expected to be active against drug-resistant HBV.

Thus, 4′Ed2FA could be an ideal drug for use against HBV infection.

The work described in this paper was achieved through the tremendous efforts of the many coworkers whose names appear in the cited references.

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Annexure - 4

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(54) **2'-FLUORO-4'-SUBSTITUTED NUCLEOSIDES, THE PREPARATION AND USE**

2'-FLUOR-4'-SUBSTITUIERTE NUKLEOSIDE, HERSTELLUNG UND VERWENDUNG

2'-FLUORO NUCLÉOSIDES SUBSTITUÉS EN POSITION 4', LEUR PRÉPARATION ET LEUR UTILISATION

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- **KLAUS KLUMPP ETAL: "2'-Deoxy-4'-azido nucleoside analogs are highly potent inhibitors of hepatitis C virus replication despite the lack of 2'-alpha-hydroxyl groups" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, INC, US LNKD- DOI: 10.1074/JBC.M708929200, vol. 283, no. 4, 25 January 2008 (2008-01-25), pages 2167-2175, XP002517080 ISSN: 0021-9258**

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EP 2 177 527 B1

Description

Field of the Invention

[0001] This invention relates to nucleoside analogues, preparation methods and uses thereof, and more particularly, relates to 2'-fluorine -4'substituted nucleoside analogues, preparation methods and uses thereof.

Background of the Invention

[0002] The first case of AIDS patient was discovered in the United States in 1981. It was until in 1983 that the AIDS virus (HIV) is isolated and identified by scientists, revealing that HIV composed of 9749 nucleotides only is categorized into the Lentivirinae in Retroviridae. At present, the replication cycle of HIV virus has been made generally clear. According to relevant research findings, the reproduction process of the HIV virus can be divided into the following steps: the viral adsorption, invasion and uncoating, the reverse transcription, the integration of virus as well as the synthesis, the assembly, the release and the maturing of viral RNA and protein. Each of these steps can be used as a target for screening the Anti-HIV drugs, and the protein synthesis and the viral genome DNA replication are the most critical steps. At present, the screening of anti-HIV drugs is focused on finding the inhibitors for these specific enzymes, including the reverse transcriptase (RT) inhibitors, the protein synthesis inhibitors and the reverse transcriptase initiation inhibitors. HIV-reverse transcriptase is a multifunctional enzyme, which also has the activities of RNA dependent DNA polymerase, DNA dependent DNA polymerase and Rhase H. Initially, DNA chain(-) synthesis of HIV is processed with the virus genome RNA acting as a template and the host cell RNA as a primer, and then the synthesis of DNA chain (+) is completed in the same way. After the completion of the reverse transcription, all the genetic information carried by HIV is transformed from single-stranded RNA into double-stranded mDNA. Reverse transcriptase inhibitors can prevent the extension of mDNA and interfere with the HIV reverse transcription process, and thus become the drugs for the chemotherapy of AIDS. According to different mechanisms of the inhibitors, these drugs could be divided into two categories: 1) the nucleoside reverse transcriptase inhibitors which realize anti-HIV actions by being inserted into the viral DNA to promote the viral DNA to become defective DNA, resulting in the invalid duplication after integration of HIV with host cell during HIV DNA reverse transcription, and the available drugs include Zidovudine (AZT), Didanosine (ddI), Zalcitabine (ddC), Stavudine (d4T), Lamivudine (3TC), Abacavir; 2) non-nucleoside reverse transcriptase inhibitors, and mechanisms thereof are to prevent HIV RNA from being directly connected to the RT and thus being encoded into DNA. The available drugs include Nevirapine, Delavirdine and Efavirenz.

[0003] Zidovudine, the first commercial anti-HIV drug that was put into market in 1987, is a nucleoside reverse transcriptase inhibitor. This drug can alleviate the symptoms and prolong the lives of the patients, although it has much toxicity and failed to cure any patient. Later, several anti-HIV nucleoside analogue agents have also become available in the market. Therefore, nucleoside analogues are considered as an important class of compounds with anti-HIV activity. However, these drugs have some shortcomings at present. On the one hand, their effect is limited; on the other hand, severe toxic and side effects and drug resistance will be generated in a long-term administration. Accordingly, the synthesis of new nucleoside analogues is still an important trend in the research. In order to find more effective nucleoside anti-viral agents, diversified chemical modifications have been made for the nucleosides, including the fluorin-containing nucleoside analogues (Clark, J. PCT Patent Appl., WO 2005003174; Ismaili, H.M.A. PCT Patent Appl., WO 0160315A22001). Relevant studies have shown that these compounds have anti-viral activity at different levels, and are a new type of compounds with anti-viral activities. However, in the currently available literatures, it is not found any relevant report on the synthesis of 2'-fluoro-4 '-substituted-D-and L-nucleoside analogues of the present invention as well as on their applications in the preparation of anti-HIV, anti-HBV and anti-HCV drugs.

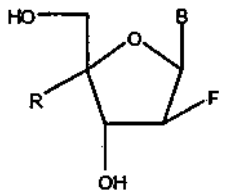
[0004] YUN-HA JIN et al (Arch. Pharm. Res., vol 18, N°5 PP 364-365 (1995) also discloses antiviral compounds. Nevertheless, said compounds only possess weak anti-HSV-1 and -2 activities.

Summary of the invention

[0005] It is the primary object of the present invention to provide 2'-fluoro-4 '-substituted nucleoside analogues. It is another object of the present invention to provide the synthesis method for such compounds. It is a further object of the present invention to provide the uses of such compounds in pharmacology.

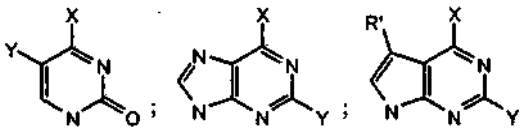
[0006] To achieve the objects of the present invention, the technical solutions are provided as follows:

2'-fluoro-4'- substituted nucleoside analogues of the present invention has the structure of the general formula (I):



(I)

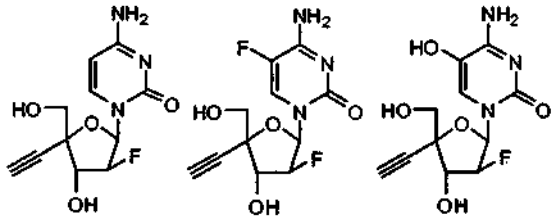
wherein :

B= 

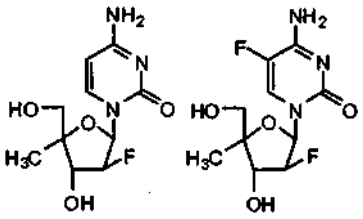
R = CH_3, CN, N_3, C≡CH;
R' = H, F;
X = F, NH_2;
Y = H, CH_3, F, OH, NH_2

[0007] The above compounds may exist in the form of salts, which are generated through the reaction between the active compounds (I) or their 5'-phosphate esters and organic or inorganic acid.

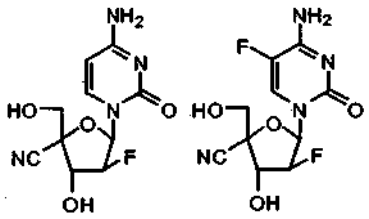
[0008] The above compounds may be one of the following compounds, but are not limited to these compounds:



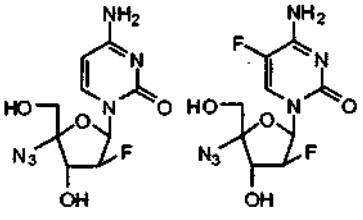
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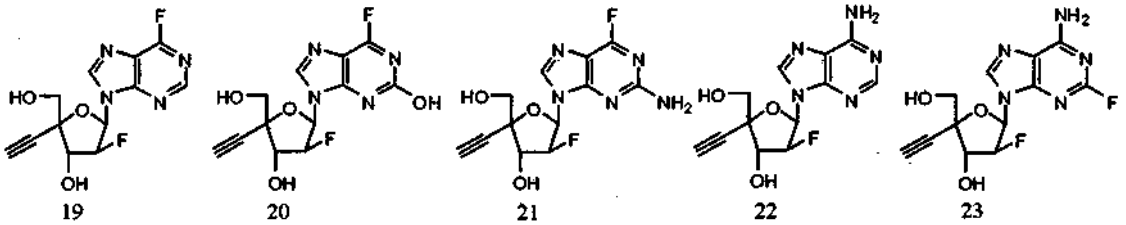
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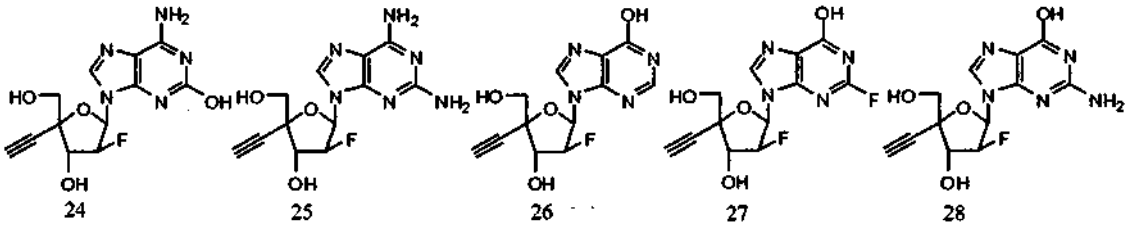


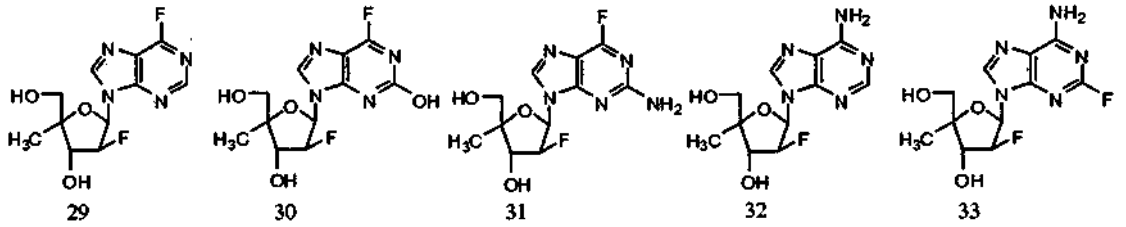
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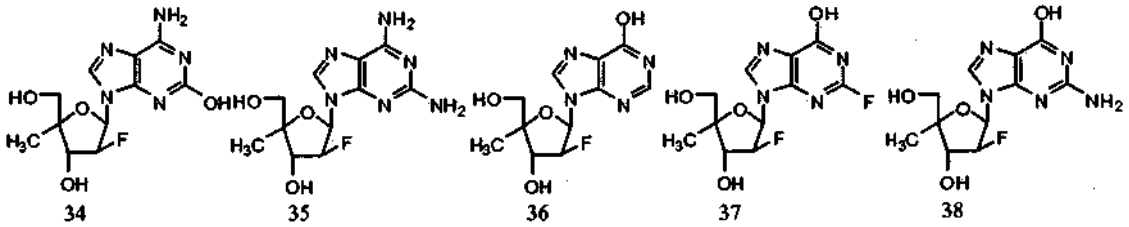


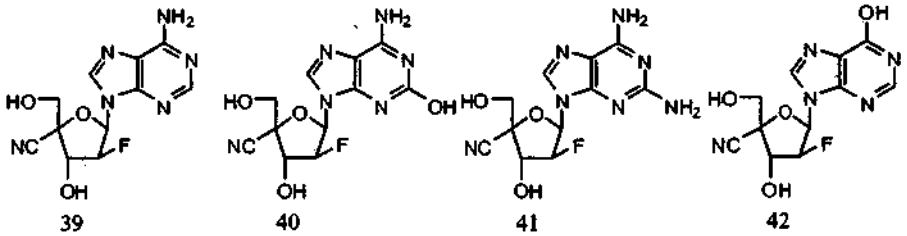
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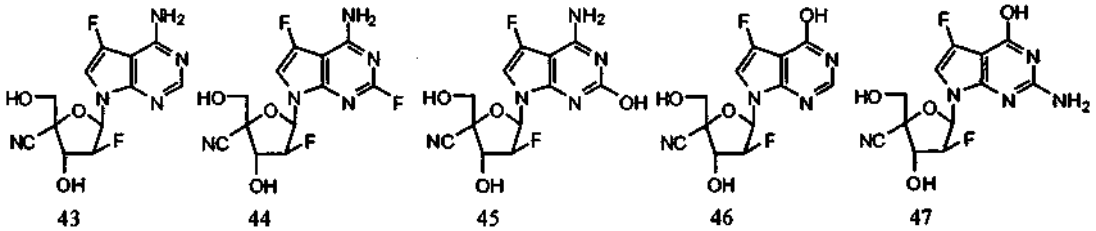

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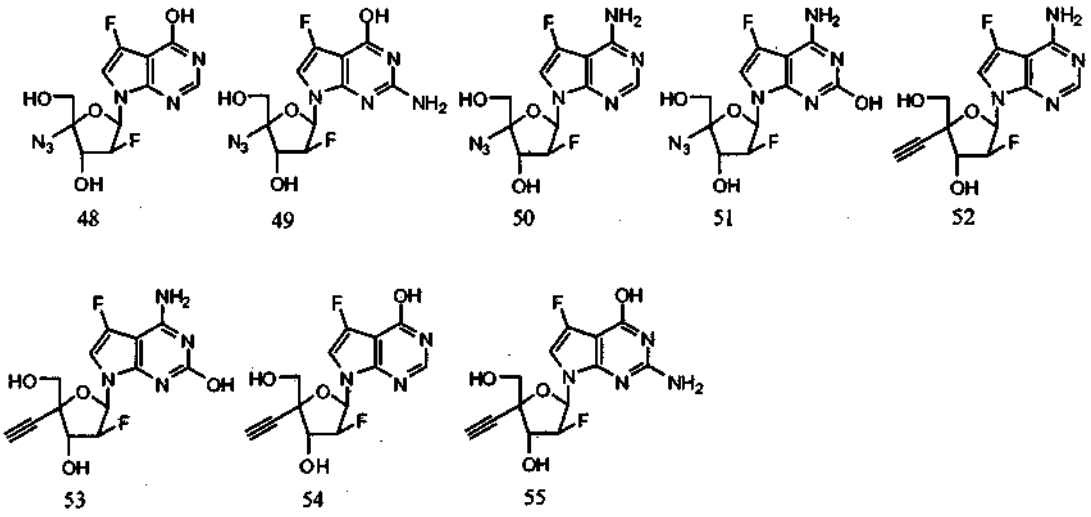

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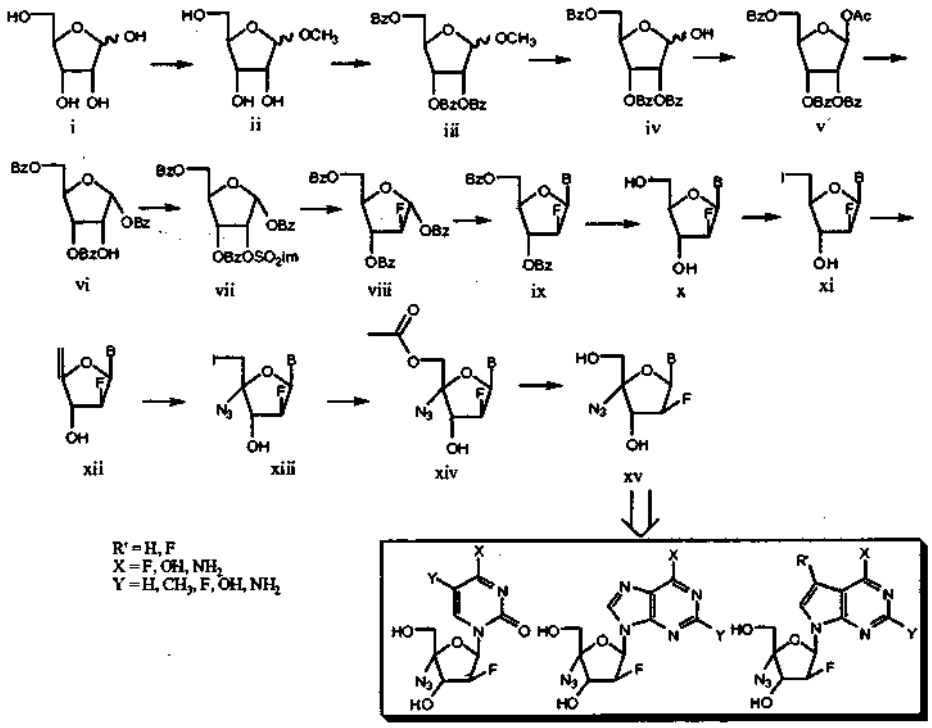

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[0009] The present invention also relates to the uses of 2'-fluoro-4 '-substituted nucleoside analogues in the preparation of medicine such as anti-viral drugs for HBV or HCV or HIV, wherein said anti-viral drugs for HBV are anti-hepatitis B virus drugs; said anti-viral drugs for HIV are anti-AIDS drugs; and said anti-viral drugs for HCV are anti-hepatitis C drugs. The present invention also relates to the preparation reaction of 2'-fluoro-4 '-substituted nucleoside analogues. Where $R=N_3$, the reaction path is as follows:



[0010] The synthesis of compound ii: The compound i (D-ribose or L-ribose) is dissolved in HCl/ MeOH. The solution is stirred in a water bath under hermetization and warm preservation. Pyridine is added into the solution to terminate the reaction, and then the solution is drawn off under a reduced pressure, so as to obtain light yellow syrup-like compound ii.

[0011] The synthesis of compound iii: The compound ii is dissolved in dry pyridine, and the solution is slowly added dropwise with benzoyl chloride in an ice-salt bath, and then stirred for reaction at a room temperature. After the reaction is completed, the reaction solution is poured into ice water, and extracted with chloroform. The organic layer is washed sequentially with ice water, pre-cooled sulfuric acid and saturated sodium bicarbonate until the water layer shows weak alkaline, then washed with ice water until the water layer shows neutral. The solution is dried over anhydrous sodium sulfate, and then drawn off under a reduced pressure, so as to obtain light yellow syrup-like compound iii.

[0012] The synthesis of compound iv: Benzoyl compound iii is dissolved in chloroform, red phosphorus is added, bromine is slowly added dropwise while stirring the solution in an ice-water bath. It is stirred for about 30 minutes. Then, the solution is slowly added dropwise with ice water and stirred at a room temperature. Broken ice is added into the solution and the solution is stirred until the ice is melted, and then the reaction solution is poured into ice water and extracted with chloroform. The organic layer is washed with saturated sodium bicarbonate until the water layer shows weak alkaline, and then washed with ice water until the water layer shows neutral. The solution is dried over anhydrous sodium sulfate, and then drawn off under a reduced pressure, so as to obtain dark yellow syrup. Finally the syrup is separated by silica gel column, so as to obtain white dry syrup compound iv.

[0013] The synthesis of compound v: The compound iv is dissolved in dry pyridine, the solution is slowly added dropwise with acetic anhydride in an ice water bath, and then stirred for about 30 minutes. Then, the ice water bath is removed, and the solution is stirred at a room temperature for about 7 hours, and then is heated up to 40°C. This temperature is kept for about 1 hour. Broken ice is added into the solution, and the solution is stirred until the broken ice is melted. Then, the reaction solution is poured into ice water and extracted with chloroform. The organic layer is washed sequentially with ice water, pre-cooled sulfuric acid and saturated sodium bicarbonate until the water layer shows weak alkaline, then washed with ice water until the water layer shows neutral, dried over anhydrous sodium sulfate, and then drawn off under a reduced pressure, so as to obtain light yellow syrup-like compound v.

[0014] The synthesis of compound vi: The compound v is dissolved in dry dichloromethane and the dry HCl gas is slowly introduced into an ice-water bath. The solution is washed with ice water so hat an organic layer is separated. The organic layer is washed with saturated sodium bicarbonate until the water layer shows weak alkaline, and then washed with ice water until the water layer shows neutral, dried over anhydrous sodium sulfate, and drawn off under a reduced pressure to obtain light yellow syrup. The syrup is recrystallized to obtain white solid compound vi.

[0015] The synthesis of compound vii: The compound vi is dissolved in dry dichloromethane and dry DMF, and SO_2Cl_2 is slowly added into the solution while stirring the solution at about -15 °C. The solution is stirred at -15°C for about 30 minutes, and naturally warmed up to a room temperature for reaction. Then, imidazole is added into the solution for three times at 0°C, and the mixed solution is stirred at a room temperature. After completion of the reaction, the reaction solution is diluted by adding CH_2Cl_2 and then is washed with ice water. The water layer is extracted with CH_2Cl_2. The organic layers are combined and then dried over anhydrous sodium sulfate, and drawn off under a reduced pressure to obtain light yellow syrup. The syrup is separated and purified by silica gel column, so as to obtain white solid compound vii.

[0016] The synthesis of compound viii: The compound vii is dissolved in ethyl acetate, and Et3N 3HF is added while stirring the solution. The solution is heated up to about 60°C and stirred for about 3h, and then heated up to 70 °C and stirred for about 1.5h. It is added with ice-salt water to terminate the reaction, and then extracted with dichloromethane. Then the organic layers are combined and then washed sequentially with saline, water and saturated sodium bicarbonate, dried over anhydrous sodium sulfate, and drawn off under a reduced pressure to obtain dark yellow syrup. The syrup is purified to obtain light yellow syrup. Finally the crude product is crystallized to obtain white crystalline compound viii.

[0017] The synthesis of compound ix: The compound viii is dissolved in anhydrous dichloromethane and a mixed solution of HBr-AcOH is added. The solution is stirred for reaction at a room temperature. After completion of the reaction, the mixture is evaporated to dryness, and the residue is dissolved by dichloromethane. The dichloromethane solution is washed with sodium bicarbonate solution, and dichloromethane is removed through evaporation to obtain a syrup-like product. Meanwhile, the protected cytosine and $(NH_4)_2SO_4$ are refluxed under nitrogen protection in HMDS. After the completion of reaction, the solvent is removed through evaporation under reduced pressure to obtain the silylated cytosine. The syrup obtained in the above reaction is dissolved in dichloroethane, and then added into the silylated cytosine. Then the mixture is refluxed under N_2 protection, and the reaction is terminated by ice. Then the solution is extracted by dichloromethane, and the dichloromethane layer is washed sequentially with saturated sodium bicarbonate and saline, and dried over anhydrous sodium sulfate. After drying the solvent is removed through evaporation to get white solid, the white solid is separated and purified by column chromatography to obtain the compound ix.

[0018] The synthesis of compound x: The compound ix is dissolved in saturated NH_3-CH_3OH and is stirred for reaction at room temperature. After completion of the reaction, the solvent is evaporated to dryness, and the obtained residue is purified by column chromatography to obtain the compound x.

[0019] The synthesis of compound xi: The compound x, imidazole and triphenylphosphine are dissolved in tetrahydrofuran, and the solution is slowly added dropwise with iodiferous tetrahydrofuran. The solution is stirred for reaction at a room temperature. After completion of the reaction, the solvent is removed through evaporation, and the residue is added with ethyl acetate and filtered. The ethyl acetate is removed through evaporation and the residue is separated by column chromatography to obtain the compound xi.

[0020] The synthesis of compound xii: The compound xi is dissolved in tetrahydrofuran, and then the solution is added with DBU, being stirred for reaction at about 60°C. After completion of the reaction, the solvent is evaporated to dryness, and the residue is separated by column chromatography to obtain the compound xii.

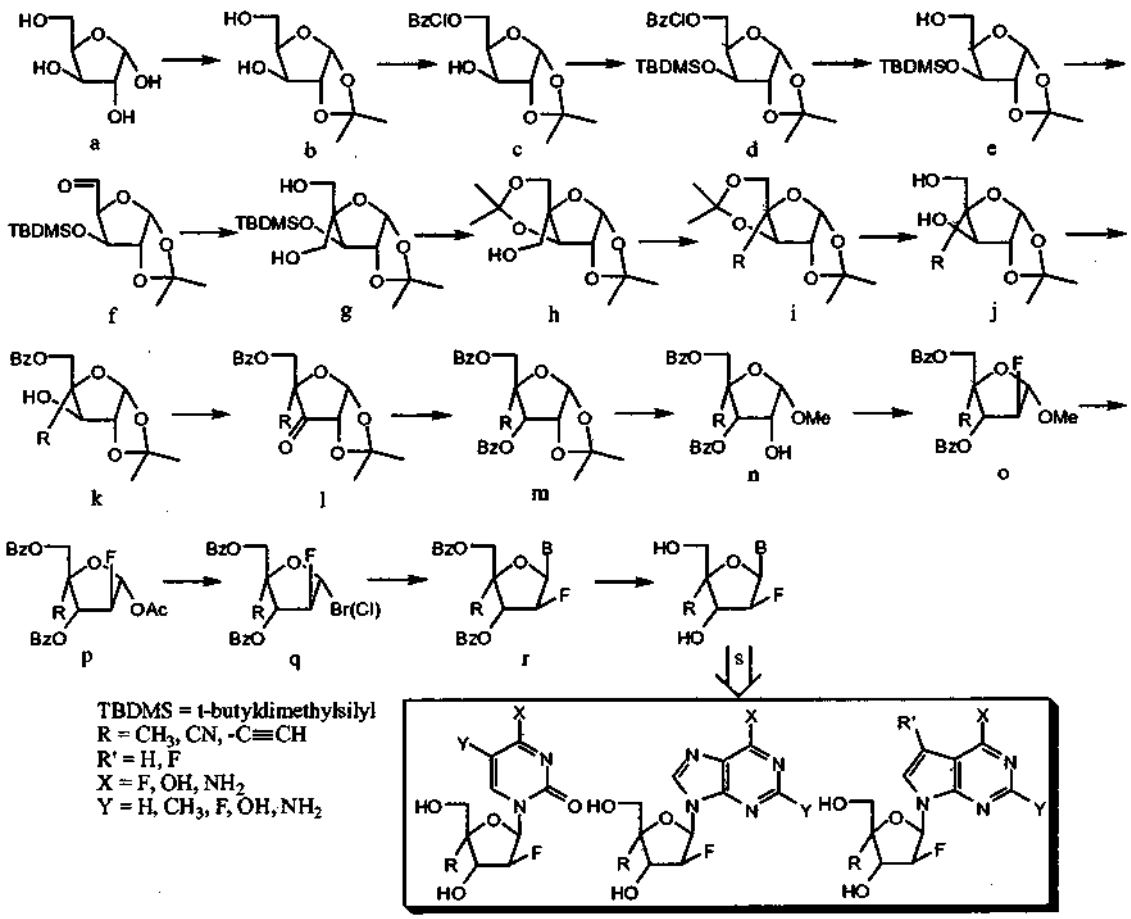
[0021] The synthesis of compound xiii: The DMF solution dissolved with ICl is added in the DMF solution dissolved with NaN_3 at about 0 °C. The solution is stirred at the freezing point for about 10 minutes and is slowly added dropwise

with DMF solution dissolved with the compound xii to continue the reaction. After completion of the reaction, the mixed solution is added with sodium sulfite until the color of iodine disappears completely. The solvent is removed through evaporation under a reduced pressure, and the residue is separated and purified by column chromatography, so as to obtain the compound xiii.

[0022] The synthesis of compound xiv: The compound xiii is dissolved in DMF, silver acetate is added while stirring, and the reaction is carried out at a room temperature. After completion of the reaction, the solution is filtered. The solvent is removed under a reduced pressure and the residue is purified by column chromatography, so as to obtain the compound xiv.

[0023] The synthesis of compound xv: The compound xiv is dissolved in the solution of methanol-triethylamine, and the solution is stirred for reaction at a room temperature. After completion of the reaction, the solvent is removed through evaporation and the residue is purified by column chromatography, so as to obtain the compound xv.

[0024] The present invention also relates to the preparation reaction for 2'-fluoro-4 '-substituted nucleoside analogues. Where R=CH_3, CN, C≡CH, the reaction path is as follows:



[0025] The synthesis of compound b: Compound a is added in acetone, the concentrated sulfuric acid is slowly added while stirring the solution at a room temperature, and the solution is stirred for reaction at a room temperature. After the reaction is detected by TLC as complete, the solution is added with the concentrated ammonia to adjust pH, filtered, and evaporated under a reduced pressure to remove most of the acetone. Then 0.4% dilute hydrochloric acid is added while stirring, and the reaction is completed under the acetone reflux condition until the compound a has been completely hydrolyzed into the compound b. Then the reaction solution is neutralized by solid $NaHCO_3$, then filtered, and evaporated to remove the solvent under a reduced pressure. The residue is dissolved by dichloromethane, dried over anhydrous Na_2SO_4 for overnight and then filtered, and then evaporated to remove the solvent, so as to obtain the yellow viscous oily compound b.

[0026] The synthesis of compound c: The compound b and triethylamine are dissolved in dichloromethane. The solution is slowly added dropwise with parachlorobenzoyl chloride in an ice salt bath, and mechanically stirred for reaction at a temperature below 0 °C. After completion of the reaction, the solution is added with a saturated $NaHCO_3$ solution. The dichloromethane layer is washed with water and saturated salt water, dried over anhydrous $MgSO_4$, filtered, and then

evaporated under a reduced pressure to remove the solvent. The residue is recrystallized to obtain the white crystalline compound c.

[0027] The synthesis of compound d: The compound c is dissolved in anhydrous dichloromethane. The solution is added with imidazole and tert-butyl-2-methyl chloride silicon (TBDMSCl) under nitrogen protection for the reaction at a room temperature. After completion of the reaction, the reaction is neutralized by hydrochloric acid, and two layers are obtained. The organic layer is washed with water and saturated salt water respectively, dried over anhydrous Na_2SO_4, and then evaporated to remove the solvent. The residue is separated and purified by column chromatography, so as to obtain the compound d.

[0028] The synthesis of compound e: The compound d is dissolved in the mixed solution of sodium methoxide and methanol for reaction at a room temperature. After completion of the reaction, the solution is neutralized by dilute acetic acid, filtered, washed with methanol and then evaporated under a reduced pressure to remove the solvent. Finally the residue is separated and purified by column chromatography, so as to obtain the compound e.

[0029] The synthesis of the compound f: DMSO is added dropwise into the dichloromethane solution of oxalyl chloride at about -60 °C. The solution is stirred at the same temperature for about 15 minutes, and then added dropwise with the dichloromethane solution of the compound e. After the solution is stirred for reaction at about -65 °C for about 30 minutes, triethylamine is added, and the solution is stirred for reaction at a room temperature. After completion of the reaction, water is added into the reaction solution so that the organic layer is separated, and the organic layer is dried over anhydrous magnesium sulfate. The solvent is removed through evaporation and the residue is separated and purified by column chromatography, so as to obtain the compound f.

[0030] The synthesis of compound g: Sodium hydroxide and water are added in the reaction bottle. The solution is stirred evenly and then added with a formaldehyde solution, 95% ethanol, and then the compound f. The solution is stirred for reaction at about 35 °C. After completion of the reaction, the reaction bottle is cooled with ice water while stirring until the product is completely precipitated. After the suction filtration, the solid product is washed with water to neutral, dried, dissolved in anhydrous methanol, and then sodium borohydride is added for reflux reaction. After completion of the reaction, the reaction solution is neutralized by dilute hydrochloric acid and then extracted with dichloromethane, dried over anhydrous Na_2SO_4. The solvent is removed by evaporation so that the compound g is obtained.

[0031] The synthesis of compound h: The compound g is dissolved in methanol, and the solution is added with Dowex H^+ (washed with methanol in advance) for reaction at a room temperature. After completion of the reaction, the resin is removed from the solution through filtration and repeatedly washed with methanol. The solution is evaporated to obtain the solid substance. The obtained solid substance is dissolved in acetone, and then concentrated sulfuric acid (catalytic amount) is slowly added into the solution for reaction at a room temperature while stirring. The reaction solution is added with concentrated ammonia water to adjust pH value, and then filtered and evaporated under a reduced pressure to remove acetone, so as to obtain the compound h.

[0032] The synthesis of compound i: DMSO is added dropwise into the dichloromethane solution of oxalyl chloride at -60 °C. Then, the solution is stirred for about 15 minutes and then is added dropwise with the dichloromethane solution of the compound h. After the reaction is carried out for 30 minutes at -60 °C, triethylamine is added in the solution to continue the reaction at a room temperature for 30 minutes. Then, water is added to terminate the reaction, and the organic layer is separated and dried over anhydrous Na_2SO_4. The solvent is removed through evaporation and the residue is separated and purified by column chromatography to obtain the solid product. $ClCH_2P(C_6H_5)_3Cl$ is added into anhydrous tetrahydrofuran. The solution is cooled to about -78 °C, added with n-BuLi hexane solution, stirred for reaction for about 1h, then added with the dry tetrahydrofuran solution of the solid product. The temperature of the solution is gradually increased to about 0 °C to continue the reaction. After completion of the reaction, saturated NH_4Cl solution is carefully added dropwise in the solution, and the solution is subsequently extracted with ethyl acetate. The organic layer is washed with saline twice, dried over anhydrous $MgSO_4$, and evaporated to remove the solvent. The residue obtained is dissolved into anhydrous tetrahydrofuran, cooled to about -78 °C, and slowly added dropwise with n-BuLi hexane solution. After the solution is stirred for about 2 hours, the reaction is carefully terminated by using saturated NH_4Cl. The organic layer is washed with saturated salt water, dried over anhydrous MgSO4, and evaporated under a reduced pressure to remove the solvent. Finally the residue is separated and purified by rapid silica gel column, so as to obtain the compound i.

[0033] The synthesis of compound j: The compound i is added in the diluted hydrochloric acid solution, the solution is stirred at a room temperature for reaction. After completion of the reaction, the solution is neutralized by solid $NaHCO_3$, and then filtered. The filtrate is evaporated under a reduced pressure to remove the solvent, and the residue is dissolved by dichloromethane and dried over anhydrous Na_2SO_4, and then filtered and evaporated to remove the solvent, so as to obtain the compound j.

[0034] The synthesis of compound k: The compound j is dissolved in pyridine, and the solution is added dropwise with BzCl for reaction at a room temperature. After completion of the reaction, the solvent is evaporated to dryness, and the residue is purified to obtain the compound k.

[0035] The synthesis of compound 1: The compound k and 2,2,6,6 - tetramethyl piperidine-N-oxide are dissolved in

dichloromethane, the solution is cooled to about 0 °C and added with the mixed solution of sodium hypochlorite solution, $NaHCO_3$ and water for reaction at about 0 °C for about 30 minutes. Isopropanol is added into the reaction solution and stirred at a room temperature. The separated dichloromethane layer is washed with water twice, dried over anhydrous $MgSO_4$, filtered, and evaporated to remove the solvent. The obtained residue is recystalized to obtain the compound 1.

[0036] The synthesis of compound m: The compound 1 is dissolved in the mixed solution of absolute ethanol and ethyl acetate. The solution is cooled to about 0 °C, and then added with sodium borohydride in batches, being stirred for reaction. After completion of the reaction, the reaction solution is neutralized with a dilute acetic acid solution, filtered, and the filtrate is evaporated. The residue is dissolved in dichloromethane and then washed with water. The dichloromethane layer is dried over anhydrous $MgSO_4$, filtered, and evaporated to remove the dichloromethane under a reduced pressure. The residue is recrystallized by the mixed solvent of dichloromethane and petroleum ether to obtain solid substance. Then the solid product is dissolved in pyridine. BzCl is added dropwise at about 0°C, and after that the reaction goes on at a room temperature. After completion of the reaction, the solvent is removed through evaporation and the residue is extracted with ethyl acetate, washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, evaporated to remove the solvent, so that the compound m is obtained.

[0037] The synthesis of compound n: The compound m is dissolved in methanol, and then the solution is added with the mixed solution of concentrated hydrochloric acid / dioxane for reaction at a room temperature. After completion of the reaction, the reaction solution is neutralized with $NaHCO_3$, and filtered. The solvent is removed from the filtrate by vacuum distillation, and the residue is dissolved in an appropriate amount of dichloromethane and dried over anhydrous Na_2SO_4 for overnight and then filtered, evaporated to dryness, and finally separated and purified by column chromatography to obtain the compound n.

[0038] The synthesis of compound o: The compound n is dissolved in dichloromethane, and the solution is added with DAST under a room temperature and stirred for reaction. After completion of the reaction, the solution is poured into saturated $NaHCO_3$ solution and the organic layer is separated. The organic layer is washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and separated and purified by column chromatography to obtain the compound o.

[0039] The synthesis of compound p: The compound o is added in formic acid solution, and the solution is stirred at a room temperature for reaction. After completion of the reaction, the solution is evaporated under a reduced pressure to remove the solvent. The residue and toluene are evaporated together to obtain the solid product. The obtained solid product is dissolved in the solution of dry pyridine and acetic anhydride for reaction at a room temperature. After the reaction is completed, the reaction solution is concentrated and then evaporated together with toluene, so as to obtain the compound p.

[0040] The synthesis of compound q: The compound p is dissolved in anhydrous dichloromethane and cooled to about 0°C. The HCl gas is slowly introduced into the solution to a saturation level and the solvent is removed by vacuum evaporation at a room temperature. The residue is dried in vacuum to obtain the compound q.

[0041] The synthesis of compound r: With the existence of $(NH_4)_2SO_4$, 2,6-diamino-purine and Hexamethyldisilazane (HMDS) are heated and refluxed to be a transparent solution. The solvent is removed under vacuum to obtain a white solid product, and the solid product is subsequently dissolved in 1, 2-dichloroethane. Then, 1,2-dichloroethane solution of compound q and molecular sieve are added, the solution is stirred for reaction under nitrogen protection at a room temperature. After the reaction is detected by TLC as complete, the reaction solution is added with dichloromethane and filtered by diatomite. The filtrate is washed with saturated Na_2SO_4 and saline respectively. The organic layer is dried over anhydrous Na_2SO_4 overnight and the solvent is removed through evaporation to obtain the residue. The residue is separated under a reduced pressure through silica gel column, so as to obtain the compound r.

[0042] The synthesis of compound s: The compounds r is dissolved in saturated NH_3-CH_3OH solution, and the solution is stirred for reaction at a room temperature. After completion of the reaction, the solvent is removed through evaporation and the residue is separated and purified by column chromatography, so as to obtain the compound s.

[0043] The beneficial effects of the present invention lie in that, by means of chemical modifications made to the glycosyl and base, D-and L-nucleoside analogue would contain special structures such as fluoric group, alkynyl, azide and cyano etc. and thus can overcome the deficiencies of the present D-and L - nucleoside analogues, including more toxic side effect and less activity. Furthermore, the synthesis method is simple and feasible with high yield ratio, having a good value when it is used in the preparation of anti-HBV or anti-HCV or anti-HIV drugs.

Specific Embodiments

[0044] The present invention is further described hereinafter according to the synthetic route of the compound expressed in the general formula I in combination with the preferred embodiments. However, these preferred embodiments provided are not to limit the scope of the present invention.

Example 1. The synthesis of the compound 15 wherein the base is cytosine

[0045] The synthesis of compound ii: The compound i (D-ribose or L-ribose) (8.66mmol) was dissolved in 31.2ml HCl/MeOH (0.2mmol/L), and the solution was stirred in a water bath at 27°C for 3 hours under hermetization and warm preservation. The solution was added with 7.8ml pyridine to terminate the reaction, and then drawn off under a reduced pressure, so as to obtain the light yellow syrup-like compound ii (96%);

[0046] The synthesis of compound iii: The compound ii (7.92mmol) was dissolved in 21 ml dry pyridine, and 5.2 ml benzoyl chloride (0.0447mol) was slowly added dropwise in the solution in an ice-salt bath. The solution was stirred at a room temperature for 17 hours. The reaction solution was poured into 39 ml ice water, and extracted with chloroform. The organic layer was washed sequentially with ice water, pre-cooled sulfuric acid (3mol/L) and saturated sodium bicarbonate solution until the water layer shows weak alkaline, and then washed with ice water until the water layer shows neutral, dried over anhydrous sodium sulfate for more than 4 hours, and drawn off under a reduced pressure, so as to obtain the light yellow syrup-like compound iii (80.0%). ^{1}H NMR($CDCl_3$) δppm: 7.28~8.10 (m, 15H, OBz), 5.87 (m, 1H, H-3), 5.68 (d, 1H, H-2), 5.16 (s, 1H, H-1), 4.72 m, 2H, H-5), 4.52 (q, 1H, H-4), 3.42 (s, 3H, OCH_3).

[0047] The synthesis of compound iv: Benzoyl compound iii (0.0027mol) was dissolved in chloroform (13mol), red phosphorus (390mg, 0.0126mol) was added, and bromine (1.3ml., 0.025mmol) was slowly added dropwise while stirring the solution in an ice-water bath. After stirring it for 30 minutes, 1.3 ml ice water was slowly added dropwise and the solution was stirred at a room temperature for 4 hours. 7g broken ice was added and stirred to melt the broken ice. Then the reaction solution was poured into 14 ml ice water and extracted with chloroform. The organic layer was washed with saturated sodium bicarbonate until the water layer shows weak alkaline, and then washed with ice water until the water layer shows neutral, and then dried over anhydrous sodium sulfate for over 4 hours, and then drawn off under a reduced pressure to obtain the dark yellow syrup. Finally the syrup was separated by silica gel column (gradient elution, petroleum ether: acetone), so as to obtain the white dry syrup compound iv (64.2%). ^{1}H NMR($CDCl_3$) δppm: 7.32~8.10 (m, 15H, OBz), 5.90 (m, 1H, H-3), 5.70 (d, 1H, H-2), 5.63 (s, 1H, H-1), 4.55~4.79 (m, 3H, H-5, H-4).

[0048] The synthesis of compound v: The compound iv (2.73mmol) was dissolved in 5ml dry pyridine, and the solution was slowly added dropwise with acetic anhydride (0.0276mol) in an ice water bath, then stirred for 30 minutes. Then, the ice water bath was removed, and the solution was stirred at a room temperature for 7 hours, and then heated up to 40°C. This temperature was kept for an hour. The solution was added with 6.5g broken ice and then stirred until the broken ice was melted. The reaction solution was poured into 13 ml ice water and extracted with chloroform. The organic layer was washed sequentially with ice water, pre-cooled sulfuric acid (3mol/L) and saturated sodium bicarbonate until the water layer shows weak alkaline, then washed with ice water until the water layer shows neutral, dried over anhydrous sodium sulfate for more than 4 hours, and then drawn off under a reduced pressure, so as to obtain the light yellow syrup-like compound v (92.1%). ^{1}H NMR($CDCl_3$) δppm: 7.30~8.10 (m, 15H, OBz), 6.43 (s, 1H, H-1), 5.90 (m, 1H, H-3), 5.80 (d, 1H, H-2), 4.50~4.80 (m, 2H, H-5), 2.00 (s, 3H, CH_3COO-).

[0049] The synthesis of compound vi: The compound v (2.57mmol) was dissolved in 26 ml dry dichloromethane and dry HCl gas was slowly introduced into an ice-water bath for 2.5 hours.

[0050] The solution was washed with 19.5ml ice water and the organic layer was separated. The organic layer was washed with saturated sodium bicarbonate until the water layer shows weak alkaline, and then washed with ice water until the water layer shows neutral, dried over anhydrous sodium sulfate for more than 4 hours, and then drawn off under a reduced pressure to obtain light yellow syrup. The syrup was recrystallized using the mixed solvent of hexane and dichloromethane, so as to obtain the white solid compound vi (65.1%). ^{1}H NMR($CDCl_3$) δppm: 7.36~8.14 (m, 15H, OBz), 6.68 (d, *J*=4.4Hz, 1H, H-1), 5.59 (dd, 1H, H-3), 4.64-4.80 (m, 4H, H-2, H-4 and H-5). M.p.139~140°C.

[0051] The synthesis of compound vii: The compound vi (2.81mmol) was dissolved in 13 ml dry dichloromethane and 3.5ml dry DMF, and SO_2Cl_2 (0.0079mmol) was slowly added while stirring the solution at -15°C. After it was stirred at -15°C for about 30 minutes, the solution was naturally warmed up to a room temperature, reacting for 3 hours. Then the solution was added with imidazole (0.0407mmol) for three times at 0°C. The mixed solution was stirred at a room temperature for 15 hours. The reaction solution was diluted with CH_2Cl_2 (26ml) added, and washed with ice water (35ml). The water layer was extracted with CH_2Cl_2. Then the organic layers were combined and then dried over anhydrous sodium sulfate for over 4 hours, and then drawn off under a reduced pressure to obtain light yellow syrup. The syrup was separated and purified by silica gel column (gradient elution, petroleum ether: acetone) to obtain the white solid compound vii (76.0%). ^{1}H NMR($CDCl_3$) δppm: 7.00~8.10 (m, 15H, OBz), 6.71 (d, *J*=4.4Hz, 1H, H-1), 5.59 (dd, 1H, H-3), 5.25 (dd, 1H, H-2), 4.56~4.81 (m, 3H, H-4, H-5). M.p.128~129°C.

[0052] The synthesis of compound viii: The compound vii (2.2mmol) was dissolved in ethyl acetate (54ml), and Et_3N • 3HF (2.08ml, 0.013mmol) was added while stirring the solution. The solution was heated up to 60°C, stirred for 3h, and then heated to 70 °C and stirred for 1.5h. The solution was added with ice-salt (10 ml) to terminate the reaction, and extracted with dichloromethane. The organic layers were combined and then washed sequentially with saline, water and saturated sodium bicarbonate, and dried over anhydrous sodium sulfate for over 4 hours. The solvent was drawn off under a reduced pressure to obtain dark yellow syrup. The syrup was purified (dichloromethane elution) by means

of a silica gel funnel (5cm × 5cm) to yield light yellow syrup(86.8%). Finally the crude product was crystallized in 95% ethanol solution to obtain the white compound viii crystalline (66.4%). ^{1}H NMR($CDCl_3$) δppm: 7.31~8.10 (m, 15H, OBz), 6.71 (d, *J*=9.0Hz, 1H, H-1), 5.68 (dd, *J*=19.44Hz, 1H, H-3), 5.32 (d, *J*=48.2Hz, 1H, H-2), 4.65-4.77 (m, 3H, H-4, H-5). M.p.80~82°C.

[0053] The synthesis of compound ix: The compound viii (6.0mmol) was dissolved in anhydrous dichloromethane (20ml), and a mixed solution of HBr-AcOH (45%, V/V, 4.6ml, 25mmol) was added in the solution. The solution was stirred for reaction at a room temperature for 20 hours. The mixture was evaporated to dryness. The residue was dissolved by dichloromethane (50ml). The dichloromethane solution was washed with sodium bicarbonate solution (3×30ml), and dichloromethane was removed through evaporation to obtain a syrup-like product. Meanwhile, the protected cytosine (15mmol) and $(NH_4)_2SO_4$ (0.1g) were refluxed for 17 hours under nitrogen protection in HMDS (30ml). After completion of reaction, the solvent was removed through evaporation under a reduced pressure to obtain silylated cytosine. The syrup obtained in the above reaction was dissolved in dichloroethane (25ml), and added into the silylated cytosine. Then the mixture was refluxed for 15 hours under N_2 protection and the reaction was terminated by ice. Then, the solution was extracted with dichloromethane (3×45ml) and the dichloromethane layer was washed sequentially with saturated sodium bicarbonate and saline, and dried over anhydrous sodium sulfate. After drying, the solvent was removed through evaporation to yield white solid, and the solid was separated and purified by column chromatography (1% MeOH-$CHCl_3$) to obtain the compound ix(71%). ^{1}H NMR($CDCl_3$) δppm: 8.07 (d, J=9.86Hz, 1H), 7.45 (d, J=9.82Hz, 1H), 7.26~8.10 (m, 10H, OBz), 6.03 (d, *J*=9.0Hz, 1H, H-1), 5.64 (dd, *J*=19.44Hz, 1H, H-3), 5.26 (d, *J*=48.2Hz, 1H, H-2), 4.65~4.77 (m, 3H, H-4, H-5).

[0054] The synthesis of compound x: The compound ix (3.60mmol) was dissolved in saturated NH_3-CH_3OH (30ml), and the solution was stirred for reaction at a room temperature for 15 hours. After completion of the reaction, the solvent was evaporated to dryness to obtain the residue, and the residue was purified by column chromatography (15:1 $CHCl_3$-MeOH) to obtain the compound x(80%). ^{1}H NMR($CDCl_3$) δppm: 8.10 (d, J=9.86Hz, 1H), 7.40(d, J=9.82Hz, 1H), 6.03 (d, *J*=9.0Hz, 1H, H-1), 5.64 (dd, *J*=19.44Hz, 1H, H-3), 5.26 (d, *J*=48.2Hz, 1H, H-2), 4.50 (m, 1H, H-4), 3.70~3.77 (m, 2H, H-5).

[0055] The synthesis of compound xi: The compound x (9.46mmol), imidazole (18.93mmol) and triphenylphosphine (14.19mmol) were dissolved in tetrahydrofuran (50ml). The solution was slowly added dropwise with 15ml iodiferous tetrahydrofuran (14.18mmol). The solution was stirred for reaction at a room temperature for 3 hours. After completion of the reaction, the solvent was removed through evaporation to obtain the residue, and the residue was added with ethyl acetate (100ml) and filtered. The ethyl acetate was removed through evaporation and the residue was separated by column chromatography to obtain the compound xi(83.9%). ^{1}H NMR($CDCl_3$) δppm: 8.06 (d, J=9.86Hz, 1H), 7.43 (d, J=9.82Hz, 1H), 6.01 (d, *J*=9.0Hz, 1H, H-1), 5.66 (dd, *J*=19.44Hz, 1H, H-3), 5.22 (d, *J*=48.2Hz, 1H, H-2), 4.57 (m, 1H, H-4), 3.58~3.69 (m, 2H, H-5).

[0056] The synthesis of compound xii: The compound xi (5.88mmol) was dissolved in tetrahydrofuran (50ml), and the solution was added with DBU (6.44mmol) and then stirred at 60°C for 3 hours. The solvent was evaporated to dryness, and the residue was separated by column chromatography to obtain the compound xii(75.1%) which is directly used in the next reaction.

[0057] The synthesis of compound xiii: The 15ml DMF solution dissolved with ICl (13.9mmol) was added in the DMF (15ml) solution dissolved with NaN_3 (9.75mmol) at 0°C. The solution was stirred at 0°C for 10 minutes and was slowly added dropwise with DMF (20ml) dissolved with the compound xii (6.8mmol) to continue the reaction at 0°C for an hour. After completion of the reaction, the mixed solution was added with sodium sulfite until the color of iodine disappears completely. The solvent was removed through evaporation under a reduced pressure, and the residue was separated and purified by column chromatography to obtain the compound xii (77.6%) which is directly applied in the next reaction.

[0058] The synthesis of compound xiv: The compound xiii (5mmol) was dissolved in DMF (15ml), and the solution was added with silver acetate (6mmol) and stirred. After the reaction was carried out at a room temperature for 8 hours, the solution was filtered and the solvent was removed under a reduced pressure (at a temperature below 50°C). The residue was purified by column chromatography to obtain the compound xiv (71.3%). ^{1}H NMR(DMSO-d_6, 300 MHz) δ: 8.12 (d, 1H), 7.40 (d, 1H), 7.26 (br, 2H), 6.12 (dd, 1H), 5.87 (d, 1H), 5.09 (t, 1H), 4.90 (dt, 1H), 4.18 (dt, 1H), 3.74-3.87 (m, 2H), 2.12 (s, 3H, CH_3).

[0059] The synthesis of compound xv: The compound xiv (5mmol) was dissolved in 5% methanol-triethylamine (100ml), and the solution was stirred at a room temperature for 12 hours. After completion of the reaction, the solvent was removed through evaporation and the residue was purified by column chromatography to obtain the compound xv (89.0%).ESI-MS: 287[M+H]. ^{1}H NMR(DMSO-d_6, 300 MHz)δ: 8.14 (d, 1H), 7.32 (d, 1H), 7.21 (br, 2H), 6.08 (dd, 1H), 5.82 (d, 1H), 5.11 (t, 1H), 4.92 (dt, 1H), 4.16 (dt, 1H), 3.62-3.69 (m, 2H).

Example 2. The synthesis of the compounds 25, wherein the 4'-substitued group is alkynes and the base is 1,6 - diamino-purine

[0060] The synthesis of compound b: Compound a (60g) was added in acetone (2L) and the concentrated sulfuric acid (40ml) was slowly added while stirring. The solution was stirred for reaction at a room temperature for 40 minutes. After the reaction was detected by TLC as complete, the solution was added with the concentrated ammonia to adjust pH=7-8, filtered, and evaporated under a reduced pressure to remove most of the acetone. The remaining acetone and water were about 150 ml. Then the solution was added with 0.4% dilute hydrochloric acid (150m1) while being stirred, and the reaction was completed under the acetone reflux condition for 20 minutes until the compound a has been completely hydrolyzed into the compound b. After completion of the reaction, the reaction solution was neutralized to pH=7-8 by solid $NaHCO_3$, filtered, and evaporated to remove the solvent under a reduced pressure. The residue was dissolved with dichloromethane (200ml), dried over anhydrous Na_2SO_4 overnight and then filtered. Finally the solvent was removed through evaporation to obtain the yellow viscous oily b (73g, 95%), ESI-MS: 191 [M+H].

[0061] The synthesis of compound c: The compound b (154g, 0.81mol) and triethylamine (339ml, 2.43mol) were dissolved in dichloromethane (1.50L). The solution was cooled to 0 °C in an ice salt bath, slowly added dropwise with parachlorobenzoyl chloride (113ml, 0.891mol) and mechanically stirred for reaction at a temperature below 0 °C for 4 hours. After completion of the reaction, the solution was added with a saturated $NaHCO_3$ solution (500ml). The dichloromethane layer was washed with water and saturated salt water, dried over anhydrous $MgSO_4$, filtered, and evaporated under a reduced pressure to remove the solvent. The residue was recrystallized to obtain white crystalline compound c (196g, yield=74.6%), ESI-MS: 330[M+H].

[0062] The synthesis of compound d: The compound c (0.114ml) was dissolved in anhydrous dichloromethane (600ml), and the solution was added with imidazole (15.5g, 0.228mol) and tert-butyl-dimethyl chloride silicon (TBDMSCl) (18.9g, 0.125ml) under nitrogen protection for the reaction for 3 hours at a room temperature. After completion of the reaction, 1N hydrochloric acid was added to neutralize the reaction and two reaction layers were obtained. The organic layer was washed with water and saturated salt water, dried over anhydrous Na_2SO_4, and evaporated to remove the solvent. The residue was separated and purified by column chromatography to obtain compound d (yield=69.2%), ESI-MS: 444[M+H].

[0063] The synthesis of compound e: The compound d (0.64mmol) was dissolved in the mixed solution (0.2N, 15 ml) of sodium methoxide and methanol for reaction for 4 hours at a room temperature. After completion of the reaction, the solution was neutralized by dilute acetic acid, filtered, washed with methanol and evaporated under a reduced pressure to remove the solvent. Finally the residue was separated and purified by column chromatography to obtain compound e (yield=75%), ESI-MS: 305[M+H].

[0064] The synthesis of compound f: DMSO (5.5ml, 77.5mmol) was added dropwise into the dichloromethane (80.0ml) solution of oxalyl chloride (3.4ml, 38.7mmol) at -60 °C. The solution was stirred at the same temperature for 15 minutes and then added dropwise with the dichloromethane solution (100ml) of the compound e (25.7mmol). After the solution was stirred for reaction at -65°C for 30 minutes, triethylamine (10.9ml, 78.2mmol) was added to continue the reaction for 30 minutes and the solution was stirred at a room temperature. After completion of the reaction, water was added in the reaction mixture so that the organic layer is separated. The organic layer was dried over anhydrous magnesium sulfate. Finally the solvent was removed through evaporation and the residue was separated and purified by column chromatography to obtain the compound f (yield=94.6%), ESI-MS: 303[M+H].

[0065] The synthesis of compound g: Sodium hydroxide (1.3g) and water (11.5ml) were added in the reaction bottle. The solution was stirred evenly and added with formaldehyde solution (30%), 7.2 ml 95% ethanol, and then the compound f (25mmol). Then, the solution was stirred for reaction at a controlled reaction temperature of 30~35°C for 2 hours. After completion of the reaction, the reaction bottle was cooled with ice water while stirring until the product was completely precipitated. After the suction filtration, the solid product was washed with water to neutral, dried, dissolved in anhydrous methanol, then added into sodium borohydride (0.925g, 25mmol) and refluxed for an hour. After completion of the reaction, the reaction solution was neutralized by dilute hydrochloric acid and extracted with dichloromethane (3×50ml), dried over anhydrous Na_2SO_4. The solvent was removed by evaporation, so that the compound g was obtained (yield= 90.5%, ESI-MS: 335[M+H]).

[0066] The synthesis of compound h: The compound g (9.7mmol) was dissolved in methanol (260ml), and the solution was added with 40ml Dowex H^+ (washed with methanol in advance) for reaction at a room temperature for 4 hours. The resin was removed from the solution through filtration and repeatedly washed with methanol. The solvent was dried to obtain solid substance. The obtained solid substance was dissolved in acetone (200ml). Then concentrated sulfuric acid (catalytic amount, 2 ml) was slowly added dropwise for reaction at a room temperature for about 0.5 hours. Then the solution was added with concentrated ammonia water to adjust pH value to 7-8, filtered, evaporated under a reduced pressure to remove most of acetone. The reaction solution was added with diluted hydrochloric acid (0.4%, 40ml) and reacted 20 minutes under acetone refluxing. Then the reaction solution was neutralized by $NaHCO_3$ to pH=7-8, filtered, evaporated under a reduced pressure to remove the solvent. The residue was dissolved by appropriate amount of dichloromethane, dried over anhydrous Na_2SO_4, and evaporated to remove the solvent, so as to obtain the compound

h (yield=80.2%, ESI-MS: 261 [M+H])

[0067] The synthesis of compound i: DMSO (5.5ml, 77.5mmol) was added dropwise into the dichloromethane(80ml) solution of oxalyl chloride(3.4ml, 38.7mmol) at -60 °C. Then, the solution was stirred for 15 minutes and added dropwise with the dichloromethane (100ml) solution of compound h (25.7mmol). After the reaction was carried out for 30 minutes at -60 °C, triethylamine (10.9ml, 78.2mmol) was added in to continue the reaction at a room temperature for 30 minutes. Then, water was added to terminate the reaction, and the organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed through evaporation and the residue was separated and purified by column chromatography to obtain a solid product. $ClCH_2P(C_6H_5)_3Cl$ (2.15g, 6.19mmol) was added into anhydrous tetrahydrofuran (50ml). The solution was cooled to -78 °C, added with n-BuLi hexane solution(1.6M, 4.0ml), stirred for reaction for an hour, and added with the dry tetrahydrofuran solution(50ml) of the solid product (1.51mmol). The cooling temperature was gradually increased to 0 °C to continue the reaction for 3 hours. After completion of the reaction, a saturated NH_4Cl solution (10ml) was carefully added dropwise, and the solution was subsequently extracted with ethyl acetate (2×100ml). The organic layer was washed with saline twice (2×75ml), fmally dried over anhydrous $MgSO_4$, and evaporated under a reduced pressure to remove the solvent. The residue produced was dissolved into anhydrous tetrahydrofuran (40ml), cooled to -78 °C, and slowly added dropwise with the hexane solution of n-BuLi (1.6M, 20ml). After the solution was stirred for reaction for 2 hours, the reaction was terminated by carefully adding a saturated NH_4Cl solution (20ml). The organic layer was washed with saturated salt water, dried over anhydrous MgSO4, and evaporated under a reduced pressure to remove the solvent. Finally the residue was separated and purified by rapid silica gel column to obtain the compound i (yield=48.5%, ESI-MS: 255[M+H]).

[0068] The synthesis of compound j: The compound i (10mmol) was added into the diluted hydrochloric acid solution (0.2%, 50ml) and stirred for reaction for 6 hours at a room temperature. After completion of the reaction, the solution was neutralized by solid $NaHCO_3$ to pH=7-8 and then filtered. The filtrate was evaporated under a reduced pressure to remove the solvent and the residue was dissolved with dichloromethane and dried over anhydrous Na_2SO_4, and then filtered and evaporated to remove the solvent, so as to obtain the compound j (yield=99.0%, ESI-MS: 215[M+H]).

[0069] The synthesis of compound k: The compound j (5mmol) was dissolved in pyridine (20ml), and the solution was added dropwise with BzCl (5mmol) for reaction at a room temperature for 6 hours. After completion of the reaction, the solvent was removed through evaporation and the residue was purified to obtain the compound k (yield= 85.3%, ESI-MS: 319[M+H]).

[0070] The synthesis of compound 1: The compound k (59.3mmol) and 2,2,6,6-tetramethyl piperidine-N-oxide (0.059mmol) were dissolved in dichloromethane(99ml), the solution was cooled to 0 °C and added with the mixed solution of sodium hypochlorite solution (33.6ml., 8.5~13.5% active chlorine), $NaHCO_3$ (11.2g) and water (190ml) for reaction at 0 °C. After 30 minutes, the reaction solution was added with isopropanol (1.95ml) and stirred for reaction at a room temperature for 10 minutes. The dichloromethane layer separated was washed with water twice, dried over anhydrous $MgSO_4$, filtered, and then evaporated to remove the solvent. The residue obtained was recystalized to obtain the crystalline compound 1 (yield= 87.8%, ESI-MS: 317[M+H]).

[0071] The synthesis of compound m: The compound 1 ((0.05mol) was dissolved in the mixed solution of absolute ethanol (60ml) and ethyl acetate (30ml); the solution was cooled to 0 °C, and then added with sodium borohydride (23.3g, 612mmol) in batches, being stirred for reaction at 0 °C for an hour. After completion of the reaction, the reaction solution was neutralized with dilute acetic acid solution to pH=7-8, filtered, and the filtrate was evaporated. The residue was dissolved in dichloromethane and washed with water. The dichloromethane layer was dried over anhydrous $MgSO_4$, filtered, and evaporated to remove the dichloromethane under a reduced pressure. The residue was recrystallized by the mixed solvent of dichloromethane and petroleum ether to produce a solid product. Then the solid product was dissolved in pyridine. BzCl (50mmol) was added dropwise at 0°C, and after that, the reaction goes on at a room temperature for 3 hours. After completion of the reaction, the solvent was removed through evaporation and the residue was extracted with ethyl acetate, washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, evaporated to remove the solvent, so that the compound m was obtained (yield=89.7%, ESI-MS: 423[M+H]).

[0072] The synthesis of compound n: The compound m (4mmol) was dissolved in methanol (22ml), and then the solution was added with the mixed solution (4.0M, 20ml) of concentrated hydrochloric acid/dioxane for reaction at a room temperature for 10 hours. After completion of the reaction, the reaction solution was neutralized with $NaHCO_3$ to pH=7-8 and filtered. The filtrate was distilled under a reduced pressure to remove the solvent, and the residue was dissolved in an appropriate amount of dichloromethane, dried over anhydrous Na_2SO_4 for overnight, then filtered, evaporated to dryness, and finally separated and purified by column chromatography to obtain the compound n (yield=89.5%, ESI-MS: 397[M+H]).

[0073] The synthesis of compound o: The compound n (1.76mmol) was dissolved in dichloromethane, and the solution was added with DAST (0.4ml., 3.03mmol) under a room temperature and stirred for 12 hours. After completion of the reaction, the solution was poured into 10 ml saturated $NaHCO_3$ solution and the organic layer was separated. The organic layer was washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and separated and purified by column chromatography to obtain the compound o (yield=11.5%, ESI-MS: 399[M+H]).

[0074] The synthesis of compound p: The compound o (10mmol) was added in a 20 ml formic acid (80%) solution for reaction and the solution was stirred at a room temperature for 12 hours. After completion of the reaction, the solution was evaporated under a reduced pressure to remove the solvent. The residue and toluene were evaporated together to obtain a solid product. The obtained solid product was dissolved in the solution of dry pyridine and acetic anhydride for reaction at a room temperature for 4 hours. After the reaction was completed, the reaction solution was concentrated and then evaporated together with toluene to obtain the compound p (90.3%, ESI-MS: 427[M+H]). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.07 (s, 3H), 2.85 (s, 1H), 4.62-4.77 (m, 2H), 4.89-5.07 (m, 3H), 7.32-8.17 (m, 10H).

[0075] The synthesis of compound q: The compound p (4.76mmol) was dissolved in anhydrous dichloromethane (40ml) and cooled to 0°C. The HCl gas was slowly introduced into the solution to saturation level for about 3 hours, and the solvent was removed through vacuum evaporation at a room temperature. The residue was dried in vacuum for 2 hours to obtain the compound q (yield=87.1%) which is directly applied in the next reaction.

[0076] The synthesis of compound r: With the existence of $(NH_4)_2SO_4$(50mg), 2,6-diamino-purine (4.78mmol)and Hexamethyldisilazane (HMDS, 9ml) were heated and refluxed to obtain transparent solution (in about 5 hours).The solvent was removed under vacuum to obtain a white solid product, and the solid product was dissolved in 1,2-dichloroethane(35 ml). Then, the 1,2-dichloroethane solution of compound q (4.56mmol, 30ml) and 0.4nm molecular sieve (2.6g) were added, and the solution was stirred for reaction at a room temperature for 6 days under nitrogen protection. After the reaction was detected by TLC as complete, the reaction solution was added with dichloromethane (80 ml) and filtered by diatomite. The filtrate was washed with saturated Na_2SO_4 and saline respectively. The organic layer was dried over anhydrous Na_2SO_4 overnight and the solvent was removed through evaporation to obtain the residue. The residue was separated under a reduced pressure by silica gel column, so as to obtain the compound r (yield=78.5%, ESI-MS: 517[M+H]). ^{1}H NMR($CDCl_3$, 300 MHz)δ: 2.85 (s, 1H), 4.67-4.79 (m, 2H), 4.92-5.13 (m, 3H), 5.79 (br, 1H), 7.32-8.17 (m, 9H), 8.35 (s, 1H).

[0077] The synthesis of compound **25:** The compound r (3.60mmol) was dissolved in the saturated NH_3-CH_3OH solution (30ml), and the solution was stirred for reaction at a room temperature for 15 hours. After completion of the reaction, the solvent was removed through evaporation and the residue was separated and purified by column chromatography to obtain the compound 25(yield=78.9%, ESI-MS: 309[M+H]). ^{1}H NMR($CDCl_3$, 300 MHz)δ: 2.85 (s, 1H), 3.69-3.79 (m, 2H), 4.79-5.07 (m, 3H), 5.60 (br, 1H), 5.79 (br, 1H), 7.32-8.17 m, 4H), 8.35 (s, 1H).

[0078] **In Vitro test on the anti-HIV activity of the present invention:** The anti-HIV activities of the compounds 1, 9, 15, 19, 36, 39, 46, 50 and 52 in the cell culture.

[0079] **Drugs to be tested:** The compounds 1, 9, 15, 19, 36, 39, 46, 50 and 52, no batch number, water-insoluble, DMSO-soluble. During the test, the solution was dissolved by DMSO to suitable concentration, diluted by culture medium, and immediately added into cell culture.

Positive control drugs:

[0080] Zidovudine (AZT), a known HIV-1 nucleoside reverse transcriptase inhibitors that has been put into clinical application, in the form of white powder, purchased from Shanghai Desano Chemical & Pharmaceutical Co., Ltd., with batch number: 040201b;

[0081] Nevirapine (NVP), one of known non-nucleoside reverse transcriptase inhibitors in clinical application, purchased from Nanjing Zezhong Medical & Chemical Information Research Center (Batch No: 0301001).

[0082] **HIV-1IIIB Virus strain for test** was donated by Dr.Jiang Jiandong, Mount Sinai Medical Center, USA, amplified in Cell H9 and under cryopreservation at -196°C.

[0083] **Human T-lympbocyte subculture MT-4,** donated by Professor Zhang Xingquan, Medical Research Center, University of Colorado (USA), subcultured in our laboratory, under cryopreservation at -196 °C. Cell culture medium: RPMI Medium 1640 culture medium containing 10% fetal bovine serum, 100IU/ml penicillin, 100μg/ml streptomycin and kanamycin, and L-glutamine. The cells would be cultured at 37°C, in 5% CO_2 incubator, passaging once every three days.

[0084] **Principal reagents:** RPMI Medium 1640 culture medium, GIBCO(US); Fetal Bovine Serum(FBS), Tianjin Chuanye Biochemical Products Co., Ltd.; penicillin and streptomycin, North China Pharmaceutical Company; kanamycin, Shanghai Xudong Haipu Pharmaceutical Co., Ltd; tetrazolium (Thiazolyl blue, MTT) and citric acid, Sigma(U.S.) ; Triton X-100, KEBO AB STOCKHOLM (Sweden); N, N-dimethyl-Formamide, Beijing Chemical Plant; HIV-1 P24 antigen kit, BioMerieux (Netherlands).

[0085] **Main instruments:** Emax™ (Enzyme-labeled meter), MolICular Devices Inc. (United States);

Method:

[0086] With a 96-well culture plate, a test was conducted on the drug and the positive control drug group of drug-inhibited virus. Each well was added with 100ul DMSO solution of different concentrations of the drugs or the positive control drugs AZT and NVP or oral drugs for rats, different doses of different groups, and then at different time blood

sampling, and making different diluted group, 100ul per well. At the same time, a test was conducted on cell control group, virus control group, positive drugs AZT and NVP control group. MT-4 cells were infected by 100$TCID_{50}$ HIV-1 IIIB for 1.5 hours, washed with medium once, and prepared to solution of 2×10^5cells/ml that were inoculated in 96-well culture plates of the test groups of drug-inhibited virus, positive control drug group and virus control group. Cell control groups were added in equivalent medium and cultured for 4 days. Cytopathic changes were observed by microscopy, and toxicities were measured by MTT staining method. The P24 antigens in supernatant were measured according to the kits' instructions. Accordingly, the inhibition ratios, the median toxic concentrations (CC_{50}) and the median effective concentrations (IC_{50}) and SI were calculated respectively.

The inhibition effects of the compounds 1, 9, 15, 19, 36, 39, 46, 50 and 52 on HIV-1 in MT-4 cell culture

[0087] The cytotoxicities and anti-HIV-1 activities of the compounds 1, 9, 15, 19, 36, 39, 46, 50 and 52 were determined by MTT, and their cytotoxicities on CC_{50} and SI were compared by using the HIV-1 infected and uninfected cells, as shown in Table 1.

Table 1 Anti-HIV-1 activities of the compound 1, 9, 15, 19, 36, 39, 46, 50, 52 and positive drugs AZT and NVP, in the MT-4 cell culture

Drug	CC_{50}(μg/ml)*		IC_{50}(μg/ml)*	SI	
	HIV-infected	HIV-uninfected		HIV-infecte d	HIV-uninfected
AZT	>0.625	>0.625	0.000182	>3434	>3434
NVP	>12.5	>12.5	0.00364	>3434	>3434
1	43.6	40.9	0.101	432	405
9	31.23	30.05	0.162	298	291
15	>100	>100	0.00001	>100000	>100000
19	43.8	39.8	0.09	487	422
36	35.12	37.54	0.127	276	296
39	38.26	36.77	1.49	26	25
46	48.52	55.35	0.131	370	423
50	28.3	27.9	2.30	12	12
52	41.3	38.6	0.133	311	290
*: Cytotoxicity: MTT method					

Table 2. The Pharmacological screening results of the compounds 1, 5 and 19 that were obtained by means of the pharmacological model of wild type HIV-1 replication

Compound	Pharmacological model	cell	Administration Routine	Dose (mol/L)	Inhibition ratio (%)	solvent	Remarks IC_{50} (μM)
1	VSVG/HIV	Cell 293	Proir to infection	$1x10^{-5}$	99.4±0.3	DMSO	
				$1x10^{-6}$	90±0	DMSO	0.5μM
				$1x10^{-7}$	28±0	DMSO	
				$1x10^{-8}$	0±3	DMSO	
15	VSVG/HIV-luc	Cell 293	Proir to infection	$1x10^{-5}$	100	DMSO	
				$1x10^{-6}$	100	DMSO	1.5nM
				$1x10^{-7}$	100	DMSO	
				$1x10^{-8}$	72±6	DMSO	

(continued)

Compound	Pharmacological model	cell	Administration Routine	Dose (mol/L)	Inhibition ratio (%)	solvent	Remarks IC_{50} (μM)
19	VSVG/HIV-luc	Cell 293	Proir to infection	$1x10^{-5}$	92.7±0.9	DMSO	
				$1x10^{-6}$	48±0	DMSO	1.0μM
				$1x10^{-7}$	4±1	DMSO	
				$1x10^{-8}$	0±2	DMSO	
AZT	VSVG/HIV-luc	Cell 293	Proir to infection	$1x10^{-7}$	97.2±1.1	DMSO	
				$1x10^{-8}$	54.1±2.0	DMSO	
3TC	VSVG/HIV-luc	Cell 293	Proir to infection	$1x10^{-6}$	86.3±4.3	DMSO	
				$1x10^{-7}$	50.9±2.4	DMSO	
d4T	VSVG/HIV-luc	Cell 293	Proir to infection	$1x10^{-6}$	92.9±1.6	DMSO	
				$1x10^{-7}$	24.0±1.1	DMSO	
EFV	VSVG/HIV-luc	Cell 293	Proir to infection	$1x10^{-8}$	100	DMSO	
				$1x10^{-9}$	55.2±0.6	DMSO	
NVP	VSVG/HIV-luc	Cell 293	Proir to infection	$1x10^{-7}$	73.1±3.2	DMSO	
				$1x10^{-8}$	18.8±2.8	DMSO	

[0088] As can be known from Table 1 and Table 2, all the new 2'-fluoro-4'- substituted nucleosides have good anti-HIV activities, especially the compounds 1, 15 and 19 have good prospects in terms of application and development. Therefore, the development of such compounds will bring good news to patients with AIDS.

In vitro screening of anti-hepatitis B virus drugs in the present invention

[0089] **Object:** HBV genome transfected Hep G2.2.15 cell is used for screening of anti-hepatitis B virus drugs to provide theoretical and experimental basis in research and development of novel anti-HBV drugs.

[0090] **Method:** Lamivudine, compounds **15, 17** with concentration less than TC_{50} were selected to treat cells. Cell culture supernatant was collected at the time point of 144h and 216h respectively, for fluorescent quantitative-PCR detection of HBV DNA levels.

[0091] **Results:** Both Lamivudine and the compound 15 can significantly reduce the HBV DNA copy number after administration.

[0092] **Conclusion:** The compound 15 can significantly reduce HBV DNA activity in vitro tests, with less toxicity. This experiment provided a basis for further study on anti-HBV effects of the compounds.

[0093] Cell **lines:** Hep G2.2.15 cell lines, the HBV DNA cloning transfected human liver cancer HepG2 developed by Mount Sinai Medical Center (United States) in 1986, characterized by the stable expressions of e antigen and surface antigen, donated by the Wuhan Institute of Virology, Chinese Academy of Sciences. Passage and G418 screening were made by our laboratory in the process of cell culture.

[0094] **Test drugs:** synthetic drugs of compound 15 and 17 in the present invention;

[0095] **Control drugs:** Lamivudine (3TC), GlaxoSmithKline, Suzhou, China.

[0096] **Main reagents and instruments:** DMEM culture medium, Hyclone; FBS, Hangzhou Sijiqing; 24-well plates and culture bottles, Coming (USA); HBV PCR Fluorescence quantitative detection kit, Shenzhen PG Biotech; G418, Introvegen.

In vitro studies on Anti-HBV

[0097] Hep G2.2.15 cell culture: culture cell by DMEM medium containing 10% fetal bovine serum and 500mg / L G418, digestion by 0.25% trypsin and 0.02% EDTA; 1: 3 subculture; one passage every three days.

The studies on the Anti-HBV effects of drugs:

[0098] The cells with good growth conditions were selected, and the cell concentration was adjusted to $2X10^4$ cells/ml. Each well was inoculated by 1ml in a 24-well plate. Then the cells were cultured at 37 °C in 5% CO_2 incubator for 48 hours. The test was started as soon as the cells have shown good adhesive growth. Grouping was made based on different concentrations of the drugs (Table 1). Each concentration was set for two wells. The cell culture control group without drug was set as a blank control. On the 3rd, 6th and 9th days after administration, the drug-containing medium and blank medium in the same concentration were renewed once, and the supernatant of each well was sucked into EP tube and stored at -20 °C for inspection.

Table 1. The concentrations of Lamivudine and the compounds15 and 17

Drug	concentration (μg/L)				
Lamivudine (3TC)	20,000	2000	200	20	2
Compound **15**	10	2	0.4	0.08	0.016
Compound **17**	1000	500	100		

[0099] Fluorescence quantification detection on the drugs for HBV PCR:

the inhibition ratio of HBV DNA (%)=(HBV DNA copy numbers in the blank group - HBV DNA copy numbers in the test group) / HBV DNA copy numbers in the blank group X 100%.

[0100] **Results:** The inhibition effects of drugs on HBV DNA in Hep G2.2.15 cells supernatant are shown in Tables 2 and 3

Table 2. The effects of Lamivudine on the copy numbers of HBV DNA (copy numbers, x $\times 10^5$) in the cell supernatant

Group	Concentartion(μg/ml)	HBV DNA Level	Inhibition ratio
Normal	/	99.79	/
3TC	20μg /ml	12.15	87.82
	2μg /ml	25.32	74.63
	0.2μg /ml	39.59	60.33
	0.02μg /ml	55.98	43.9
3TC EC_{50} is 38.78μg/L			

Table 3. The effects of the compounds 15 and 17 on the copy numbers of HBV DNA (copy numbers, x $\times 10^5$) in the cell supernatant

Group	Dose	HBV DNA copy number (*10^5)	Inhibition ratio (%)
Normal	/	32.01	/
3TC	10,000μg/L	5.49	82.8
15	2μg/L	5.21	83.7
	0.4μg/L	10.3	67.8
	0.08μg/L	13.5	57.8
	0.016μg/L	18.9	41.0
17	1000μg/L	10.2	67.9
	500μg/L	14.3	52.2

(continued)

Group	Dose	HBV DNA copy number ($*10^5$)	Inhibition ratio (%)
	100μg/L	19.2	40.6

For the compound **15, EC50** is 0.19μg/L.

Table 4. The cytotoxicity of the compound 15 on Hep G2.2.15 (MTT)

Group	Dose	OD Value	Cell Survival Rate
Normal	/	0.644	/
15	1000μg/ml	0.520	82.6%
	200μg/ml	0.552	85.5%
	40μg/ml	0.606	96.7%
	8μg/ml	0.626	98.7%

In Vitro test on the anti- HCV activity of the present invention

1. Materials and methods

[0101] The HCV replication cells (Avva.5) were cultivated in the Dulbecco's improved Eagle medium containing 10% fetal calf serum and 1mg/ml G418. 293 - Sip - L cells were cultivated in the Dulbecco's improved Eagle medium containing 10% fetal calf serum , 250 μg / ml G418 and 150μg / ml hygromycin B.

1.2 Method for determining HCV infection (RT - PCR method)

[0102] Using Petri culture dish in a diameter of 60mm, the cells were cultivated in a positive culture medium containing 100μl of HCV for 12 hours. Then, the cells were cultivated in the fresh medium containing on HCV and the medium was replaced once everyday. When detecting HCV - RNA 7 days after the cells have been infected with HCV, it is necessary to clean the cells using the Dulbecco's improved Eagle medium in trypsinization and centrifugation methods. The upper layer part cleaned for the second time (used for comparison) and the washed cells were collected for RNA extraction and RT - PCR detection. β-actin mRNA was simultaneously determined for the purpose of comparison.

1.3 Quantitative determination on HCV - RNA

[0103] The quantitative determination on HCV - RNA is performed in automatic PCR Enzyme-linked immunosorbent assay (ELISA). (Edition 2.0, Roche Diagnostics, Branchburg, NJ)

2. Results

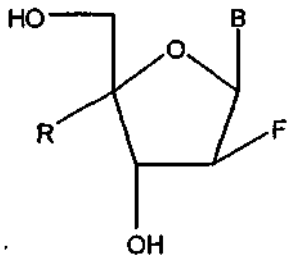
[0104] The compound 17 had inhibition effect when its concentration is 6 μg/ ml, 0.6 μg/ ml or 0.06 μ g/ml (refer to the table below). HCV- RNA level was determined in automatic PCR ELISA.

The concentration of the compound 17 in the cell supernatant	Inhibition effect	
	Test 1	Test 2
0 μg/ml	5.1×10^2	4.4×10^2
0.06 μg/ml	4.9×10^2	4.1×10^2
0.6 μg/ml	0.92×10^2	0.78×10^2
6 μg/ml	1.1×10^2	0.81×10^2

[0105] As shown in this table, the compound 17 had a relatively strong inhibition effect on HCV.

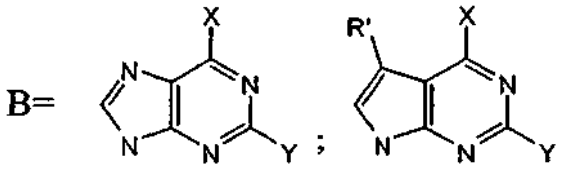
Claims

1. 2'-fluoro-4'- substituted nucleoside analogues, **characterized in that**, the compounds have the structure expressed in the general formula (I):



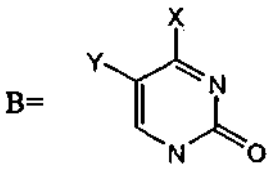
(I)

wherein :



R = CH_3, CN, N_3, C≡CH;
R' = H, F;
X = F, OH, NH_2;
Y = H, CH_3, F, OH, NH_2 -

or
wherein:

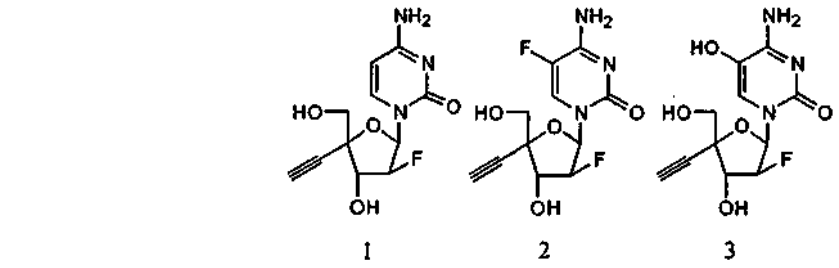


with

R = CH_3, CN, N_3, C≡CH;
X = F, NH_2;
Y = H, CH_3, F, OH, NH_2

2. The 2'-fluoro-4'- substituted nucleoside analogues of claim 1 or 5'-phosphate esters thereof, **characterized in that**, the compounds exist in the form of salts, which are generated through the reaction between the active compounds (I) and organic or inorganic acid.

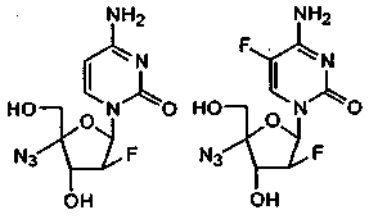
3. The 2'-fluoro-4'- substituted nucleoside analogues of claim 1, **characterized in that**, said compounds are selected from the group consisting of the following compounds:



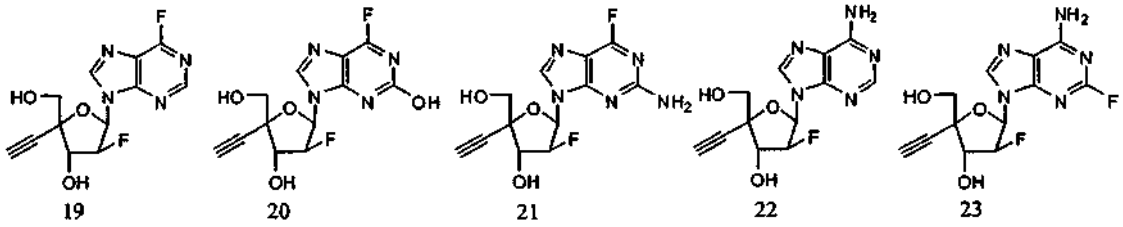
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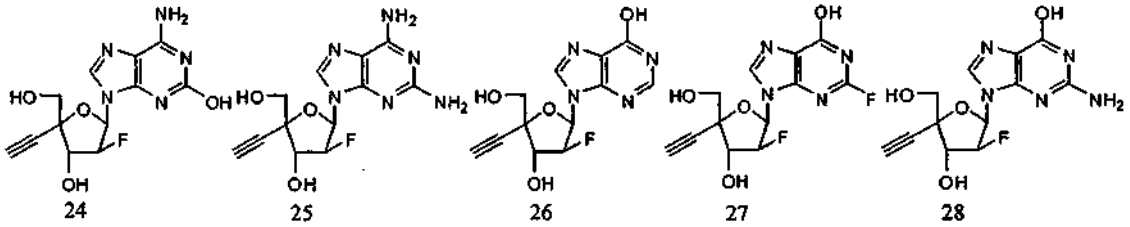
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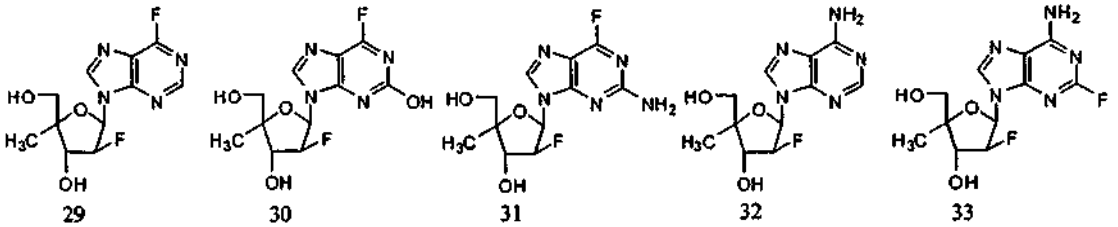
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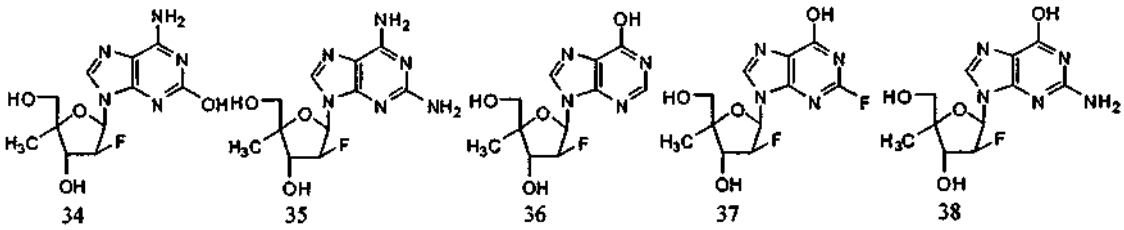
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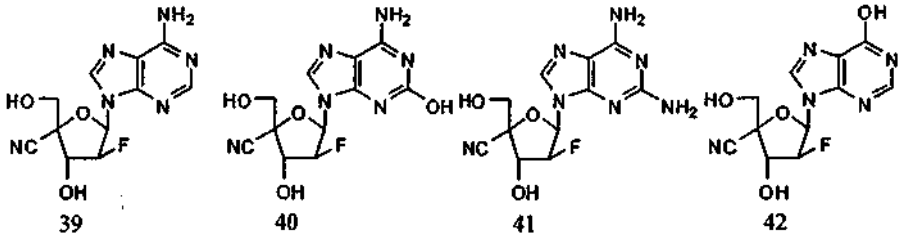


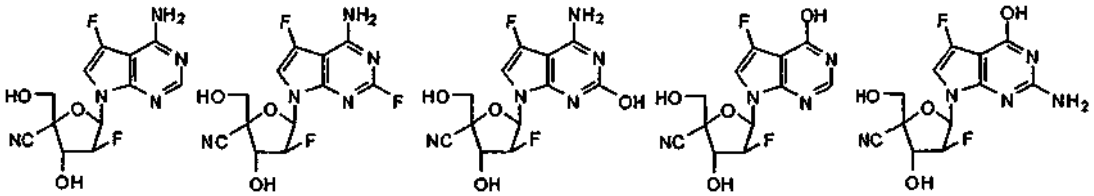
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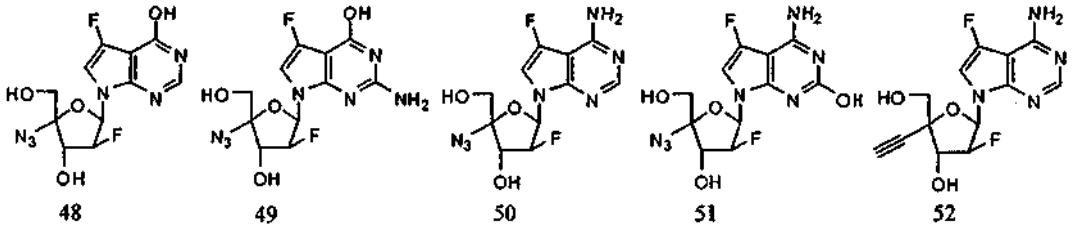


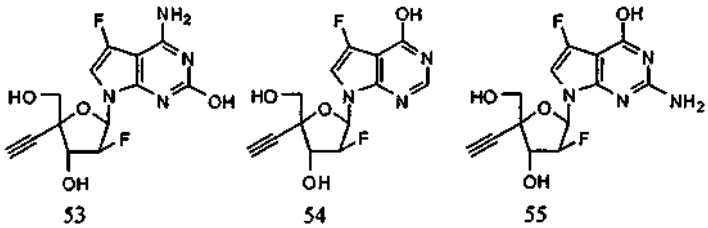
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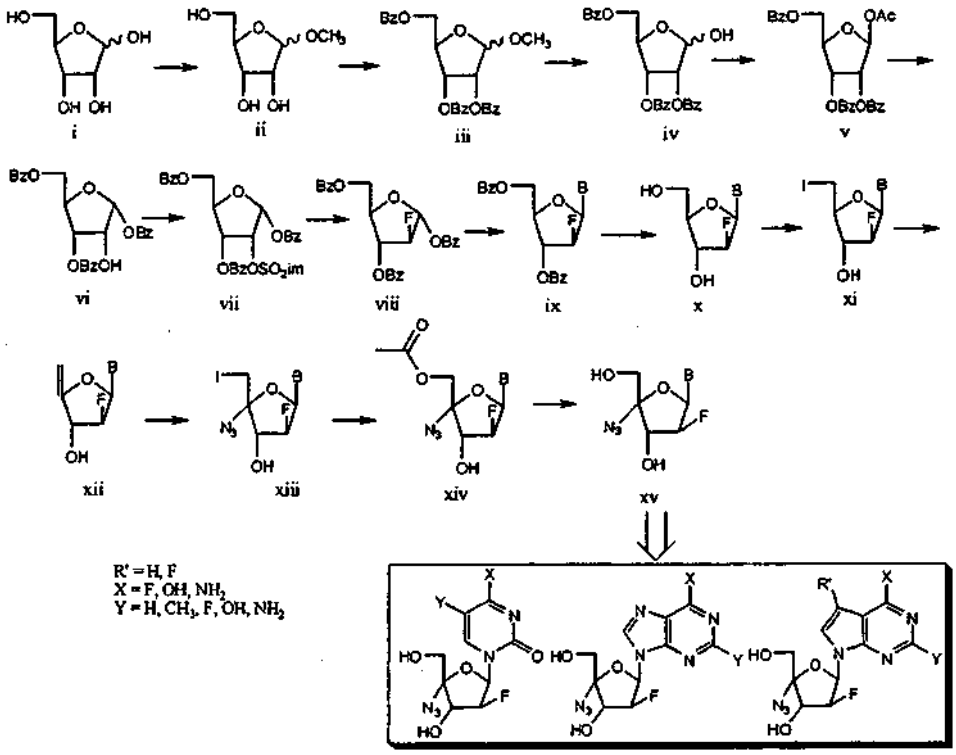

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4. A method for the preparation of the nucleoside analogues of claim 1, **characterized in that**, said method comprises the steps of:



the synthesis of compound ii: dissolving compound i (D-ribose or L-ribose) in HCl/MeOH, stirring in a water bath under hermetization and warm preservation, adding pyridine into the solution to terminate the reaction, and then drawing it off under a reduced pressure, so as to obtain light yellow syrup-like compound ii;

the synthesis of compound iii: dissolving the compound ii in dry pyridine, slowly adding benzoyl chloride dropwise in an ice-salt bath, stirring for reaction at a room temperature, pouring the reaction solution into ice-water after the reaction is completed, and extracting with chloroform; washing the organic layer sequentially with ice-water, pre-cooled sulfuric acid and saturated sodium bicarbonate until water layer shows weak alkaline, and then washing with ice-water until water layer shows neutral; drying the organic layer over anhydrous sodium sulfate, and then drawing it off under a reduced pressure, so as to obtain light yellow syrup-like compound iii;

the synthesis of compound iv: dissolving benzoyl compound iii in chloroform, adding red phosphorus, slowly adding bromine dropwise while stirring the solution in an ice-water bath, stirring for about 30 minutes, slowly adding ice water dropwise and stirring at a room temperature; then adding broken ice into the solution and stirring the solution until the ice is melted; subsequently pouring the reaction solution into ice water and extracting with chloroform; washing the organic layer with saturated sodium bicarbonate until the water layer shows weak alkaline, washing with ice-water until the water layer shows neutral, drying over anhydrous sodium sulfate, and then drawing it off under a reduced pressure to obtain dark yellow syrup; finally the syrup being separated by silica gel column, so as to obtain white dry syrup compound iv;

the synthesis of compound v: dissolving the compound iv in dry pyridine, slowly adding acetic anhydride dropwise in an ice water bath, and then stirring for about 30 minutes; removing the ice water bath, stirring at a room temperature for about 7 hours, and then the solution being heated up to 40°C and kept for an hour; after adding broken ice and stirring it to be melted, pouring the reaction solution into ice water and extracting with chloroform; washing the organic layer sequentially with ice water, pre-cooled sulfuric acid and saturated sodium bicarbonate until the water layer shows weak alkaline, then washing with ice water until the water layer shows neutral, drying over anhydrous sodium sulfate, and then drawing it off under a reduced pressure, so as to obtain light yellow syrup-like compound v;

the synthesis of compound vi: dissolving the compound v in dry dichloromethane and slowly introducing the dry HCl gas into an ice-water bath, washing with ice-water so that an organic layer is separated; washing the organic layer with saturated sodium bicarbonate until the water layer shows weak alkaline, washing the organic layer with ice-water until the water layer shows neutral, drying over anhydrous sodium sulfate, and drawing it off under a reduced pressure to obtain light yellow syrup; recrystallizing the syrup to obtain white solid compound vi;

the synthesis of compound vii: dissolving the compound vi in dry dichloromethane and dry DMF, slowly adding SO_2Cl_2 into the solution with stirring at about -15 °C for about 30 minutes, naturally warming up to a room temperature for reaction; adding imidazole into the solution for three times at 0°C, and stirring the mixture at a room temperature,

diluting the reaction solution by adding CH_2Cl_2 after the completion of the reaction, then washing the solution with ice water; extracting the water layer with CH_2Cl_2; combining the organic layers and then drying over anhydrous sodium sulfate, and drawing it off under a reduced pressure to obtain light yellow syrup; separating and purifying the syrup by silica gel column, so as to obtain white solid compound vii;

the synthesis of compound viii: dissolving the compound vii in ethyl acetate, and adding Et3N • 3HF with stirring; heating up to about 60°C with stirring for about 3h, and then heating up to 70 °C with stirring for 1.5h; adding ice-salt water to terminate the reaction, extracting with dichloromethane; combining the organic layers and then washing sequentially with saline, water and saturated sodium bicarbonate, drying over anhydrous sodium sulfate, and drawing it off under a reduced pressure to obtain dark yellow syrup; purifying the syrup to obtain light yellow syrup, and finally crystallizing the crude product to obtain the white crystalline compound viii;

the synthesis of compound ix: dissolving the compound viii in anhydrous dichloromethane , adding a mixed solution of HBr-AcOH, and stirring for reaction at a room temperature; after completion of the reaction, evaporating the mixture to dryness; dissolving the residue with dichloromethane, washing the dichloromethane solution with sodium bicarbonate solution, and removing dichloromethane through evaporation to obtain a syrup-like product; at the same time, the protected cytosine and $(NH_4)_2SO_4$ refluxing under nitrogen protection in the HMDS; after the completion of reaction, evaporating the solvent under a reduced pressure to obtain the silylated cytosine; dissolving the syrup obtained from the above reaction into dichloroethane, which is added into the silylated cytosine, and then refluxing for reaction under N_2 protection; adding ice to terminate the reaction; extracting with dichloromethane; and washing sequentially the dichloromethane layer with saturated sodium bicarbonate and saline, and drying over anhydrous sodium sulfate; evaporating the solvent to obtain white solid, separating and purifying by column chromatography to obtain the compound ix;

the synthesis of compound x: dissolving the compound ix in saturated NH_3-CH_3OH and stirring for reaction at a room temperature; after completion of the reaction, evaporating the solvent to dryness to obtain the residue; and purifying the residue by column chromatography to obtain the compound x;

the synthesis of compound xi: dissolving the compound x, imidazole and triphenylphosphine in tetrahydrofuran, slowly adding iodiferous tetrahydrofuran; stirring for reaction at a room temperature; removing the solvent through evaporation after completion of the reaction, adding ethyl acetate in the residue and filtering; evaporating ethyl acetate and separating the residue by column chromatography to obtain the compound xi;

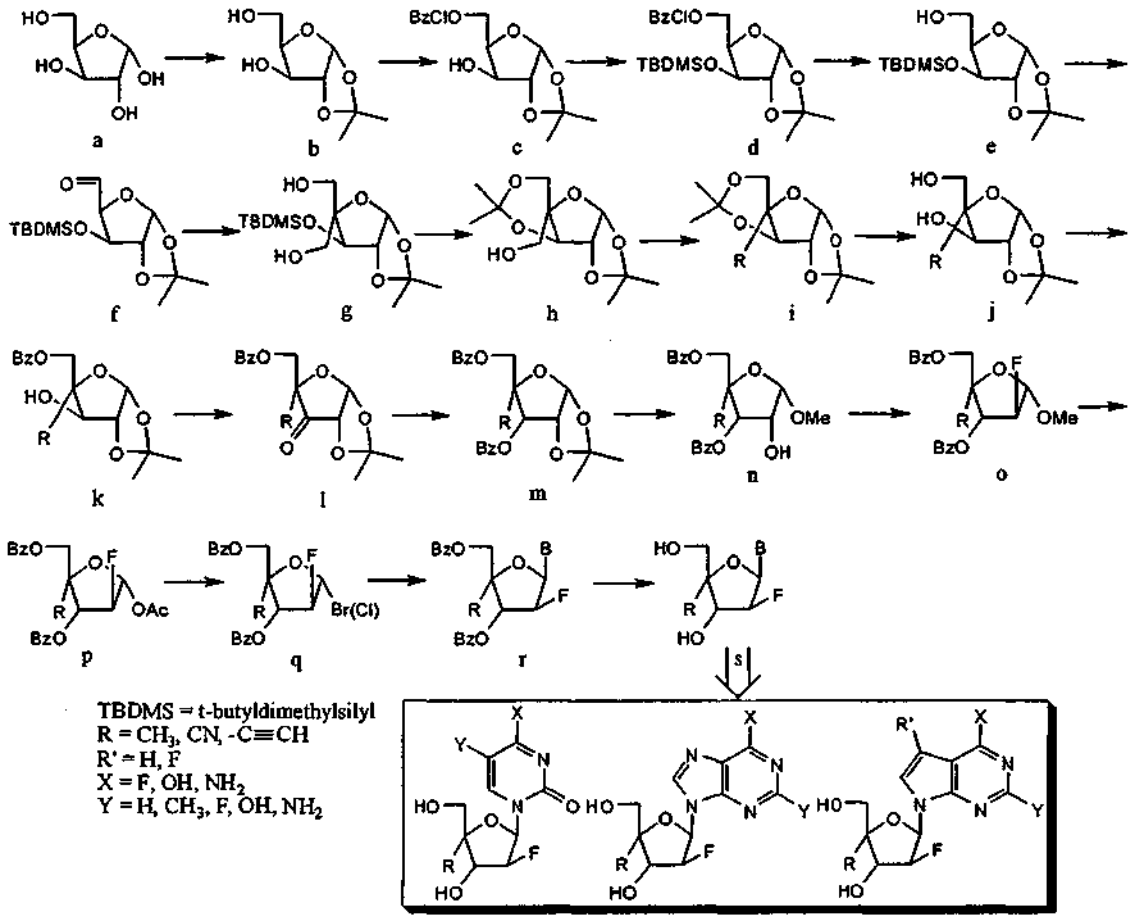
the synthesis of compound xii: dissolving the compound ix in tetrahydrofuran and adding DBU; stirring for reaction at about 60°C; evaporating the solvent to dryness after completion of the reaction; separating the residue by column chromatography to obtain the compound xii;

the synthesis of compound xiii: adding the DMF solution dissolved with ICl in the DMF solution dissolved with NaN_3 at 0 °C; stirring at the freezing point for about 10 minutes and slowly adding DMF dissolved with the compound xii to continue the reaction; a after completion of the reaction, adding sodium sulfite in the mixed solution until the color of iodine disappears completely; removing the solvent through evaporation under a reduced pressure; separating and purifying the residue by column chromatography, so as to obtain the compound xiii;

the synthesis of compound xiv: dissolving the compound xiii in DMF, and adding silver acetate with stirring; reacting at a room temperature; filtering after completion of the reaction; removing the solvent under a reduced pressure; purifying the residue by column chromatography, so as to obtain the compound xiv;

the synthesis of compound xv: dissolving the compound xiii in the solution of methanol-triethylamine, stirring for reaction at a room temperature; removing the solvent through evaporation after completion of the reaction, and purifying the residue by column chromatography, so as to obtain the compound xv.

5. A method for preparation of the nucleoside analogues of claim 1, **characterized in that**: said preparation method comprises the following steps:



the synthesis of compound b: adding compound a in acetone, slowly adding the concentrated sulfuric acid while stirring at a room temperature, and stirring for reaction at a room temperature; after the reaction is detected by TLC as complete, adding the concentrated ammonia to adjust pH=7-8, filtering and evaporating under a reduced pressure to remove most of the acetone; then adding 0.4% dilute hydrochloric acid with stirring, and reacting under the acetone reflux condition until the compound a has been completely hydrolyzed into the compound b; neutralizing the reaction solution by solid $NaHCO_3$, filtering, and evaporating to remove the solvent under a reduced pressure; dissolving the residue by dichloromethane, drying over anhydrous Na_2SO_4 for overnight, filtering, and then evaporating to remove the solvent, so as to obtain the yellow viscous oily compound b;

the synthesis of compound c: dissolving the compound b and triethylamine in dichloromethane; slowly adding dropwise parachlorobenzoyl chloride in an ice salt bath, and mechanically stirring for reaction at a temperature below 0 °C; after completion of the reaction, adding a saturated $NaHCO_3$ solution; washing the dichloromethane layer with water and saturated salt water, drying over anhydrous $MgSO_4$, filtering, and then evaporating under a reduced pressure to remove the solvent; recrystallizing the residue to obtain the white crystalline compound c;

the synthesis of compound d: dissolving the compound c in anhydrous dichloromethane; adding imidazole and tert-butyl-2-methyl chloride silicon (TBDMSCI) under nitrogen protection for the reaction at a room temperature; after completion of the reaction, neutralizing the reaction by hydrochloric acid, and obtaining two layers; washing the organic layer with water and saturated salt water respectively, drying over anhydrous Na_2SO_4, and then evaporating to remove the solvent; separating and purifying the residue by column chromatography, so as to obtain the compound d;

the synthesis of compound e: dissolving the compound xiii in the mixed solution of sodium methoxide and methanol for reaction at a room temperature; after completion of the reaction, neutralizing the solution by dilute acetic acid, filtering, washing with methanol and then evaporating under a reduced pressure to remove the solvent; finally separating and purifying the residue by column chromatography, so as to obtain the compound e;

the synthesis of compound f: adding dropwise DMSO into the dichloromethane solution of oxalyl chloride at about -60 °C; after stirring at the same temperature for about 15 minutes, adding dropwise the dichloromethane solution of the compound e; after stirring for reaction at about -65 °C for 30 minutes, adding triethylamine and stirring for the reaction at a room temperature; after completion of the reaction, adding water into the reaction solution so that an

organic layer is separated; drying the organic layer over anhydrous magnesium sulfate, removing the solvent through evaporation; and separating and purifying the residue by column chromatography, so as to obtain the compound xiii;

the synthesis of compound g: adding sodium hydroxide and water in the reaction bottle; after stirring evenly, adding formaldehyde solution, 95% ethanol, and then the compound f, with stirring for reaction at about 35 °C; after completion of the reaction, cooling the reaction bottle with ice water while stirring until the product is completely precipitated; after the suction filtration, washing the solid product with water to neutral, drying, and dissolving it in anhydrous methanol, and then adding sodium borohydride for reflux reaction; after completion of the reaction, neutralizing the reaction solution by dilute hydrochloric acid and then extracting with dichloromethane, drying over anhydrous Na_2SO_4 , and removing the solvent, so as to obtain the compound g;

the synthesis of compound h: dissolving the compound g in methanol, and adding Dowex H^+ (washed with methanol in advance) for reaction at a room temperature; after completion of the reaction, removing the resin from the solution through filtration and repeatedly washing it with methanol, and evaporating the solution to obtain the solid substance; dissolving the obtained solid substance in acetone, then slowly adding concentrated sulfuric acid (a catalytic amount) into the solution for reaction at a room temperature while stirring; adding concentrated ammonia water to adjust pH value, and then filtering and evaporating under a reduced pressure to remove acetone, so as to obtain the compound h;

the synthesis of compound i: adding dropwise DMSO into the dichloromethane solution of oxalyl chloride at about -60 °C, and stirring for about 15 minutes after dripping off; adding dropwise the dichloromethane solution of the compound h, reacting for about 30 minutes at -60 °C after dripping off; adding triethylamine in the solution; after the reaction is carried out at a room temperature for about 30 minutes, adding water to terminate the reaction; separating the organic layer and drying over anhydrous Na_2SO_4; removing the solvent through evaporation and separating and purifying the residue by column chromatography to obtain the solid product; adding $ClCH_2P(C_6H_5)_3Cl$ into anhydrous tetrahydrofuran, cooling to about -78 °C, adding n-BuLi hexane solution, and stirring for reaction for about an hour; then adding the dry tetrahydrofuran solution of the solid product; increasing the temperature of the solution gradually to about 0 °C to continue the reaction; after completion of the reaction, adding a saturated NH_4Cl solution carefully dropwise in the solution, and subsequently extracting with ethyl acetate; washing the organic layer with saline twice, drying over anhydrous $MgSO_4$, and evaporating to remove the solvent; dissolving the residue obtained into anhydrous tetrahydrofuran, cooling to -78 °C, and slowly adding dropwise n-BuLi hexane solution; after stirring the solution for 2 hours, terminating the reaction carefully by using saturated NH_4Cl; washing the organic layer with saturated salt water, drying over anhydrous MgSO4, and evaporating under a reduced pressure to remove the solvent; finally separating and purifying the residue by rapid silica gel column, so as to obtain the compound i;

the synthesis of compound j: adding the compound i in the diluted hydrochloric acid solution, stirring the solution at a room temperature for reaction; after completion of the reaction, neutralizing the solution with solid $NaHCO_3$, and then filtering; evaporating the filtrate under a reduced pressure to remove the solvent; dissolving the residue in dichloromethane; filtering after drying over anhydrous Na_2SO_4, and evaporating to remove the solvent, so as to obtain the compound j;

the synthesis of compound k: dissolving the compound j in pyridine, adding BzCl dropwise for reaction at a room temperature; after completion of the reaction, evaporating the solvent to dryness, and purifying the residue to obtain the compound k;

the synthesis of compound 1: dissolving the compound k and 2,2,6,6-tetramethyl piperidine-N-oxide in dichloromethane, cooling to 0 °C and adding the mixed solution of sodium hypochlorite solution, $NaHCO_3$ and water in the reaction solution for reaction at 0 °C for about 30 minutes; adding isopropanol and stirring at a room temperature; washing the separated dichloromethane layer with water twice, drying over anhydrous $MgSO_4$, filtering, and evaporating to remove the solvent; recystalizing the residue to obtain the compound 1;

the synthesis of compound m: dissolving the compound 1 in the mixed solution of absolute ethanol and ethyl acetate and cooling to about 0 °C; adding sodium borohydride in batches and stirring for reaction; after completion of the reaction, neutralizing with dilute acetic acid solution, filtering, and evaporating the filtrate to dryness; dissolving the residue in dichloromethane and then washing with water; drying the dichloromethane layer over anhydrous $MgSO_4$, filtering, and evaporating to remove the dichloromethane under a reduced pressure; recrystallizing the residue by the mixed solvent of dichloromethane and petroleum ether to obtain solid substance; then dissolving the solid product in pyridine, and adding dropwise BzCl at about 0°C; after that, the reaction is carried out at a room temperature; after completion of the reaction, removing the solvent through evaporation and extracting the residue with ethyl acetate, washing with saturated $NaHCO_3$ solution, drying over anhydrous Na_2SO_4, filtering, and evaporating to remove the solvent, so as to obtain the compound m;

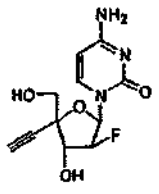
the synthesis of compound n: dissolving the compound m in methanol, adding the mixed solution of concentrated hydrochloric acid/dioxane for reaction at a room temperature; after completion of the reaction, neutralizing the reaction solution with $NaHCO_3$, and filtering; removing the solvent from the filtrate by vacuum distillation; dissolving the residue in appropriate amount of dichloromethane, drying over anhydrous Na_2SO_4 for overnight , filtering, and

evaporating to dryness; separating and purifying by column chromatography to obtain the compound n;
the synthesis of compound o: dissolving the compound n in dichloromethane, and adding DAST under a room temperature and stirring for reaction; after completion of the reaction, pouring the mixture into the saturated $NaHCO_3$ solution and separating the organic layer; washing the organic layer with the saturated $NaHCO_3$ solution, drying over anhydrous Na_2SO_4, and separating and purifying by column chromatography to obtain the compound o;
the synthesis of compound p: dissolving the compound o in formic acid solution, and stirring at a room temperature for reaction; after completion of the reaction, removing the solvent under a reduced pressure; evaporating the residue and toluene together to obtain the solid product; dissolving the obtained a solid product in the solution of dry pyridine and acetic anhydride for reaction at a room temperature; after the reaction is completed, concentrating the reaction solution and then evaporating together with toluene, so as to obtain the compound p;
the synthesis of compound q: dissolving the compound p in anhydrous dichloromethane and cooling to 0°C; slowly introducing the HCl gas into the solution to a saturation level and removing the solvent by vacuum evaporation at a room temperature; drying the residue in vacuum to obtain the compound q;
the synthesis of compound r: with the existence of $(NH_4)_2SO_4$, refluxing 2,6-diamino-purine and Hexamethyldisilazane (HMDS) to be a transparent solution; removing the solvent under vacuum to obtain a white solid product, and dissolving the solid product in 1,2-dichloroethane; adding 1,2-dichloroethane solution of compound q and molecular sieve , and stirring for reaction under nitrogen protection at a room temperature; after the reaction is detected by TLC as complete, adding dichloromethane in the reaction solution and filtering by diatomite; washing the filtrate with saturated Na_2SO_4 and saline respectively; drying the organic layer over anhydrous Na_2SO_4 overnight and removing the solvent through evaporation to obtain the residue; separating the residue under a reduced pressure through silica gel column, so as to obtain the compound r;
the synthesis of compound s: dissolving the compound r in a saturated NH_3-CH_3OH solution, and stirring for reaction at a room temperature; after completion of the reaction, removing the solvent through evaporation and separating and purifying the residue by column chromatography, so as to obtain the compound s.

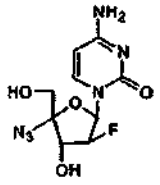
6. The uses of 2'-fluoro-4'- substituted nucleoside analogues of claim 1, 2, 3 in pharmaceutical preparation, **characterized in that**, said compounds are applied in the preparation of anti-HBV, anti-HCV or anti-HIV drugs.

7. The uses of 2'-fluoro-4'- substituted nucleoside analogue of claim 6 in pharmaceutical preparation, **characterized in that**, HBV antiviral drugs are anti-HBV drugs; HIV antiviral drugs are anti-HIV drugs; and HCV antiviral drugs are anti-HCV drugs.

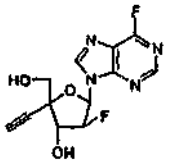
8. The 2'-fluoro-4'-substituted nucleoside analogues of claim 1, **characterized in that**, said compounds are selected from the group consisting of the following compounds:



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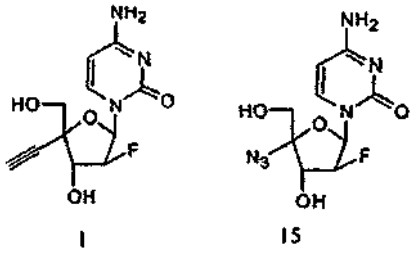


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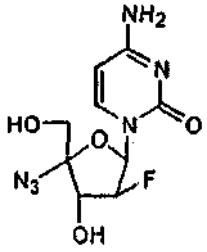
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9. The 2'-fluoro-4'-substituted nucleoside analogues of claim 1, **characterized in that**, said compounds are selected from the group consisting of the following compounds:



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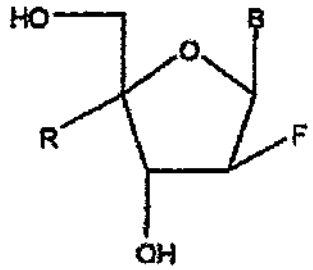
10. The 2'-fluoro-4'-substituted nucleoside analogue of claim 1, **characterized in that**, said compound is



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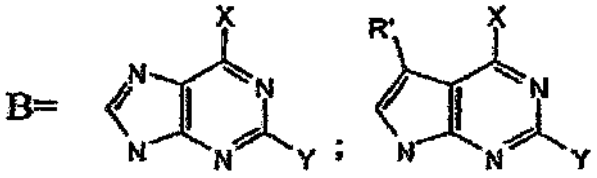
Patentansprüche

1. 2'-Fluor-4'-substituierte Nucleosidanaloga, **dadurch gekennzeichnet, dass** die Verbindungen die in der allgemeinen Formel (I) ausgedrückte Struktur aufweisen:



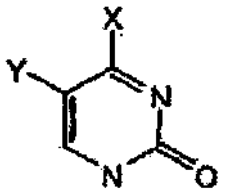
(I)

wobei:



R=CH_3, CN, N_3, C≡CH;
R' = H, F;
X = F, OH, NH_2;
Y = H, CH_3, F, OH, NH_2-

oder wobei:

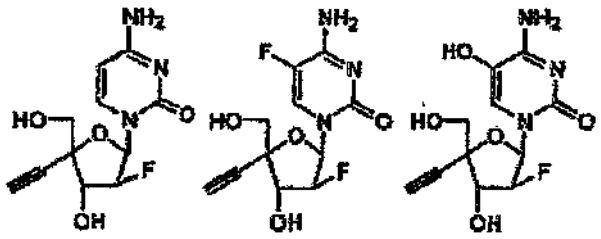
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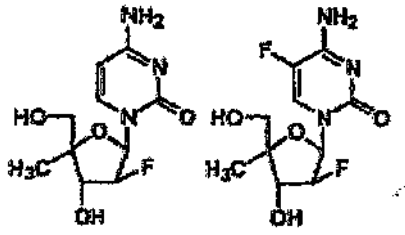
R = CH_3, CN, N_3, C≡CH;
X = F, NH_2;
Y = H, CH_3, F, OH, NH_2.

2. 2'-Fluor-4'-substituierte Nucleosidanaloga gemäß Anspruch 1 oder 5'-Phosphorsäureester davon, **dadurch gekennzeichnet, dass** die Verbindungen in Form von Salzen vorliegen, die durch die Reaktion zwischen den aktiven Verbindungen (I) und einer organischen oder anorganischen Säure entstehen.

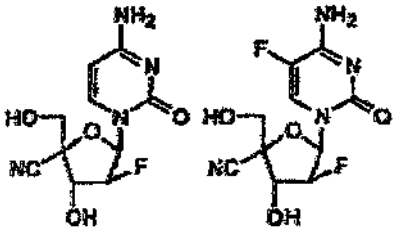
3. 2'-Fluor-4'-substituierte Nucleosidanaloga gemäß Anspruch 1, **dadurch gekennzeichnet, dass** die Verbindungen aus der Gruppe ausgewählt sind, die aus den folgenden Verbindungen besteht:



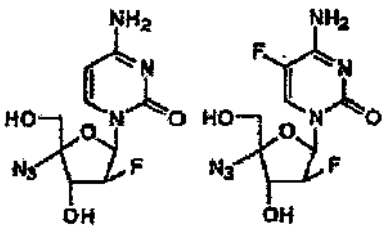
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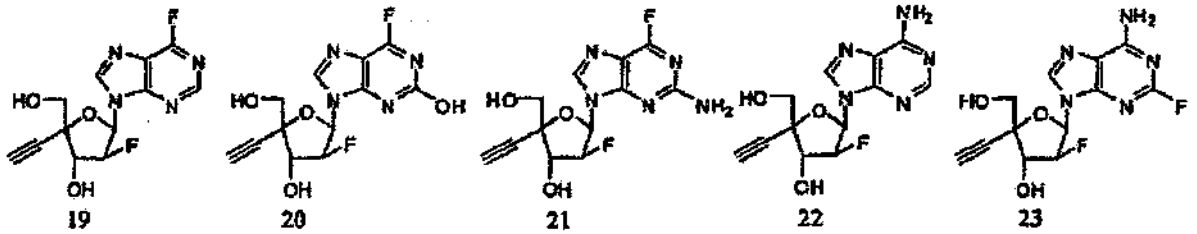
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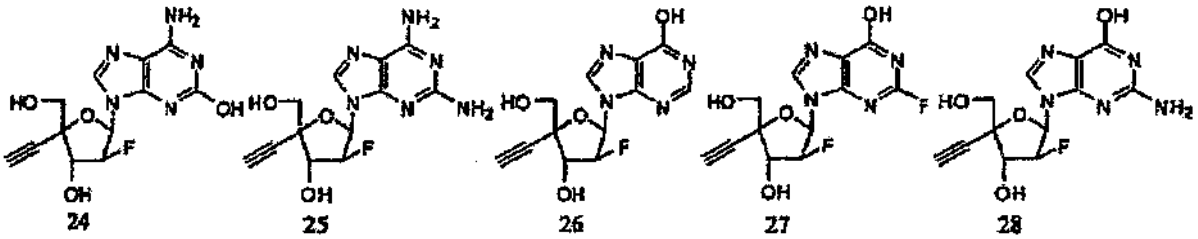


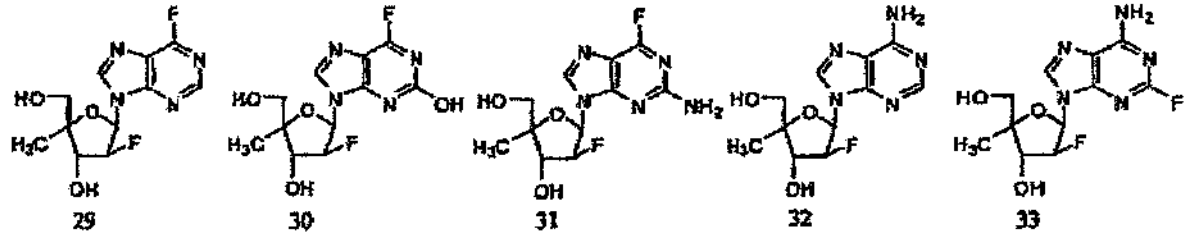
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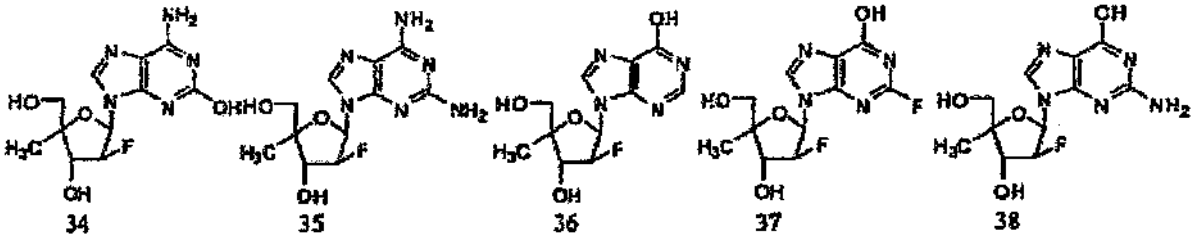


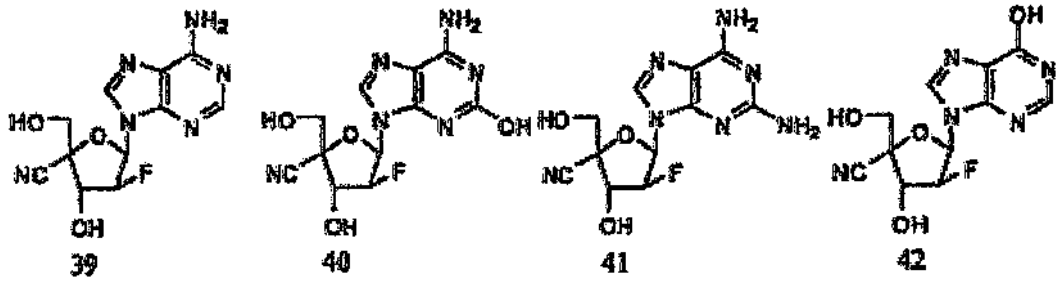
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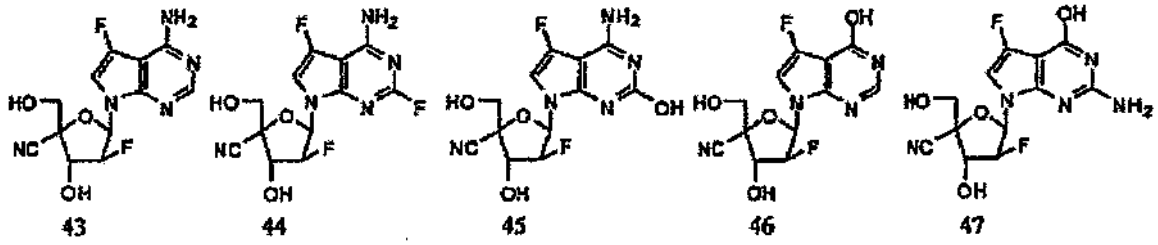

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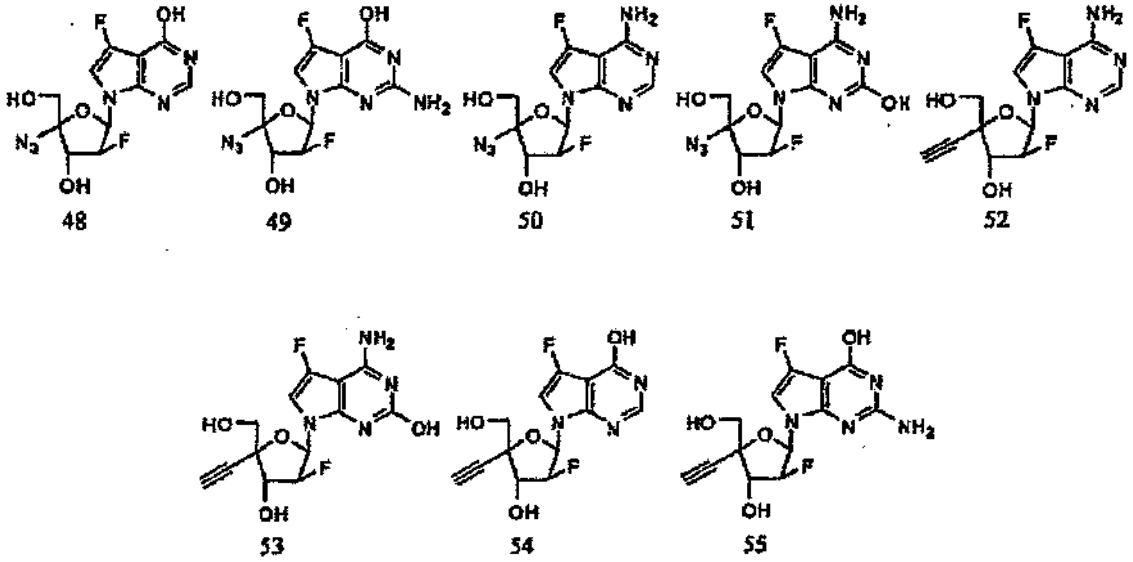

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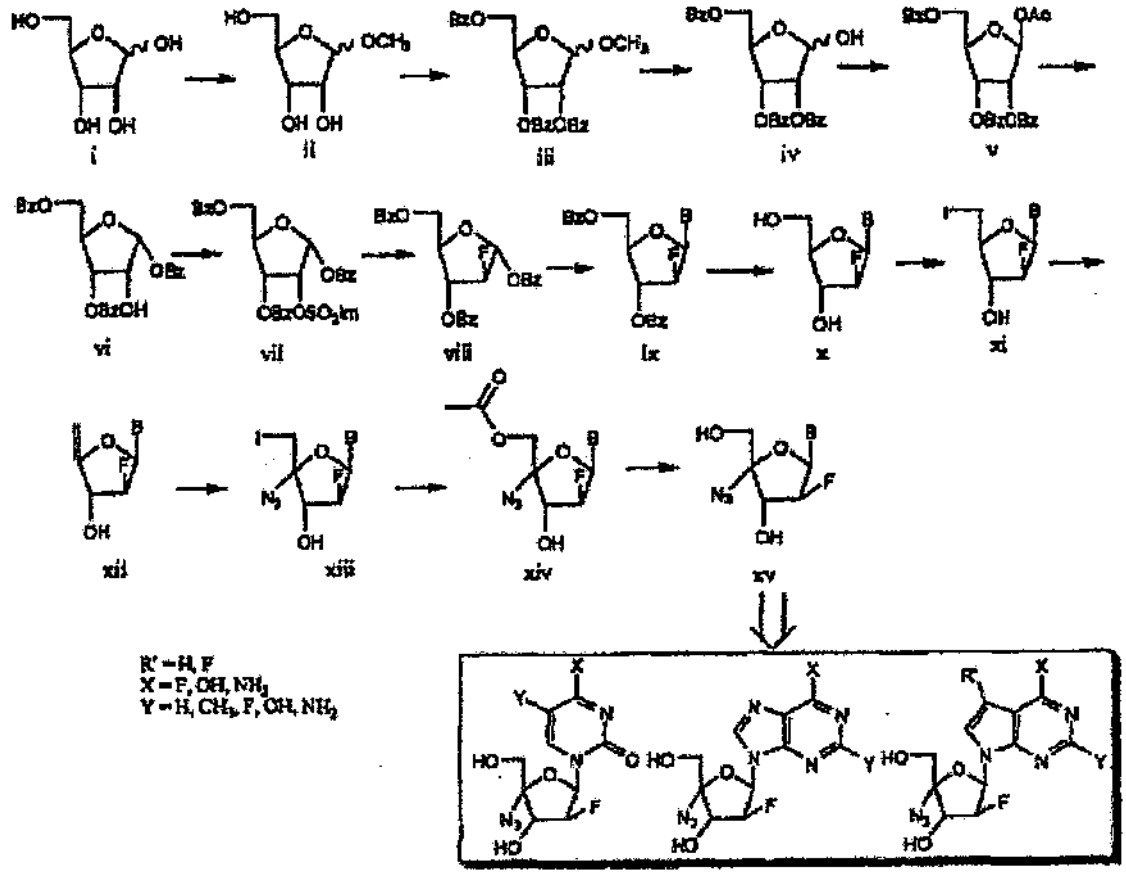

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4. Verfahren zur Herstellung der Nucleosidanaloga gemäß Anspruch 1, **dadurch gekennzeichnet, dass** das Verfahren die folgenden Schritte umfasst:



Synthese von Verbindung ii: Auflösen der Verbindung i (D-Ribose oder L-Ribose) in HCl/MeOH, Rühren in einem Wasserbad unter Luftausschluss und Warmhalten, Hinzufügen von Pyridin zu der Lösung zum Abbruch der Reaktion und dann Absaugen unter reduziertem Druck, wobei man die hellgelbe sirupartige Verbindung ii erhält;
Synthese von Verbindung iii: Auflösen der Verbindung ii in trockenem Pyridin, langsames Hinzufügen von Benzoylchlorid tropfenweise in einem Eis-Kochsalz-Bad, Rühren für eine Reaktion bei Raumtemperatur, Gießen der Re-

aktionslösung in Eiswasser, nachdem die Reaktion beendet ist, und Extrahieren mit Chloroform; Waschen der organischen Schicht nacheinander mit Eiswasser, vorgekühlter Schwefelsäure und gesättigter Natriumhydrogencarbonat-Lösung, bis die Wasserschicht schwach alkalisch ist, und dann Waschen mit Eiswasser, bis die Wasserschicht neutral ist;

Trocknen der organischen Schicht über wasserfreiem Natriumsulfat und dann Absaugen unter reduziertem Druck, wobei man die hellgelbe sirupartige Verbindung iii erhält;

Synthese von Verbindung iv: Auflösen der Benzoylverbindung iii in Chloroform, Hinzufügen von rotem Phosphor, langsames Hinzufügen von Brom tropfenweise und unter Rühren der Lösung in einem Eiswasserbad, Rühren während etwa 30 Minuten, langsames Hinzufügen von Eiswasser tropfenweise und Rühren bei Raumtemperatur; dann Hinzufügen von zerstoßenem Eis zu der Lösung und Rühren der Lösung, bis das Eis geschmolzen ist; anschließend Gießen der Reaktionslösung in Eiswasser und Extrahieren mit Chloroform; Waschen der organischen Schicht mit gesättigter Natriumhydrogencarbonat-Lösung, bis die Wasserschicht schwach alkalisch ist, Waschen mit Eiswasser, bis die Wasserschicht neutral ist, Trocknen über wasserfreiem Natriumsulfat, und dann Absaugen unter reduziertem Druck, wobei man einen dunkelgelben Sirup erhält; schließlich wird der Sirup über eine Silicagel-Säule aufgetrennt, wobei man Verbindung iv als weißen trockenen Sirup erhält;

Synthese von Verbindung v: Auflösen der Verbindung iv in trockenem Pyridin, langsames Hinzufügen von Essigsäureanhydrid tropfenweise in einem Eiswasserbad und dann Rühren während etwa 30 Minuten; Entfernen des Eiswasserbads, Rühren bei Raumtemperatur während etwa 7 Stunden, und dann wird die Lösung auf 40 °C erhitzt und eine Stunde lang gehalten; nach Hinzufügen von zerstoßenem Eis und Rühren, bis es geschmolzen ist, Gießen der Reaktionslösung in Eiswasser und Extrahieren mit Chloroform; Waschen der organischen Schicht nacheinander mit Eiswasser, vorgekühlter Schwefelsäure und gesättigter Natriumhydrogencarbonat-Lösung, bis die Wasserschicht schwach alkalisch ist, und dann Waschen mit Eiswasser, bis die Wasserschicht neutral ist, Trocknen über wasserfreiem Natriumsulfat und dann Absaugen unter reduziertem Druck, wobei man die hellgelbe sirupartige Verbindung v erhält;

Synthese von Verbindung vi: Auflösen der Verbindung v in trockenem Dichlormethan und langsames Einleiten von trockenem HCl-Gas in ein Eiswasserbad, Waschen mit Eiswasser, so dass sich eine organische Schicht abscheidet; Waschen der organischen Schicht mit gesättigter Natriumhydrogencarbonat-Lösung, bis die Wasserschicht schwach alkalisch ist, Waschen der organischen Schicht mit Eiswasser, bis die Wasserschicht neutral ist, Trocknen über wasserfreiem Natriumsulfat und Absaugen unter reduziertem Druck, wobei man einen hellgelben Sirup erhält; Umkristallisieren des Sirups, wobei man die Verbindung vi als weißen Feststoff erhält;

Synthese von Verbindung vii: Auflösen der Verbindung vi in trockenem Dichlormethan und trockenem DMF, langsames Hinzufügen von SO_2Cl_2 zu der Lösung unter Rühren bei etwa -15 °C während etwa 30 Minuten, Aufwärmenlassen auf Raumtemperatur für die Reaktion; dreimaliges Hinzufügen von Imidazol zu der Lösung bei 0 °C und Rühren des Gemischs bei Raumtemperatur, Verdünnen der Reaktionslösung durch Hinzufügen von CH_2Cl_2, nachdem die Reaktion beendet ist, dann Waschen der Lösung mit Eiswasser; Extrahieren der Wasserschicht mit CH_2Cl_2; Vereinigen der organischen Schichten und dann Trocknen über wasserfreiem Natriumsulfat und Absaugen unter reduziertem Druck, wobei man einen hellgelben Sirup erhält; Auftrennen und Reinigen des Sirups durch eine SilicagelSäule, wobei man Verbindung vii als weißen Feststoff erhält;

Synthese von Verbindung viii: Auflösen der Verbindung vii in Ethylacetat und Hinzufügen von $Et_3N{\cdot}3HF$ unter Rühren; Erhitzen auf etwa 60 °C unter Rühren während etwa 3 h und dann Erhitzen auf 70 °C unter Rühren während 1,5 h; Hinzufügen von Eis-Salzwasser zum Abbruch der Reaktion, Extrahieren mit Dichlormethan; Vereinigen der organischen Schichten und dann nacheinander Waschen mit Kochsalzlösung, Wasser und gesättigter Natriumhydrogencarbonat-Lösung, Trocknen über wasserfreiem Natriumsulfat und Absaugen unter reduziertem Druck, wobei man einen dunkelgelben Sirup erhält; Reinigen des Sirups, wobei man einen hellgelben Sirup erhält, und schließlich Kristallisieren des Rohprodukts, wobei man die weiße kristalline Verbindung viii erhält;

Synthese von Verbindung ix: Auflösen der Verbindung viii in wasserfreiem Dichlormethan, Hinzufügen einer gemischten Lösung von HBr-AcOH und Rühren für eine Reaktion bei Raumtemperatur; nachdem die Reaktion beendet ist, Verdampfen des Gemischs zur Trockne; Auflösen des Rückstands mit Dichlormethan, Waschen der Dichlormethan-Lösung mit Natriumhydrogencarbonat-Lösung und Entfernen des Dichlormethans durch Verdampfen, wobei man ein sirupartiges Produkt erhält; gleichzeitig Erhitzen von geschütztem Cytosin und $(NH_4)_2SO_4$ unter Rückfluss und unter Stickstoff als Schutzgas in HMDS; nachdem die Reaktion beendet ist, Verdampfen des Lösungsmittels unter reduziertem Druck, wobei man silyliertes Cytosin erhält; Auflösen des aus der obigen Reaktion erhaltenen Sirups in Dichlorethan, Hinzufügen der Lösung zu silyliertem Cytosin und dann Erhitzen unter Rückfluss für eine Reaktion unter N_2 als Schutzgas; Hinzufügen von Eis zum Abbruch der Reaktion; Extrahieren mit Dichlormethan; und nacheinander Waschen der Dichlormethan-Schicht mit gesättigter Natriumhydrogencarbonat-Lösung und Kochsalzlösung und Trocknen über wasserfreiem Natriumsulfat; Verdampfen des Lösungsmittels, wobei man einen weißen Feststoff erhält, Auftrennen und Reinigen durch Säulenchromatographie, wobei man Verbindung ix erhält;

Synthese von Verbindung x: Auflösen der Verbindung ix in gesättigtem NH_3-CH_3OH und Rühren für eine Reaktion

bei Raumtemperatur; nachdem die Reaktion beendet ist, Verdampfen des Lösungsmittels zur Trockne, wobei man einen Rückstand erhält; und Reinigen des Rückstands durch Säulenchromatographie, wobei man Verbindung x erhält;

Synthese von Verbindung xi: Auflösen von Verbindung x, Imidazol und Triphenylphosphin in Tetrahydrofuran, langsames Hinzufügen von iodhaltigem Tetrahydrofuran; Rühren für eine Reaktion bei Raumtemperatur; Entfernen des Lösungsmittels durch Verdampfen, nachdem die Reaktion beendet ist, Hinzufügen von Ethylacetat zu dem Rückstand und Filtrieren; Verdampfen von Ethylacetat und Auftrennen des Rückstands durch Säulenchromatographie, wobei man Verbindung xi erhält;

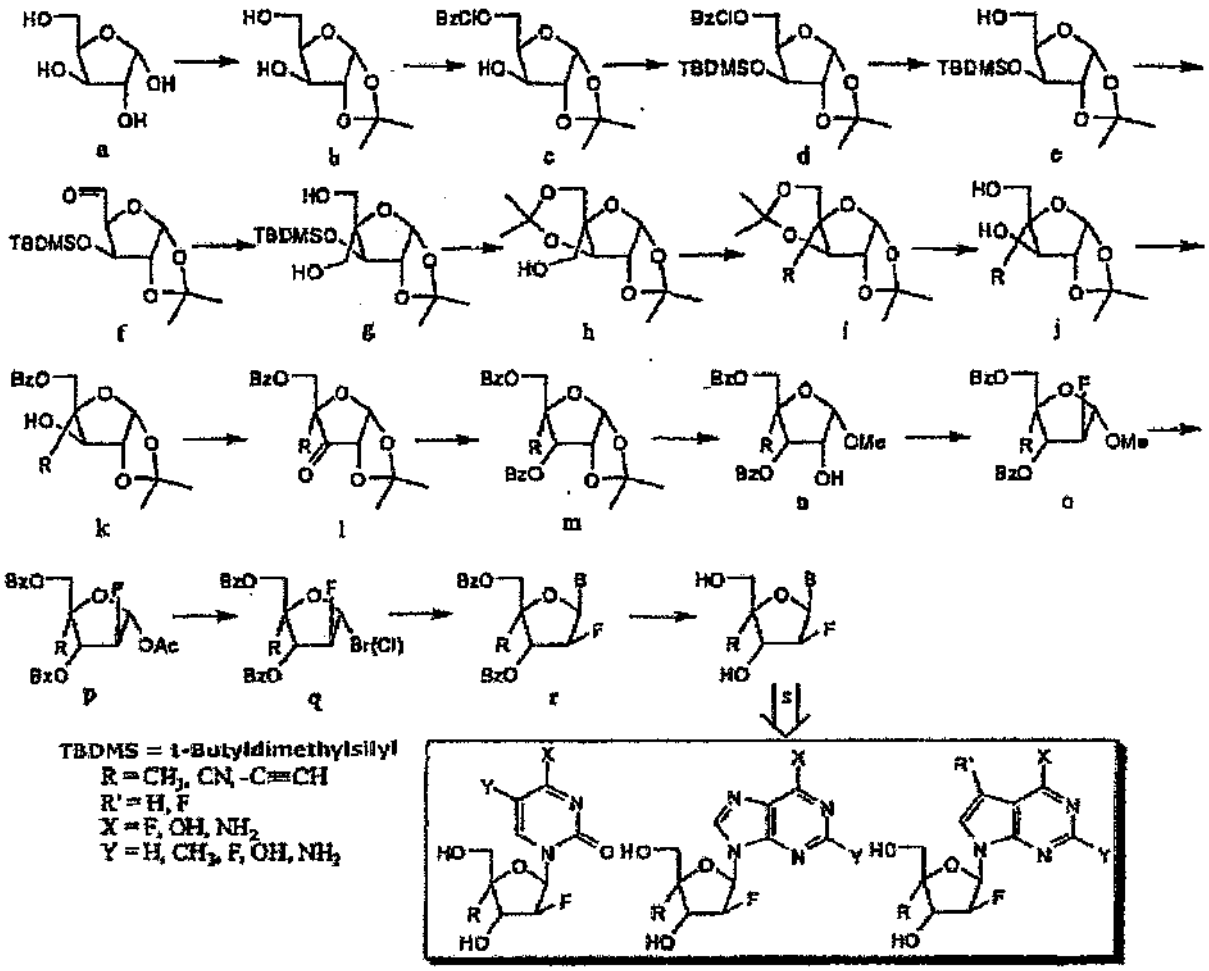
Synthese von Verbindung xii: Auflösen der Verbindung ix in Tetrahydrofuran und Hinzufügen von DBU; Rühren für eine Reaktion bei etwa 60 °C; Verdampfen des Lösungsmittels zur Trockne, nachdem die Reaktion beendet ist; Auftrennen des Rückstands durch Säulenchromatographie, wobei man Verbindung xii erhält;

Synthese von Verbindung xiii: Hinzufügen einer DMF-Lösung von ICl zu einer DMF-Lösung von NaN_3 bei 0 °C; Rühren am Gefrierpunkt während etwa 10 Minuten und langsames Hinzufügen einer DMF-Lösung der Verbindung xii, um die Reaktion fortzusetzen; nachdem die Reaktion beendet ist, Hinzufügen von Natriumsulfit zu der gemischten Lösung, bis die Farbe des Iods vollständig verschwunden ist; Entfernen des Lösungsmittels durch Verdampfen unter reduziertem Druck; Auftrennen und Reinigen des Rückstands durch Säulenchromatographie, wobei man Verbindung xiii erhält;

Synthese von Verbindung xiv: Auflösen der Verbindung xiii in DMF und Hinzufügen von Silberacetat unter Rühren; Umsetzen bei Raumtemperatur; Filtrieren, nachdem die Reaktion beendet ist; Entfernen des Lösungsmittels unter reduziertem Druck; Reinigen des Rückstands durch Säulenchromatographie, wobei man Verbindung xiv erhält;

Synthese von Verbindung xv: Auflösen der Verbindung xiii in einer Lösung von Methanol-Triethylamin, Rühren für eine Reaktion bei Raumtemperatur; Entfernen des Lösungsmittels durch Verdampfen, nachdem die Reaktion beendet ist, und Reinigen des Rückstands durch Säulenchromatographie, wobei man die Verbindung xv erhält.

5. Verfahren zur Herstellung der Nucleosidanaloga gemäß Anspruch 1, **dadurch gekennzeichnet, dass** das Herstellungsverfahren die folgenden Schritte umfasst:



Synthese von Verbindung b: Hinzufügen von Verbindung a in Aceton, langsames Hinzufügen von konzentrierter Schwefelsäure unter Rühren bei Raumtemperatur und Rühren für eine Reaktion bei Raumtemperatur; nachdem durch DC nachgewiesen wurde, dass die Reaktion beendet ist, Hinzufügen von konzentriertem Ammoniak, um den pH-Wert auf 7-8 einzustellen, Filtrieren und Verdampfen unter reduziertem Druck, um den größten Teil des Acetons zu entfernen; dann Hinzufügen von 0,4% verdünnter Salzsäure unter Rühren und Umsetzen unter Aceton-Rückflussbedingungen, bis Verbindung a vollständig zu Verbindung b hydrolysiert ist; Neutralisieren der Reaktionslösung durch festes $NaHCO_3$, Filtrieren und Verdampfen zur Entfernung des Lösungsmittels unter reduziertem Druck; Auflösen des Rückstands in Dichlormethan, Trocknen über wasserfreiem Na_2SO_4 über Nacht, Filtrieren und dann Verdampfen zur Entfernung des Lösungsmittels, wobei man Verbindung b als gelbes viskoses Öl erhält;

Synthese von Verbindung c: Auflösen von Verbindung b und Triethylamin in Dichlormethan; langsames tropfenweises Hinzufügen von Parachlorbenzoylchlorid in einem Eis-Kochsalz-Bad und mechanisches Rühren für eine Reaktion bei einer Temperatur unter 0°C; nachdem die Reaktion beendet ist, Hinzufügen von gesättigter $NaHCO_3$-Lösung; Waschen der Dichlormethan-Schicht mit Wasser und gesättigtem Salzwasser, Trocknen über wasserfreiem $MgSO_4$, Filtrieren und dann Verdampfen unter reduziertem Druck zur Entfernung des Lösungsmittels; Umkristallisieren des Rückstands, wobei man die weiße kristalline Verbindung c erhält;

Synthese von Verbindung d: Auflösen von Verbindung c in wasserfreiem Dichlormethan; Hinzufügen von Imidazol und tert-Butyldimethylsilylchlorid (TBDMSCl) unter Stickstoff als Schutzgas für eine Reaktion bei Raumtemperatur; nachdem die Reaktion beendet ist, Neutralisieren der Reaktion durch Salzsäure und Gewinnen von zwei Schichten; Waschen der organischen Schicht mit Wasser und gesättigtem Salzwasser, Trocknen über wasserfreiem Na_2SO_4 und dann Verdampfen zur Entfernung des Lösungsmittels; Auftrennen und Reinigen des Rückstands durch Säulenchromatographie, wobei man Verbindung d erhält;

Synthese von Verbindung e: Auflösen von Verbindung xiii in einer gemischten Lösung von Natriummethoxid und Methanol für eine Reaktion bei Raumtemperatur; nachdem die Reaktion beendet ist, Neutralisieren der Lösung mit verdünnter Essigsäure, Filtrieren, Waschen mit Methanol und dann Verdampfen unter reduziertem Druck zur Entfernung des Lösungsmittels; schließlich Auftrennen und Reinigen des Rückstands durch Säulenchromatographie,

wobei man Verbindung e erhält;

Synthese von Verbindung f: tropfenweises Hinzufügen von DMSO zu der Dichlormethan-Lösung von Oxalylchlorid bei etwa -60 °C; nach etwa 15 Minuten Rühren bei derselben Temperatur tropfenweises Hinzufügen einer Dichlormethan-Lösung von Verbindung e; nach 30 Minuten Rühren für eine Reaktion bei etwa -65 °C Hinzufügen von Triethylamin und Rühren für eine Reaktion bei Raumtemperatur; nachdem die Reaktion beendet ist, Hinzufügen von Wasser zu der Reaktionslösung, so dass sich eine organische Schicht abscheidet; Trocknen der organischen Schicht über wasserfreiem Magnesiumsulfat, Entfernen des Lösungsmittels durch Verdampfen; und Auftrennen und Reinigen des Rückstands durch Säulenchromatographie, wobei man Verbindung xiii erhält;

Synthese von Verbindung g: Hinzufügen von Natriumhydroxid und Wasser in einen Reaktionskolben; nach gleichmäßigem Rühren Hinzufügen einer Formaldehyd-Lösung, 95% Ethanol und dann Verbindung f unter Rühren für eine Reaktion bei etwa 35 °C; nachdem die Reaktion beendet ist, Abkühlen des Reaktionskolbens mit Eiswasser unter Rühren, bis das Produkt vollständig ausgefallen ist; nach dem Abnutschen Waschen des festen Produkts mit Wasser, bis es neutral ist, Trocknen und Auflösen in wasserfreiem Methanol und dann Hinzufügen von Natriumborhydrid für eine Reaktion unter Rückfluss; nachdem die Reaktion beendet ist, Neutralisieren der Reaktionslösung mit verdünnter Salzsäure und dann Extrahieren mit Dichlormethan, Trocknen über wasserfreiem Na_2SO_4 und Entfernen des Lösungsmittels, wobei man Verbindung g erhält;

Synthese von Verbindung h: Auflösen von Verbindung g in Methanol und Hinzufügen von Dowex H^+ (im Voraus mit Methanol gewaschen) für eine Reaktion bei Raumtemperatur; nachdem die Reaktion beendet ist, Entfernen des Harzes aus der Lösung durch Filtration und wiederholtes Waschen desselben mit Methanol und Eindampfen der Lösung, wobei man eine feste Substanz erhält; Auflösen der erhaltenen festen Substanz in Aceton, dann langsames Hinzufügen von konzentrierter Schwefelsäure (eine katalytische Menge) zu der Lösung für eine Reaktion bei Raumtemperatur unter Rühren; Hinzufügen von konzentriertem Ammoniakwasser, um den pH-Wert einzustellen, und dann Filtrieren und Verdampfen unter reduziertem Druck, um Aceton zu entfernen, wobei man Verbindung h erhält;

Synthese von Verbindung i: tropfenweises Hinzufügen von DMSO zu der Dichlormethan-Lösung von Oxalylchlorid bei etwa -60 °C und Rühren während etwa 15 Minuten nach dem Abtropfen; tropfenweises Hinzufügen einer Dichlormethan-Lösung von Verbindung h, Umsetzen während etwa 30 Minuten bei -60 °C nach dem Abtropfen; Hinzufügen von Triethylamin zu der Lösung; nachdem die Reaktion etwa 30 Minuten lang bei Raumtemperatur durchgeführt wurde, Hinzufügen von Wasser, um die Reaktion abzubrechen; Abtrennen der organischen Schicht und Trocknen über wasserfreiem Na_2SO_4; Entfernen des Lösungsmittels durch Verdampfen sowie Auftrennen und Reinigen des Rückstands durch Säulenchromatographie, wobei man ein festes Produkt erhält; Hinzufügen von $ClCH_2P(C_6H_5)_3Cl$ zu wasserfreiem Tetrahydrofuran, Abkühlen auf etwa -78 °C, Hinzufügen einer n-BuLi-Hexan-Lösung und Rühren für eine Reaktion während etwa einer Stunde; dann Hinzufügen einer Lösung des festen Produkts in trockenem Tetrahydrofuran; allmähliches Erhöhen der Temperatur der Lösung auf etwa 0 °C, um die Reaktion fortzusetzen; nachdem die Reaktion beendet ist, vorsichtiges tropfenweises Hinzufügen einer gesättigten NH_4Cl-Lösung zu der Lösung und anschließend Extrahieren mit Ethylacetat; zweimal Waschen der organischen Schicht mit Kochsalzlösung, Trocknen über wasserfreiem $MgSO_4$ und Verdampfen zur Entfernung des Lösungsmittels; Auflösen des erhaltenen Rückstands in wasserfreiem Tetrahydrofuran, Abkühlen auf -78 °C und langsames tropfenweises Hinzufügen einer n-BuLi-Hexan-Lösung; nach 2 Stunden Rühren der Lösung vorsichtiges Abbrechen der Reaktion unter Verwendung von gesättigtem NH_4Cl; Waschen der organischen Schicht mit gesättigtem Salzwasser, Trocknen über wasserfreiem $MgSO_4$ und Verdampfen unter reduziertem Druck zur Entfernung des Lösungsmittels; schließlich Auftrennen und Reinigen des Rückstands durch eine schnelle Silicagel-Säule, wobei man Verbindung i erhält;

Synthese von Verbindung j: Hinzufügen von Verbindung i zu verdünnter Salzsäure, Rühren der Lösung bei Raumtemperatur für eine Reaktion; nachdem die Reaktion beendet ist, Neutralisieren der Lösung mit festem $NaHCO_3$ und dann Filtrieren; Eindampfen des Filtrats unter reduziertem Druck zum Entfernen des Lösungsmittels; Auflösen des Rückstands in Dichlormethan; Filtrieren nach Trocknen über wasserfreiem Na_2SO_4 und Verdampfen zur Entfernung des Lösungsmittels, wobei man Verbindung j erhält;

Synthese von Verbindung k: Auflösen von Verbindung j in Pyridin, tropfenweises Hinzufügen von BzCl für eine Reaktion bei Raumtemperatur; nachdem die Reaktion beendet ist, Verdampfen des Lösungsmittels zur Trockne und Reinigen des Rückstands, wobei man Verbindung k erhält;

Synthese von Verbindung l: Auflösen von Verbindung k und 2,2,6,6Tetramethylpiperidin-N-oxid in Dichlormethan, Abkühlen auf 0 °C und Hinzufügen einer gemischten Lösung von Natriumhypochlorit, $NaHCO_3$ und Wasser zu der Reaktionslösung für eine Reaktion bei 0 °C während etwa 30 Minuten; Hinzufügen von Isopropanol und Rühren bei Raumtemperatur; zweimal Waschen der abgetrennten Dichlormethan-Schicht mit Wasser, Trocknen über wasserfreiem $MgSO_4$, Filtrieren und Verdampfen zur Entfernung des Lösungsmittels; Umkristallisieren des Rückstands, wobei man Verbindung I erhält;

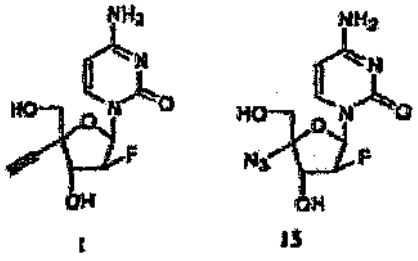
Synthese von Verbindung m: Auflösen von Verbindung I in einer gemischten Lösung von absolutem Ethanol und Ethylacetat und Abkühlen auf etwa 0 °C; Hinzufügen von Natriumborhydrid in Chargen und Rühren für eine Reaktion;

nachdem die Reaktion beendet ist, Neutralisieren mit verdünnter Essigsäurelösung, Filtrieren und Eindampfen des Filtrats zur Trockne;
Auflösen des Rückstands in Dichlormethan und dann Waschen mit Wasser; Trocknen der Dichlormethan-Schicht über wasserfreiem $MgSO_4$, Filtrieren und Verdampfen zum Entfernen des Dichlormethans unter reduziertem Druck; Umkristallisieren des Rückstands aus einem Lösungsmittelgemisch von Dichlormethan und Petrolether, wobei man eine feste Substanz erhält; dann Auflösen des festen Produkts in Pyridin und tropfenweises Hinzufügen von BzCl bei etwa 0 °C; danach wird die Reaktion bei Raumtemperatur durchgeführt; nachdem die Reaktion beendet ist, Entfernen des Lösungsmittels durch Verdampfen und Extrahieren des Rückstands mit Ethylacetat, Waschen mit gesättigter $NaHCO_3$-Lösung Trocknen über wasserfreiem Na_2SO_4 Filtrieren und Verdampfen zur Entfernung des Lösungsmittels, wobei man Verbindung m erhält;
Synthese von Verbindung n: Auflösen von Verbindung m in Methanol, Hinzufügen einer gemischten Lösung von konzentrierter Salzsäure/Dioxan für eine Reaktion bei Raumtemperatur; nachdem die Reaktion beendet ist, Neutralisieren der Reaktionslösung mit $NaHCO_3$ und Filtrieren; Entfernen des Lösungsmittels aus dem Filtrat durch Vakuumdestillation; Auflösen des Rückstands in einer geeigneten Menge Dichlormethan, Trocknen über wasserfreiem Na_2SO_4 über Nacht, Filtrieren und Eindampfen zur Trockne; Auftrennen und Reinigen durch Säulenchromatographie, wobei man Verbindung n erhält;
Synthese von Verbindung o: Auflösen von Verbindung n in Dichlormethan und Hinzufügen von DAST bei Raumtemperatur und Rühren für eine Reaktion; nachdem die Reaktion beendet ist, Gießen des Gemischs in die gesättigte $NaHCO_3$-Lösung und Abtrennen der organischen Schicht; Waschen der organischen Schicht mit gesättigter $NaHCO_3$-Lösung, Trocknen über wasserfreiem Na_2SO_4 sowie Auftrennen und Reinigen durch Säulenchromatographie, wobei man Verbindung o erhält;
Synthese von Verbindung p: Auflösen von Verbindung o in Ameisensäurelösung und Rühren bei Raumtemperatur für eine Reaktion; nachdem die Reaktion beendet ist, Entfernen des Lösungsmittels unter reduziertem Druck; Eindampfen des Rückstands zusammen mit Toluol, wobei man ein festes Produkt erhält; Auflösen des erhaltenen festen Produkts in einer Lösung von trockenem Pyridin und Essigsäureanhydrid für eine Reaktion bei Raumtemperatur; nachdem die Reaktion beendet ist, Einengen der Reaktionslösung und dann Eindampfen zusammen mit Toluol, wobei man Verbindung p erhält;
Synthese von Verbindung q: Auflösen von Verbindung p in wasserfreiem Dichlormethan und Abkühlen auf 0 °C; langsames Einleiten von HCl-Gas in die Lösung bis zur Sättigung und Entfernen des Lösungsmittels durch Eindampfen im Vakuum bei Raumtemperatur; Trocknen des Rückstands im Vakuum, wobei man Verbindung q erhält;
Synthese von Verbindung r: in Gegenwart von $(NH_4)_2SO_4$ Erhitzen von 2,6-Diaminopurin und Hexamethyldisilazan (HMDS) unter Rückfluss, bis man eine klare Lösung erhält; Entfernen des Lösungsmittels im Vakuum, wobei man ein weißes festes Produkt erhält, und Auflösen des festen Produkts in 1,2-Dichlorethan; Hinzufügen einer 1,2-Dichlorethan-Lösung von Verbindung q und Molekularsieb und Rühren für eine Reaktion unter Stickstoff als Schutzgas bei Raumtemperatur; nachdem durch DC nachgewiesen wurde, dass die Reaktion beendet ist, Hinzufügen von Dichlormethan zu der Reaktionslösung und Filtrieren über Diatomit; Waschen des Filtrats mit gesättigtem Na_2SO_4 und Kochsalzlösung; Trocknen der organischen Schicht über wasserfreiem Na_2SO_4 über Nacht und Entfernen des Lösungsmittels durch Verdampfen, wobei man einen Rückstand erhält; Auftrennen des Rückstands unter reduziertem Druck über eine Silicagel-Säule, wobei man Verbindung r erhält;
Synthese von Verbindung s: Auflösen von Verbindung r in einer gesättigten NH_3-CH_3OH-Lösung und Rühren für eine Reaktion bei Raumtemperatur; nachdem die Reaktion beendet ist, Entfernen des Lösungsmittels durch Verdampfen sowie Auftrennen und Reinigen des Rückstands durch Säulenchromatographie, wobei man Verbindung s erhält.

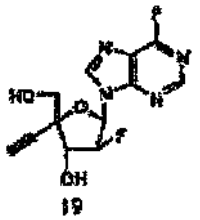
6. Verwendung der 2'-Fluor-4'-substituierten Nucleosidanaloga gemäß Anspruch 1, 2, 3 in einem pharmazeutischen Präparat, **dadurch gekennzeichnet, dass** die Verbindungen in einem Präparat von Anti-HBV-, Anti-HCV- oder Anti-HIV-Wirkstoffen angewendet werden.

7. Verwendung des 2'-Fluor-4'-substituierten Nucleosidanalogen gemäß Anspruch 6 in einem pharmazeutischen Präparat, **dadurch gekennzeichnet, dass** es sich bei den Anti-HBV-Wirkstoffen um antivirale HBV-Wirkstoffe, bei den Anti-HIV-Wirkstoffen um antivirale HIV-Wirkstoffe und bei den Anti-HCV-Wirkstoffen um antivirale HCV-Wirkstoffe handelt.

8. 2'-Fluor-4'-substituierte Nucleosidanaloga gemäß Anspruch 1, **dadurch gekennzeichnet, dass** die Verbindungen aus der Gruppe ausgewählt sind, die aus den folgenden Verbindungen besteht:

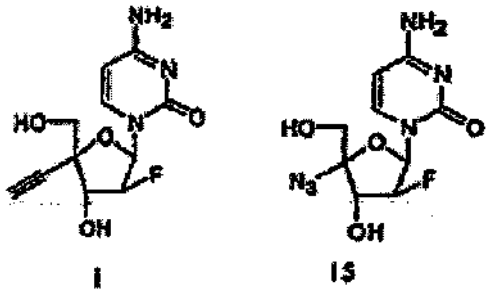


I 15



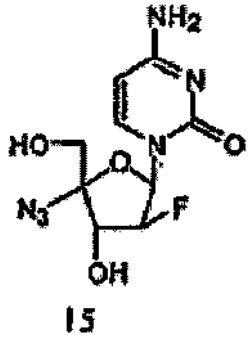
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9. 2'-Fluor-4'-substituierte Nucleosidanaloga gemäß Anspruch 1, **dadurch gekennzeichnet, dass** die Verbindungen aus der Gruppe ausgewählt sind, die aus den folgenden Verbindungen besteht:



I 15

10. 2'-Fluor-4'-substituiertes Nucleosidanalog gemäß Anspruch 1, **dadurch gekennzeichnet, dass** es sich bei der Verbindung um

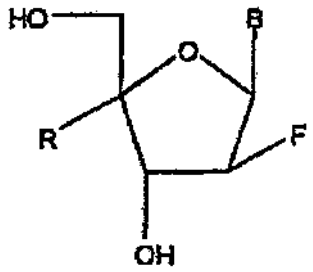


15

handelt.

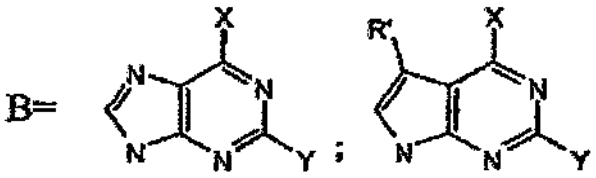
Revendications

1. Analogues de nucléosides 2'-fluoro-4'-substitués, **caractérisés en ce que** les composés possèdent une structure telle qu'exprimée dans la formule générale (I) :



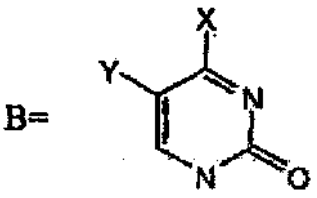
(I)

dans laquelle :



R = CH_3, CN, N_3. C≡CH;
R' = H, F;
X = F, OH, NH_2;
Y = H, CH_3, F, OH, NH_2-

ou dans laquelle :

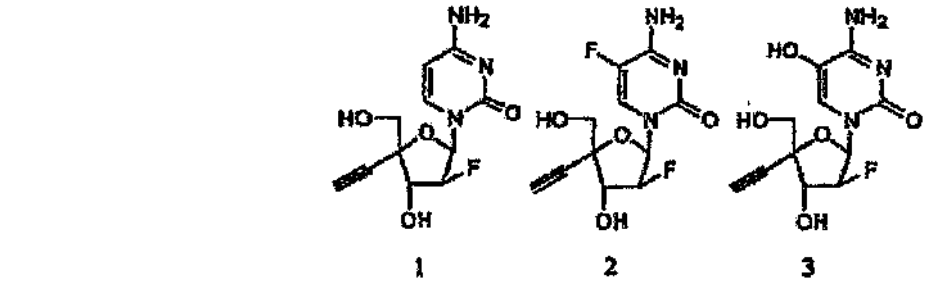


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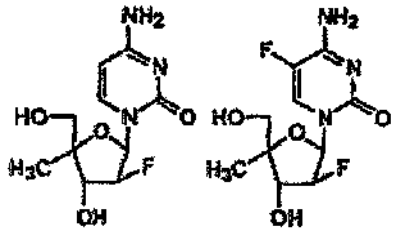
R = CH_3, CN, N_3, C≡CH;
X = F, NH_2;
Y = H, CH_3, F, OH, NH_2.

2. Analogues de nucléosides 2'-fluoro-4'-substitués selon la revendication 1 ou des esters phosphoriques en 5' de ceux-ci, **caractérisés en ce que** les composés se présentent sous la forme de sels, qui sont générés par la réaction entre les composés actifs (I) et un acide organique ou inorganique.

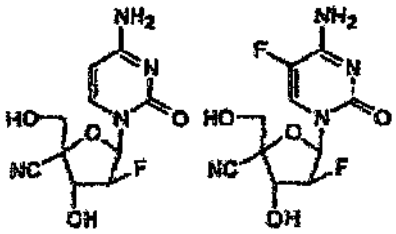
3. Analogues de nucléosides 2'-fluoro-4'-substitués selon la revendication 1, **caractérisés en ce que** lesdits composés sont choisis dans le groupe consistant en les composés suivants :



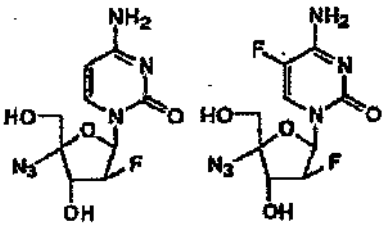
1 2 3



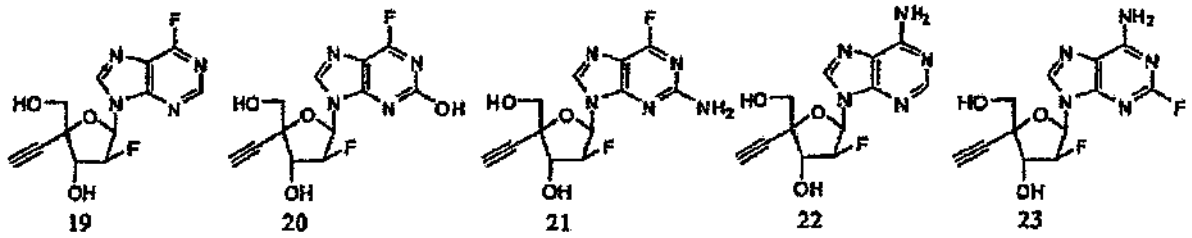
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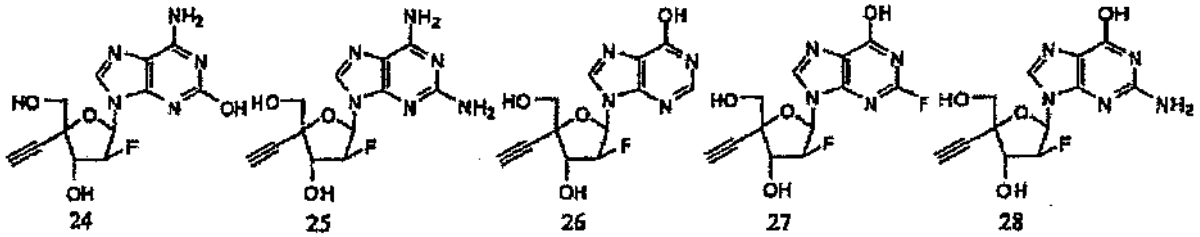
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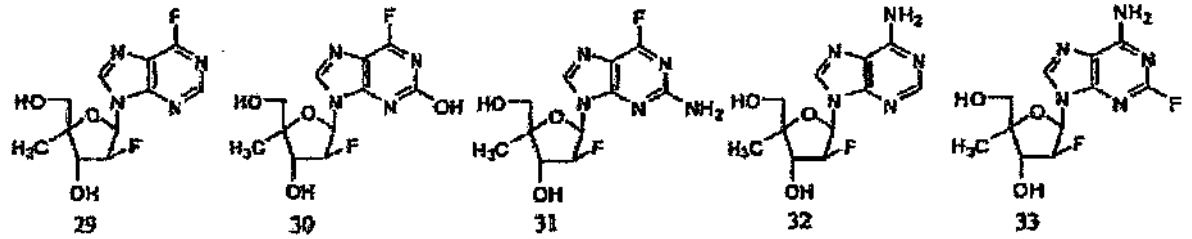
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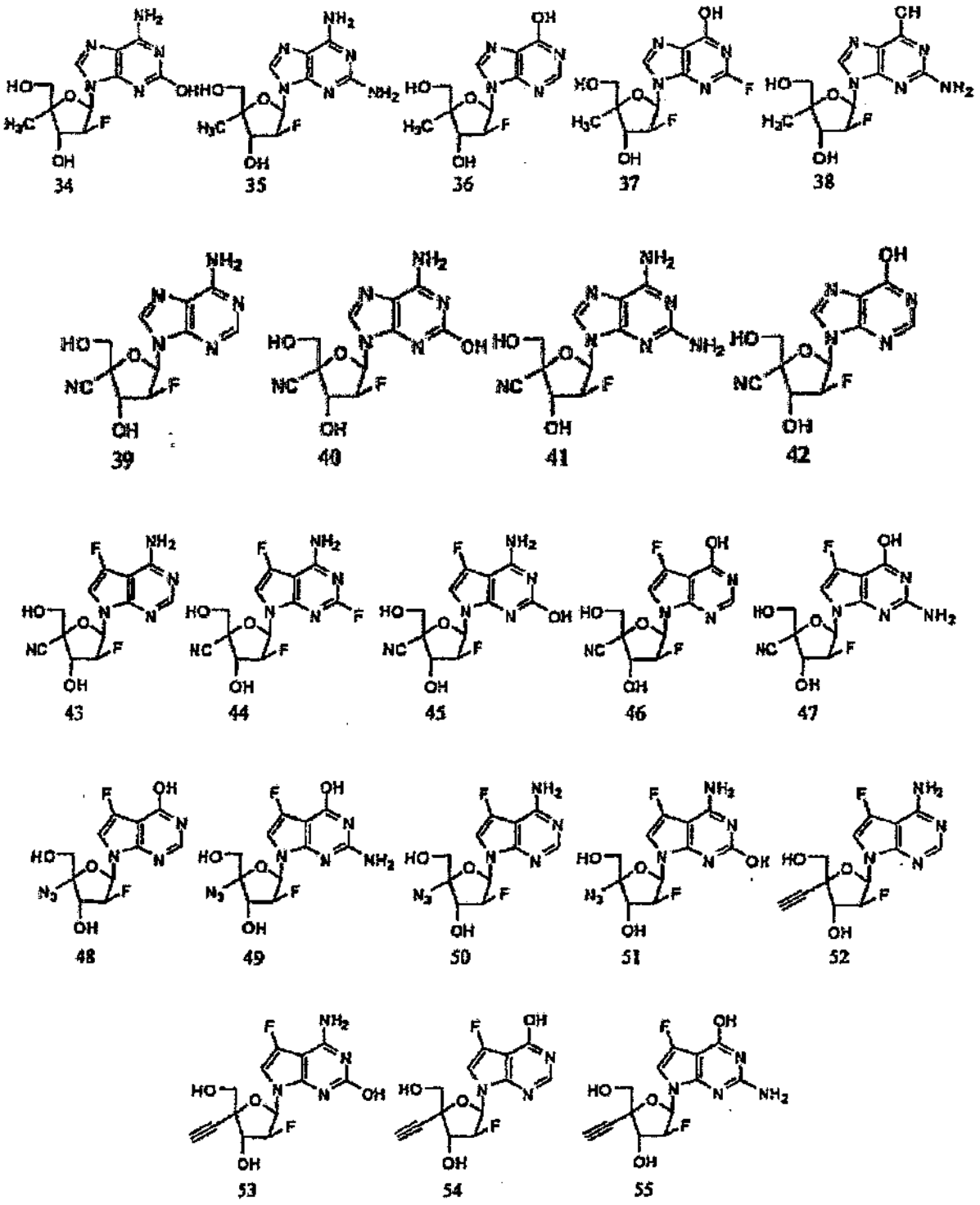
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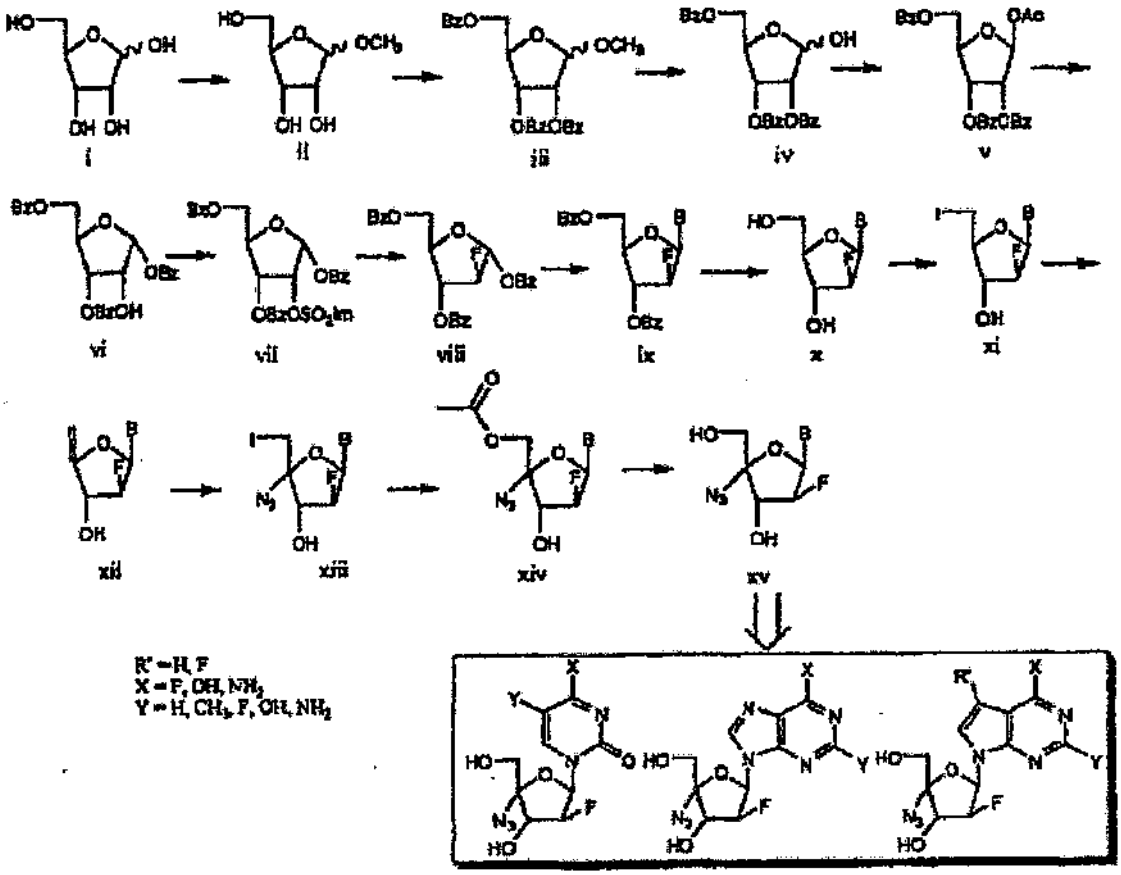
24 25 26 27 28



29 30 31 32 33



4. Procédé pour la préparation des analogues de nucléosides selon la revendication 1, **caractérisé en ce que** ledit procédé comprend les étapes suivantes :



synthèse du composé ii : dissoudre le composé i (D-ribose ou L-ribose) dans du HCl/MeOH, agiter dans un bain marie avec exclusion d'air en gardant au chaud, ajouter de la pyridine à la solution pour terminer la réaction et ensuite aspirer sous pression réduite pour obtenir le composé ii, sirupeux, jaune clair ;

synthèse du composé iii : dissoudre le composé ii dans de la pyridine sèche, ajouter lentement du chlorure de benzoyle goutte à goutte dans un bain glace/sel, agiter pour réaction à température ambiante, verser la solution réactionnelle dans l'eau glacée après la réaction est terminée, et extraire avec du chloroforme ; laver la couche organique successivement avec de l'eau glacée, de l'acide sulfurique préalablement refroidi et une solution saturée de bicarbonate de sodium, jusqu'à ce que la couche aqueuse soit faiblement alcaline, et ensuite laver avec de l'eau glacée, jusqu'à ce que la couche aqueuse soit neutre ; sécher la couche organique avec du sulfate de sodium anhydre et ensuite aspirer sous pression réduite pour obtenir le composé iii, sirupeux, jaune clair ;

synthèse du composé iv : Dissoudre le composé benzoyle iii dans du chloroforme, ajouter du phosphore rouge, ajouter lentement du brome goutte à goutte et en agitant la solution dans un bain d'eau glacée, agiter pendant environ 30 minutes, ajouter lentement de l'eau glacée goutte à goutte et agiter à température ambiante ; puis ajouter de la glace concassée à la solution et agiter la solution, jusqu'à ce que la glace soit fondue ; ensuite verser la solution réactionnelle dans de l'eau glacée et extraire avec du chloroforme ; laver la couche organique avec une solution saturée de bicarbonate de sodium, jusqu'à ce que la couche aqueuse soit faiblement alcaline, laver avec de l'eau glacée, jusqu'à ce que la couche aqueuse soit neutre, sécher avec du sulfate de sodium anhydre, et ensuite aspirer sous pression réduite pour obtenir un sirop jaune foncé ; finalement, le sirop est séparé sur une colonne de gel de silice pour obtenir le composé iv sous forme de sirop sec blanc ;

synthèse du composé v : dissoudre le composé iv dans de la pyridine sèche, ajouter lentement de l'anhydride acétique goutte à goutte dans un bain d'eau glacée et ensuite agiter pendant environ 30 minutes ; enlever le bain d'eau glacée, agiter à température ambiante pendant environ 7 heures, et ensuite la solution est chauffée à 40 °C et maintenue à cette température pendant une heure ; après avoir ajouté de la glace concassée et agité jusqu'à ce qu'elle soit fondue, verser la solution réactionnelle dans de l'eau glacée et extraire avec du chloroforme ; laver la couche organique successivement avec de l'eau glacée, de l'acide sulfurique préalablement refroidi et une solution saturée de bicarbonate de sodium, jusqu'à ce que la couche aqueuse soit faiblement alcaline, et ensuite laver avec de l'eau glacée, jusqu'à ce que la couche aqueuse soit neutre, sécher avec du sulfate de sodium anhydre et ensuite aspirer sous pression réduite pour obtenir le composé v sirupeux, jaune clair ;

synthèse du composé vi : dissoudre le composé v dans du dichlorométhane sec et introduire lentement du gaz HCl sec dans de l'eau glacée, laver avec de l'eau glacée pour séparer une couche organique ; laver la couche organique avec une solution saturée de bicarbonate de sodium, jusqu'à ce que la couche aqueuse soit faiblement alcaline, laver la couche organique avec de l'eau glacée, jusqu'à ce que la couche aqueuse soit neutre, sécher avec du sulfate de sodium anhydre et aspirer sous pression réduite pour obtenir un sirop jaune clair ; recristalliser le sirop pour obtenir le composé vi sous forme de solide blanc ;

synthèse du composé vii : dissoudre le composé vi dans du dichlorométhane sec et du DMF sec, ajouter lentement du SO_2Cl_2 à la solution en agitant à environ -15 °C pendant environ 30 minutes, laisser réchauffer naturellement à température ambiante pour réaction ; ajouter trois fois de l'imidazole à la solution à 0 °C et agiter le mélange à température ambiante, diluer la solution réactionnelle en ajoutant du CH_2Cl_2 après la réaction est terminée, puis laver la solution avec de l'eau glacée ; extraire la couche aqueuse avec du CH_2Cl_2 ; combiner les couches organiques et ensuite sécher avec du sulfate de sodium anhydre et aspirer sous pression réduite pour obtenir un sirop jaune clair ; séparer et purifier le sirop par une colonne de gel de silice pour obtenir le composé vii sous forme de solide blanc ;

synthèse du composé viii : dissoudre le composé vii dans de l'acétate d'éthyle et ajouter du $Et_3N{\cdot}3HF$ en agitant ; chauffer à environ 60 °C en agitant pendant environ 3 h et ensuite chauffer à 70 °C en agitant pendant 1,5 h ; ajouter de la glace salée pour terminer la réaction, extraire avec du dichlorométhane ; combiner les couches organiques et ensuite laver successivement avec une solution salée, de l'eau et une solution saturée de bicarbonate de sodium, sécher avec du sulfate de sodium anhydre et aspirer sous pression réduite pour obtenir un sirop jaune foncé ; purifier le sirop pour obtenir un sirop jaune clair, et finalement cristalliser le produit brut pour obtenir le composé viii, blanc, cristallin ;

synthèse du composé ix : dissoudre le composé viii dans du dichlorométhane anhydre, ajouter une solution mixte de HBr-AcOH et agiter pour réaction à température ambiante ; après la réaction est terminée, évaporer le mélange jusqu'à l'état sec ; dissoudre le résidu avec du dichlorométhane, laver la solution de dichlorométhane avec une solution de bicarbonate de sodium et éliminer le dichlorométhane par évaporation pour obtenir un produit sirupeux ; au même temps, de la cytosine protégée et du $(NH_4)_2SO_4$ sont chauffés à reflux et sous protection d'azote dans du HMDS ; après la réaction est terminée, évaporer le solvant sous pression réduite pour obtenir de la cytosine silylée ; dissoudre le sirop obtenu dans la réaction précédente dans du dichloroéthane, ajouter la solution à de la cytosine silylée, et ensuite chauffer à reflux pour réaction sous protection de N_2 ; ajouter de la glace pour terminer la réaction ; extraire avec du dichlorométhane ; et laver la couche de dichlorométhane successivement avec une solution saturée de bicarbonate de sodium et une solution salée, et sécher avec du sulfate de sodium anhydre ; évaporer le solvant pour obtenir un solide blanc, séparer et purifier par chromatographie sur colonne pour obtenir le composé ix ;

synthèse du composé x : dissoudre le composé ix dans du NH_3-CH_3OH saturé et agiter pour réaction à température ambiante ; après la réaction est terminée, évaporer le solvant jusqu'à l'état sec pour obtenir un résidu ; et purifier le résidu par chromatographie sur colonne pour obtenir le composé x ;

synthèse du composé xi : dissoudre le composé x, de l'imidazole et de la triphénylphosphine dans du tétrahydrofurane, ajouter lentement du tétrahydrofurane contenant de l'iode ; agiter pour réaction à température ambiante ; éliminer le solvant par évaporation, après la réaction est terminée, ajouter de l'acétate d'éthyle au résidu, et filtrer ; évaporer l'acétate d'éthyle, et séparer le résidu par chromatographie sur colonne pour obtenir le composé xi ;

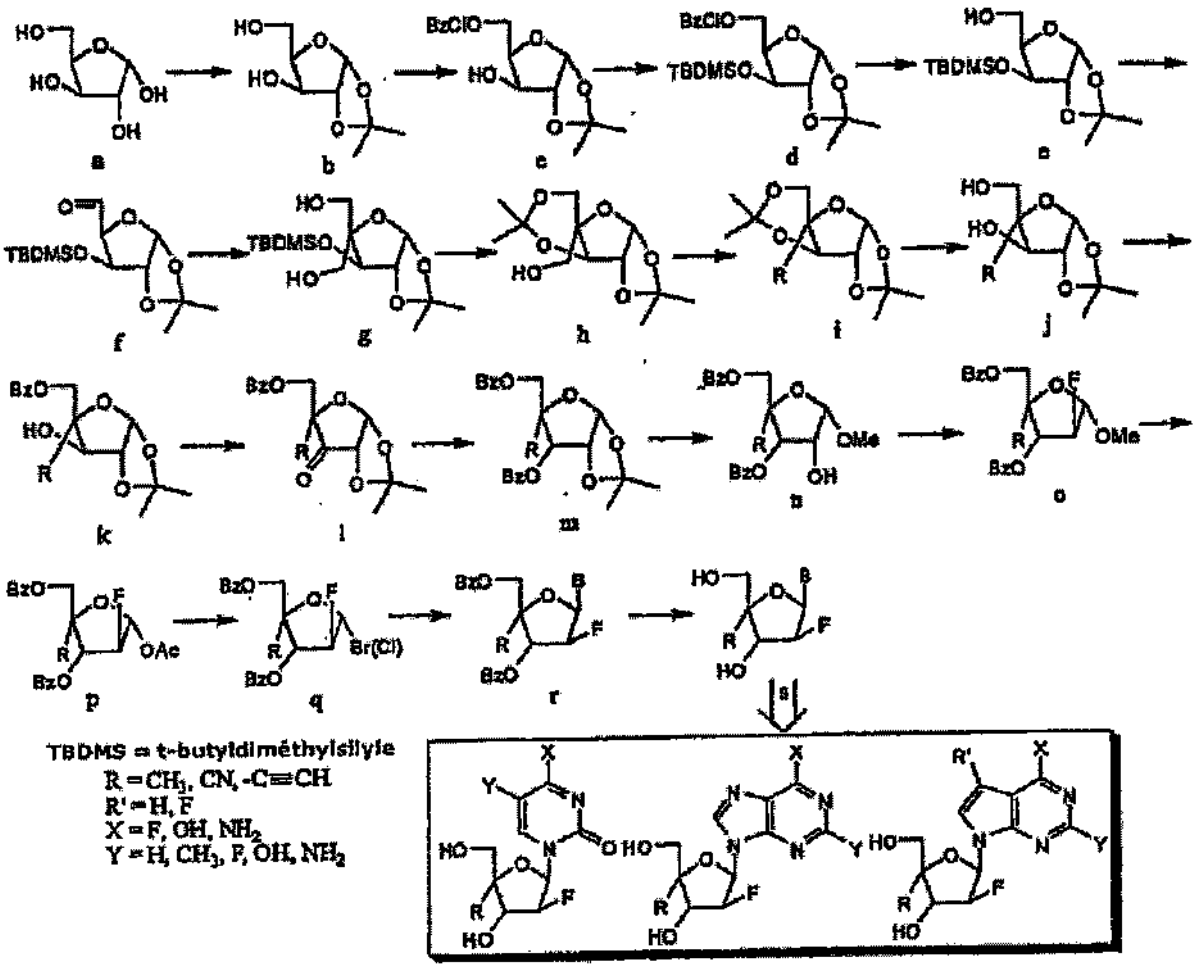
synthèse du composé xii : dissoudre le composé ix dans du tétrahydrofurane, et ajouter du DBU ; agiter pour réaction à environ 60 °C ; évaporer le solvant jusqu'à l'état sec, après la réaction est terminée ; séparer le résidu par chromatographie sur colonne pour obtenir le composé xii ;

synthèse du composé xiii : ajouter une solution de ICl dans du DMF à une solution de NaN_3 dans du DMF à 0 °C ; agiter au point de congélation pendant environ 10 minutes, et ajouter lentement une solution du composé xii dans du DMF pour continuer la réaction ; après la réaction est terminée, ajouter du sulfite de sodium à la solution mixte, jusqu'à ce que la couleur de l'iode soit complètement disparue ; éliminer le solvant par évaporation sous pression réduite ; séparer et purifier le résidu par chromatographie sur colonne pour obtenir le composé xiii ;

synthèse du composé xiv : dissoudre le composé xiii dans du DMF et ajouter de l'acétate d'argent en agitant ; faire réagir à température ambiante ; filtrer, après la réaction est terminée ; éliminer le solvant sous pression réduite ; purifier le résidu par chromatographie sur colonne pour obtenir le composé xiv ;

synthèse du composé xv : dissoudre le composé xiii dans une solution de méthanol-triéethylamine, agiter pour réaction à température ambiante ; éliminer le solvant par évaporation, après la réaction est terminée, et purifier le résidu par chromatographie sur colonne pour obtenir le composé xv.

5. Procédé pour la préparation des analogues de nucléosides selon la revendication 1, **caractérisé en ce que** ledit procédé de préparation comprend les étapes suivantes :



synthèse du composé b : ajouter du composé a dans de l'acétone, ajouter lentement de l'acide sulfurique concentré en agitant à température ambiante, et agiter pour réaction à température ambiante ; après l'achèvement de la réaction a été détecté par CCM, ajouter de l'ammoniac concentré pour ajuster le pH à 7-8, filtrer et évaporer sous pression réduite pour éliminer la plupart de l'acétone ; puis ajouter du 0,4% acide hydrochlorique en agitant, et faire réagir sous des conditions de reflux d'acétone, jusqu'à ce que le composé a ait été hydrolysé complètement en le composé b ; neutraliser la solution réactionnelle par du $NaHCO_3$ solide, filtrer et évaporer pour éliminer le solvant sous pression réduite ; dissoudre le résidu dans du dichlorométhane, sécher avec du Na_2SO_4 anhydre pendant la nuit, filtrer et ensuite évaporer pour éliminer le solvant pour obtenir le composé b sous forme d'huile visqueuse jaune ;
synthèse du composé c : dissoudre composé b et de la triéthylamine dans du dichlorométhane ; ajouter du chlorure de parachlorobenzoyle lentement goutte à goutte dans un bain glace/sel et agiter mécaniquement pour réaction à une température inférieure à 0 °C ; après la réaction est terminée, ajouter de la solution saturée de $NaHCO_3$; laver la couche de dichlorométhane avec de l'eau et de l'eau salée saturée, sécher avec du $MgSO_4$ anhydre, filtrer et ensuite évaporer sous pression réduite pour éliminer le solvant ; recristalliser le résidu pour obtenir le composé c, blanc, cristallin ;
synthèse du composé d : dissoudre composé c dans du dichlorométhane anhydre ; ajouter de l'imidazole et du chlorure de tert-butyl-2-méthylsilyle (TBDMSCl) sous protection d'azote pour réaction à température ambiante ; après la réaction est terminée, neutraliser la réaction avec de l'acide chlorhydrique pour obtenir deux couches ; laver la couche organique avec de l'eau et de l'eau salée saturée, sécher avec du Na_2SO_4 anhydre et ensuite évaporer pour éliminer le solvant ; séparer et purifier le résidu par chromatographie sur colonne pour obtenir le composé d ;
synthèse du composé e : dissoudre composé xiii dans une solution mixte de méthoxyde de sodium et de méthanol pour réaction à température ambiante ; après la réaction est terminée, neutraliser la solution avec de l'acide acétique dilué, filtrer, laver avec du méthanol et ensuite évaporer sous pression réduite pour éliminer le solvant ; finalement séparer et purifier le résidu par chromatographie sur colonne pour obtenir le composé e ;
synthèse du composé f : ajouter du DMSO goutte à goutte à une solution de chlorure d'oxalyle dans du dichloro-

méthane à environ -60 °C ; après environ 15 minutes, agiter à la même température, ajouter goutte à goutte une solution de composé e dans du dichlorométhane ; après 30 minutes, agiter pour réaction à environ -65 °C, ajouter de la triéthylamine et agiter pour réaction à température ambiante ; après la réaction est terminée, ajouter de l'eau à la solution réactionnelle pour séparer une couche organique ; sécher la couche organique avec du sulfate de magnésium anhydre, éliminer le solvant par évaporation ; et séparer et purifier le résidu par chromatographie sur colonne pour obtenir le composé xiii ;

synthèse du composé g : ajouter de l'hydroxyde de sodium et de l'eau dans un ballon à réaction ; après avoir agité uniformément, ajouter une solution de formaldéhyde, 95% éthanol et ensuite composé f en agitant pour réaction à environ 35 °C ; après la réaction est terminée, refroidir le ballon à réaction avec de l'eau glacée en agitant jusqu'à ce que le produit soit précipité complètement ; après filtration sous vide, laver le produit solide avec de l'eau jusqu'à ce qu'il soit neutre, sécher et dissoudre dans du méthanol anhydre et ensuite ajouter du borohydrure de sodium pour réaction à reflux ; après la réaction est terminée, neutraliser la solution réactionnelle avec de l'acide chlorhydrique dilué et ensuite extraire avec du dichlorométhane, sécher avec du Na_2SO_4 anhydre et éliminer le solvant pour obtenir le composé g ;

synthèse du composé h : dissoudre composé g dans du méthanol et ajouter du Dowex H^+ (lavé préalablement avec du méthanol) pour réaction à température ambiante ; après la réaction est terminée, éliminer la résine de la solution par filtration et laver plusieurs fois avec du méthanol et évaporer la solution pour obtenir une substance solide ; dissoudre dans de l'acétone le substance solide obtenu, puis ajouter lentement de l'acide sulfurique concentré (une quantité catalytique) à la solution pour réaction à température ambiante en agitant ; ajouter de l'ammoniaque concentrée pour ajuster le pH, et ensuite filtrer et évaporer sous pression réduite pour éliminer l'acétone afin d'obtenir le composé h ;

synthèse du composé i : ajouter du DMSO goutte à goutte à la solution de chlorure d'oxalyle dans du dichlorométhane à environ -60 °C et agiter pendant environ 15 minutes après drainage ; ajouter goutte à goutte une solution du composé h dans du dichlorométhane, faire réagir pendant environ 30 minutes à -60 °C après drainage ; ajouter de la triéthylamine à la

solution ; après la réaction a été réalisée pendant environ 30 minutes à température ambiante, ajouter de l'eau pour terminer la réaction ; séparer la couche organique et sécher avec du Na_2SO_4 anhydre ; éliminer le solvant par évaporation, et séparer et purifier le résidu par chromatographie sur colonne pour obtenir un produit solide ; ajouter du $ClCH_2P(C_6H_5)_3Cl$ à du tétrahydrofurane anhydre, refroidir à environ -78 °C, ajouter une solution de n-BuLi dans de l'hexane et agiter pour réaction pendant environ une heure ; puis ajouter la solution du produit solide dans du tétrahydrofurane sec ; augmenter la température de la solution graduellement à environ 0 °C pour continuer la réaction ; après la réaction est terminée, ajouter avec précaution une solution saturée de NH_4Cl goutte à goutte à la solution, et ensuite extraire avec de l'acétate d'éthyle ; laver la couche organique deux fois avec une solution salée, sécher avec du $MgSO_4$ anhydre et évaporer pour éliminer le solvant ; dissoudre le résidu obtenu dans du tétrahydrofurane anhydre, refroidir à -78 °C et ajouter lentement goutte à goutte une solution de n-BuLi dans de l'hexane ; après avoir agité la solution pendant 2 heures, terminer la réaction avec précaution en utilisant du NH_4Cl saturé ; laver la couche organique avec de l'eau salée saturée, sécher avec du $MgSO_4$ anhydre et évaporer sous pression réduite pour éliminer le solvant ; finalement séparer et purifier le résidu avec une colonne de gel de silice rapide pour obtenir le composé i ;

synthèse du composé j : ajouter du composé i à de l'acide hydrochlorique dilué, agiter la solution à température ambiante pour réaction ; après la réaction est terminée, neutraliser la solution avec du $NaHCO_3$ solide, et ensuite filtrer ; évaporer le filtrat sous pression réduite pour éliminer le solvant ; dissoudre le résidu dans du dichlorométhane ; filtrer après séchage avec du Na_2SO_4 anhydre, et évaporer pour éliminer le solvant pour obtenir le composé j ;

synthèse du composé k : dissoudre le composé j dans de la pyridine, ajouter du BzCl goutte à goutte pour réaction à température ambiante ;

après la réaction est terminée, évaporer le solvant jusqu'à l'état sec et purifier le résidu pour obtenir le composé k ;

synthèse du composé l : dissoudre le composé k et du 2,2,6,6-tétraméthylpiperidine-1-oxyle dans du dichlorométhane, refroidir à 0 °C et ajouter une solution mixte d'hypochlorite de sodium, du $NaHCO_3$ et de l'eau à la solution réactionnelle pour réaction à 0 °C pendant environ 30 minutes ; ajouter de l'isopropanol et agiter à température ambiante ; laver la couche de dichlorométhane séparée deux fois avec de l'eau, sécher avec du $MgSO_4$ anhydre, filtrer et évaporer pour éliminer le solvant ; recristalliser le résidu pour obtenir le composé l ;

synthèse du composé m : dissoudre le composé l dans une solution mixte d'éthanol absolu et d'acétate d'éthyle, et refroidir à environ 0 °C ; ajouter du borohydrure de sodium par lots et agiter pour réaction ; après la réaction est terminée, neutraliser avec une solution d'acide acétique diluée, filtrer et évaporer le filtrat jusqu'à l'état sec ; dissoudre le résidu dans du dichlorométhane et ensuite laver avec de l'eau ; sécher la couche de dichlorométhane avec du $MgSO_4$ anhydre, filtrer et évaporer pour éliminer le dichlorométhane sous pression réduite ; recristalliser le résidu dans un mélange de solvants de dichlorométhane et d'éther de pétrole pour obtenir une substance solide ; puis dissoudre le produit solide dans de la pyridine et ajouter du BzCl goutte à goutte à environ 0 °C ; ensuite, la réaction

est réalisée à température ambiante ; après la réaction est terminée, éliminer le solvant par évaporation et extraire le résidu avec de l'acétate d'éthyle, laver avec une solution saturée de $NaHCO_3$, sécher avec du Na_2SO_4 anhydre, filtrer et évaporer pour éliminer le solvant pour obtenir le composé m ;

synthèse du composé n : dissoudre le composé m dans du méthanol, ajouter une solution mixte d'acide hydrochlorique concentré/dioxane pour réaction à température ambiante ; après la réaction est terminée, neutraliser la solution réactionnelle avec du $NaHCO_3$ et filtrer ; éliminer le solvant du filtrat par distillation à vide ; dissoudre le résidu dans une quantité appropriée de dichlorométhane, sécher avec du Na_2SO_4 anhydre pendant la nuit, filtrer et évaporer jusqu'à l'état sec ; séparer et purifier par chromatographie sur colonne pour obtenir le composé n ;

synthèse du composé o : dissoudre le composé n dans du dichlorométhane et ajouter du DAST à température ambiante et agiter pour réaction ; après la réaction est terminée, verser le mélange dans une solution saturée de $NaHCO_3$ et séparer la couche organique ; laver la couche organique avec une solution saturée de $NaHCO_3$, sécher avec du Na_2SO_4 anhydre, et séparer et purifier par chromatographie sur colonne pour obtenir le composé o ;

synthèse du composé p : dissoudre le composé o dans une solution d'acide formique et agiter à température ambiante pour réaction ; après la réaction est terminée, éliminer le solvant sous pression réduite ; évaporer le résidu avec du toluène pour obtenir un produit solide ; dissoudre le produit solide obtenu dans une solution de pyridine sèche et d'anhydride acétique pour réaction à température ambiante ; après la réaction est terminée, concentrer la solution réactionnelle et ensuite évaporer avec du toluène pour obtenir le composé p ;

synthèse du composé q : dissoudre le composé p dans du dichlorométhane anhydre et refroidir à 0 °C ; introduire lentement du gaz HCl dans la solution jusqu'à saturation et éliminer le solvant par évaporation sous vide à température ambiante ; sécher le résidu sous vide pour obtenir le composé q ;

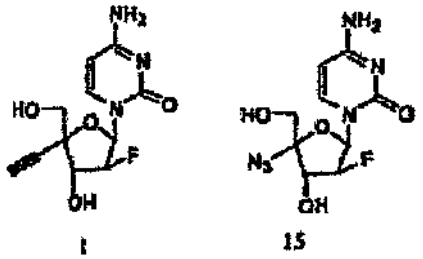
synthèse du composé r : en présence de $(NH_4)_2SO_4$, chauffer de la 2,6-diaminopurine et de l'hexaméthyldisilazane (HMDS) à reflux jusqu'à ce que la solution soit claire ; éliminer le solvant sous vide pour obtenir un produit solide blanc, et dissoudre le produit solide dans du 1,2-dichloroéthane ; ajouter une solution du composé q dans du 1,2-dichloroéthane et d'un tamis moléculaire, et agiter pour réaction sous protection d'azote à température ambiante ; après l'achèvement de la réaction a été détecté par CCM, ajouter du dichlorométhane à la solution réactionnelle et filtrer à travers de terre de diatomées ; laver le filtrat avec du Na_2SO_4 saturé et de l'eau salée ; sécher la couche organique avec du Na_2SO_4 anhydre pendant la nuit et éliminer le solvant par évaporation pour obtenir un résidu ; séparer le résidu sous pression réduite à travers une colonne de gel de silice pour obtenir le composé r ;

synthèse du composé s : dissoudre le composé r dans une solution saturée de NH_3-CH_3OH et agiter pour réaction à température ambiante ; après la réaction est terminée, éliminer le solvant par évaporation, et séparer et purifier le résidu par chromatographie sur colonne pour obtenir le composé s.

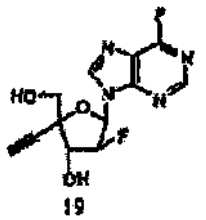
6. Utilisation des analogues de nucléosides 2'-fluoro-4'-substitués selon la revendication 1, 2, 3 dans une préparation pharmaceutique, **caractérisée en ce que** lesdits composés sont appliqués dans la préparation de médicaments anti-VHB, anti-VHC ou anti-VIH.

7. Utilisation de l'analogue de nucléosides 2'-fluoro-4'-substitué selon la revendication 6 dans une préparation pharmaceutique, **caractérisée en ce que** lesdits médicaments anti-VHB sont des médicaments antiviraux VHB, lesdits médicaments anti-VHC sont des médicaments antiviraux VHC, et lesdits médicaments anti-VIH sont des médicaments antiviraux VIH.

8. Analogues de nucléosides 2'-fluoro-4'-substitués selon la revendication 1, **caractérisés en ce que** lesdits composés sont choisis dans le groupe consistant en les composés suivants :

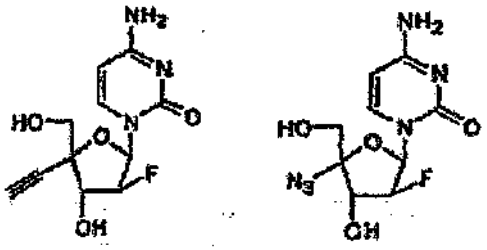


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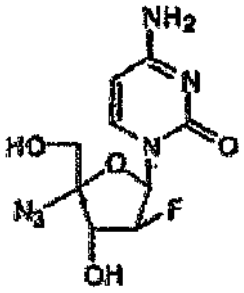
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9. Analogues de nucléosides 2'-fluoro-4'-substitués selon la revendication 1, **caractérisés en ce que** lesdits composés sont choisis dans le groupe consistant en les composés suivants :



1 15

10. Analogue de nucléosides 2'-fluoro-4'-substitué selon la revendication 1, **caractérisé en ce que** ledit composé est



15

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 2005003174 A, Clark, J. **[0003]**
- WO 0160315A22001 A, Ismaili, H.M.A. **[0003]**

Non-patent literature cited in the description

- **YUN-HA JIN et al.** *Arch. Pharm. Res.,* 1995, vol. 18 (5), 364-365 **[0004]**

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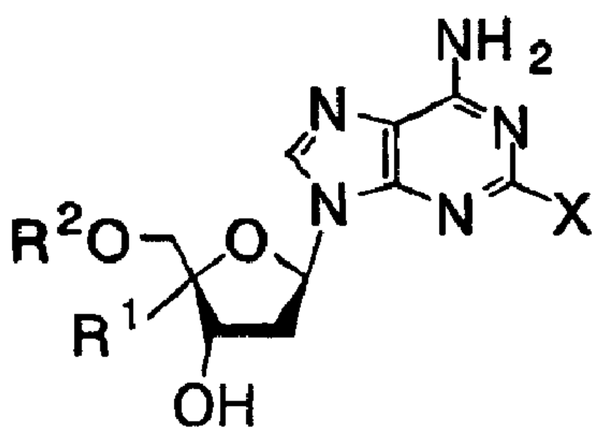
(51) Cl.Int./Int.Cl. *C07H 19/167* (2006.01),
A61K 31/7076 (2006.01), *C07H 19/16* (2006.01)

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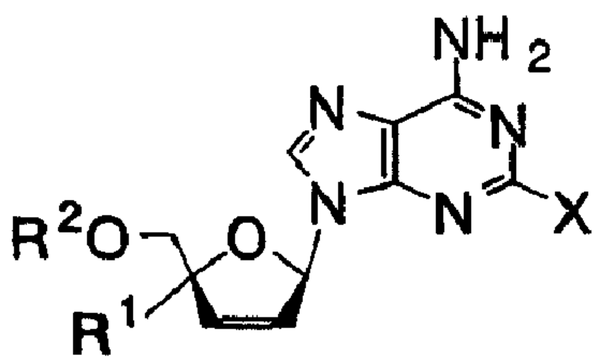
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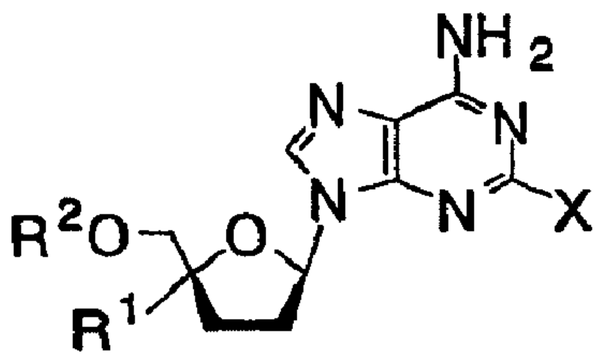
(54) Titre : DERIVE DE 2-HALOADENOSINE AVEC SUBSTITUTION SUR LE CARBONE EN POSITION 4'
(54) Title: 4'-C-SUBSTITUTED-2-HALOADENOSINE DERIVATIVE



[I]



[II]



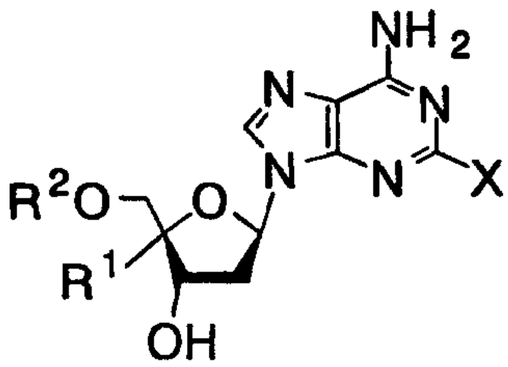
[III]

Annexure - 5

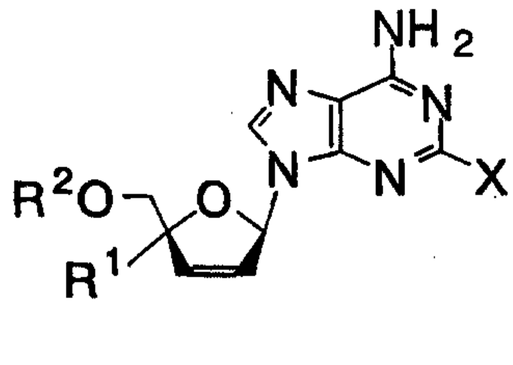
(57) Abrégé/Abstract:
The present invention provides a 4'-C-substituted-2-haloadenosine derivative represented by the following formula [I], [II], or [III]: (see formula I) (see formula II) (see formula III) (wherein X represents a halogen atom, R^1 represents an ethynyl group or a cyano group, and R^2 represents hydrogen, a phosphate residue, or a phosphate derivative residue). The present invention also provides a pharmaceutical composition containing the derivative and a pharmaceutically acceptable carrier therefor.

ABSTRACT OF THE DISCLOSURE

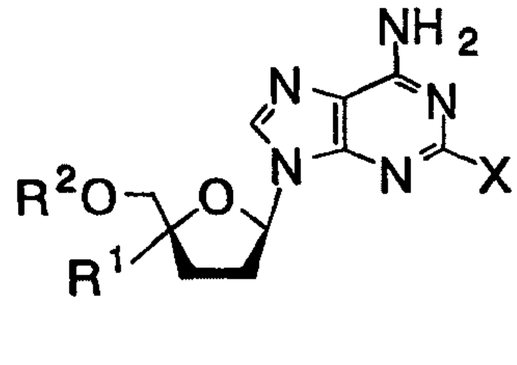
The present invention provides a 4'-C-substituted-2-haloadenosine derivative represented by the following formula [I], [II], or [III]:



[I]



[II]



[III]

(wherein X represents a halogen atom, R^1 represents an ethynyl group or a cyano group, and R^2 represents hydrogen, a phosphate residue, or a phosphate derivative residue). The present invention also provides a pharmaceutical composition containing the derivative and a pharmaceutically acceptable carrier therefor.

4'-C-Substituted-2-Haloadenosine Derivative

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to 4'-C-substituted-2-haloadenosine derivatives and use thereof as a medicine, in particular a medicine which is useful for the treatment of acquired immunodeficiency syndrome (AIDS).

Background Art

The clinical setting for AIDS has been dramatically changed by a multi-drug combination therapy, which is sometimes called highly active antiretroviral therapy, or HAART. In HAART, nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), and lamivudine (3TC), and protease inhibitors (PIs) are employed in combination.

Although HAART has drastically decreased the number of deaths caused by AIDS, there has emerged a multi-drug resistant HIV-1 (human immunodeficiency virus-1) mutant exhibiting cross-resistance to various drugs. For example, in the early 1990s patients infected with an HIV exhibiting resistance to both AZT and 3TC were very rare, whereas in 1995-1996 the percentage of AIDS patients infected with such an HIV became as high as 42%.

Ohrui, *et al*. have synthesized 2'-deoxy-4'-C-ethynyl nucleosides and assayed the anti-HIV activity thereof, and as a result, have found that a 2'-deoxy-4'-C-ethynyl nucleoside having a specific structure exhibits potent anti-HIV activity equal to or higher than that of AZT, and has effective antiviral activity against a multi-drug-resistant viral strain exhibiting resistance to various anti-HIV drugs such as AZT, ddI, ddC, d4T, and 3TC. (See, for example, Nucleic Acids Symp. Ser., Jan. 2000, (44): 105-6; J. Med. Chem., Nov. 2000, 43(23): 4516-25; Curr. Drug Targets Infect. Disord, May 2001, 1(1): 1-10; Antimicrob. Agents Chemother., May 2001, 45: 1539-1546; Nucleosides Nucleotides Nucleic Acids, May 2003, 22(5-8): 887-9; WO 00/69876; WO 00/69877; and WO 03/68796.)

The present inventors have evaluated *in vitro* toxicity of 4'-C-ethynyl purine nucleoside derivatives and 4'-C-cyano purine nucleoside derivatives, which, among a variety of 4'-C-substituted nucleosides, exhibit particularly potent anti-HIV activity. As a result, the present inventors have found that: (1) 2,6-diaminopurine derivatives and guanine derivatives, which exhibit the most potent anti-HIV activity, exhibit toxicity in vitro and in vivo; and (2) adenine derivatives, which exhibit less toxicity, are readily converted into hypoxanthine derivatives in blood by adenosine deaminase, thereby weakening the anti-HIV activity of the derivatives.

In order to attain further enhancement of selectivity

index; i.e., (concentration at which cytotoxicity is obtained)/(concentration at which anti-HIV activity is obtained) and to provide resistance to inactivation by adenosine deaminase, the present inventors have synthesized a variety of derivatives through chemical modification of 4'-C-substituted-2'-deoxyadenosine (a lead compound), which, among various 4'-C-substituted purine nucleosides, exhibits potent anti-HIV activity and less toxicity.

As has been known, when a halogen atom, which exhibits electron attraction, is introduced to the 2-position of the base moiety of an adenosine derivative, the resultant derivative exhibits a certain level of resistance to inactivation by adenosine deaminase (Chem. Pharm. Bull., 42(1994), p1688; J. Med. Chem., 39(1996), p3847). However, whether or not selectivity index can be improved through introduction of a halogen atom has remained unknown.

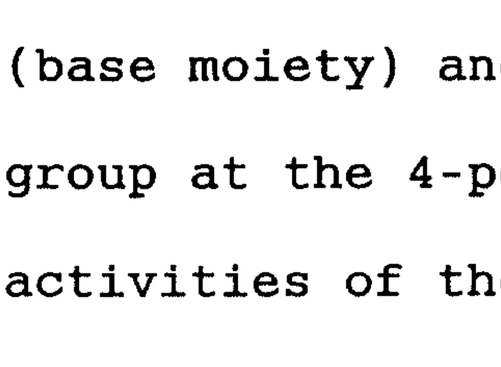
Only one literature discloses that introduction of an ethynyl group to the 4'-position of d4T (stavudine: 2',3'-didehydro-3'-deoxythymidine) enhances the selectivity index of d4T (Bioorg. Med. Chem. Lett., Nov. 2003, 13(21): 3775-7). However, effects similar to those of d4T are not expected to be obtained in an adenosine derivative, which is a purine nucleoside, whose basic skeleton differs considerably from that of d4T, and therefore, this literature does not provide useful information for the present inventors' purposes.

SUMMARY OF THE INVENTION

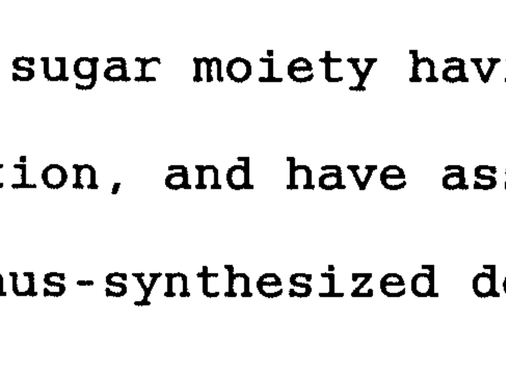
The present inventors have performed studies on the anti-HIV activity, etc. of the newly synthesized derivatives, and have found that 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine——which is obtained by introducing a fluorine atom to the 2-position of the base moiety of 2'-deoxy-4'-C-ethynyladenosine (i.e., lead compound)——exhibits resistance to inactivation by adenosine deaminase, has potent antiviral activity against a multi-drug-resistant virus strain exhibiting resistance to various anti-HIV drugs such as AZT, ddI, ddC, d4T, and 3TC, and exhibits enhanced anti-HIV activity and considerably lowered cytotoxicity.

On the basis of this finding, the present inventors have synthesized a variety of 4'-C-substituted-2-haloadenosine derivatives, each being formed of 2-haloadenine (base moiety) and a sugar moiety having an ethynyl or cyano group at the 4-position, and have assayed biological activities of the thus-synthesized derivatives. The present invention has been accomplished on the basis of the results of the assay.

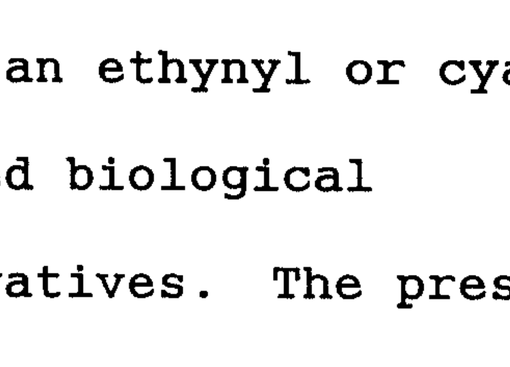
Accordingly, the present invention provides a 4'-C-substituted-2-haloadenosine derivative represented by the following formula [I], [II], or [III]:



[I]



[II]



[III]

(wherein X represents a halogen atom, R^1 represents an ethynyl group or a cyano group, and R^2 represents hydrogen, a phosphate residue, or a phosphate derivative residue).

The present invention also provides a pharmaceutical composition containing the 4'-C-substituted-2-haloadenosine derivative and a pharmaceutically acceptable carrier therefor.

The present invention also provides a method of treating AIDS, comprising administering, to a human or an animal, the 4'-C-substituted-2-haloadenosine derivative or a pharmaceutical composition containing the derivative.

As shown in the Test Examples provided hereinbelow, the compounds of the present invention (e.g., 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine) exhibit resistance to inactivation by adenosine deaminase, have potent antiviral activity against a multi-drug-resistant virus strain exhibiting resistance to various anti-HIV drugs such as AZT, ddI, ddC, d4T, and 3TC, exhibit unexpectedly enhanced anti-HIV activity; specifically, anti-HIV activity higher by a factor of 144 than that of 2'-deoxy-4'-C-ethynyladenosine (i.e., lead compound), and exhibit considerably lowered cytotoxicity. Therefore, surprisingly, the compounds of the present invention exhibit a selectivity index of 110,000, which is considerably higher than that of 2'-deoxy-4'-C-ethynyladenosine (EdAdo) (i.e., 1,630).

As described above, the compounds of the present invention exhibit excellent anti-HIV activity, particularly against a multi-drug-resistant HIV strain having resistance

to various anti-HIV drugs such as AZT, DDI, DDC, D4T, and 3TC, exhibit less cytotoxicity, and exhibit resistance to inactivation by adenosine deaminase. Therefore, the compounds of the present invention are envisaged for development for producing pharmaceuticals, particularly drugs for treating AIDS.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows stability of compounds against deamidation reaction induced by adenosine deanimase. The black squares show the results obtained from 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine (a compound of the present invention), whereas the black circles show the results obtained from 2'-deoxy-4'-C-ethynyladenosine (a known compound);

Fig. 2 shows stability of 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine (a compound of the present invention) under acidic conditions;

Fig. 3 shows stability of 2',3'-dideoxyadenosine (ddAdo; a known compound) under acidic conditions; and

Fig. 4 shows changes in body weight of mice, as measured after administration of 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine (a compound of the present invention). In Fig. 4, Graph A shows the results obtained from oral administration, and graph B shows the results obtained from intravenous injection. In both graphs, white circles show the results from placebo administration, and triangles and squares correspond to a dose of 30 mg/kg and 100 mg/kg,

respectively.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

(1) Compounds

The compounds of the present invention are represented by formulas [I], [II], and [III]. Examples of the phosphate residue represented by R^2 in these formulas include a monophosphate residue, a diphosphate residue, a triphosphate residue, and a phosphonate; and examples of the phosphate derivative residue include phosphate polyesters (e.g., a phosphate diester and a phosphate triester), phosphate amidates (e.g., a phosphate monoamidate and a phosphate diamidate), phosphorothioate, phosphoroselenoate, and phosphoroboranoate. Examples of halogen atoms represented by X include bromine, iodine, fluorine, and chlorine.

Of these compounds, preferred ones are those that satisfy one or more of the following requirements: (a) R^2 is hydrogen or phosphonate; (b) X is fluorine or chlorine; and (c) R^1 is an ethynyl group. Specific examples of preferred compounds are given below:

<compounds represented by formula [I]>

2'-deoxy-4'-C-ethynyl-2-fluoroadenosine, 4'-C-cyano-2'-deoxy-2-fluoroadenosine, 2-chloro-2'-deoxy-4'-C-ethynyladenosine, and 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine 5'-H-phosphonate;

<compounds represented by formula [II]>

2',3'-didehydro-2',3'-dideoxy-4'-C-ethynyl-2-

fluoroadenosine, 2',3'-didehydro-2',3'-dideoxy-4'-C-cyano-2-fluoroadenosine, 2',3'-didehydro-2',3'-dideoxy-4'-C-ethynyl-2-chloroadenosine, and 2',3'-didehydro-2',3'-dideoxy-4'-C-ethynyl-2-fluoroadenosine 5'-H-phosphonate; and

<compounds represented by formula [III]>

2',3'-dideoxy-4'-C-ethynyl-2-fluoroadenosine, 2',3'-dideoxy-4'-C-cyano-2-fluoroadenosine, 2',3'-dideoxy-4'-C-ethynyl-2-chloroadenosine, and 2',3'-dideoxy-4'-C-ethynyl-2-fluoroadenosine 5'-H-phosphonate.

The compounds of the present invention may be salts, hydrates, or solvates. When R^2 is hydrogen, examples of salts include acid-adducts such as hydrochlorides and sulfates; and when R^2 is a phosphate residue, examples of salts include alkali metal salts such as sodium salts, potassium salts, and lithium salts; alkaline earth metal salts such as calcium salts; and ammonium salts, and any of those salts may be used so long as they are pharmaceutically acceptable.

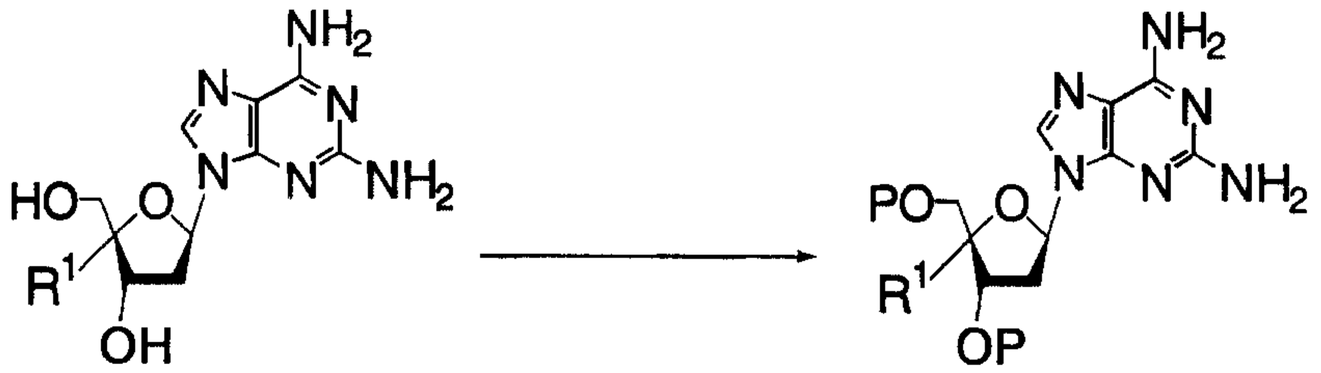
Examples of hydrates or solvates include adducts formed by combining one molecule of the compound of the present invention or a salt thereof and 0.1-3.0 molecules of water or a solvent. In addition, the compounds of the present invention encompass a variety of isomers thereof such as tautomers.

(2) Production method

The compounds [I] of the present invention can be produced through the below-described steps.

First step:

In the first step, hydroxyl groups at the 3'- and 5'-positions of a compound represented by formula [IV] are protected, to thereby yield a compound represented by formula [V]:



[IV] [V]

(wherein P represents a protective group, and R^1 represents an ethynyl group or a cyano group).

The compound [IV] (i.e., starting material) is a known compound; specifically, a compound in which R^1 is an ethynyl group (J. Med. Chem., 43, 4516-4525 (2000)), or a compound in which R^1 is a cyano group (WO 03/68796).

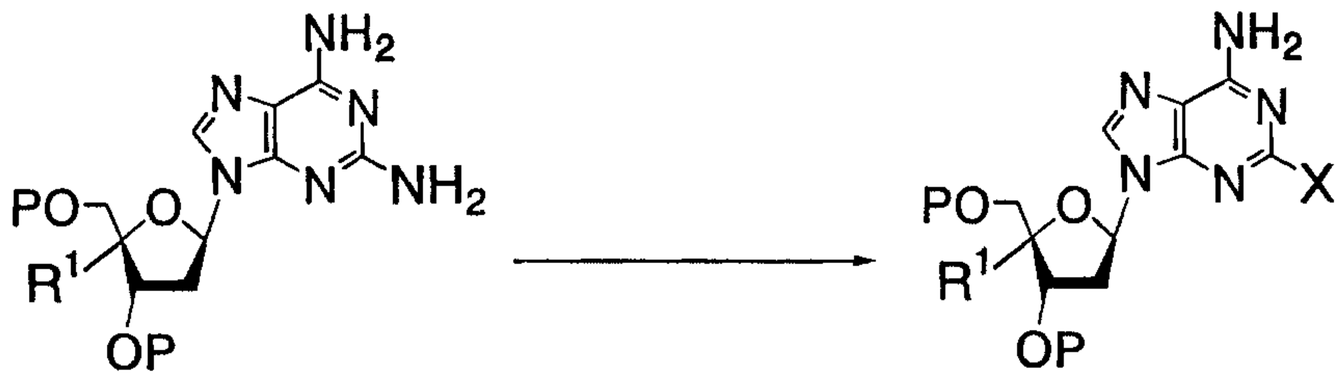
The protective groups represented by P, which protect the hydroxyl groups at the 3'- and 5'-positions, may be those groups which are generally employed for protecting a hydroxyl group. Examples of types of the protective groups include an ether type, an acyl type, a silyl type, and an acetal type. Specific examples of the protective groups which may be employed include ether-type protective groups such as methyl ether, tert-butyl ether, benzyl ether, methoxybenzyl ether, and trityl ether; acyl-type protective groups such as acetyl, benzoyl, and pivaloyl; silyl-type protective groups such as t-butyldimethylsilyl, t-butyldiphenylsilyl, trimethylsilyl,

and triethylsilyl; and acetal-type protective groups such as isopropylidene, ethylidene, methylidene, benzylidene, tetrahydropyranyl, and methoxymethyl.

Introduction of a protecting group is performed by conventional methods. For examples, in organic solvent such as pyridine, acetonitrile or dimethylformamide, compound [IV] is allowed to react with a protecting agent (alkyl halide, acid halide, acid anhydride or alkylsilyl halide) in the presence of a base such as metal alkoxide, triethylamine, 4-dimethylaminopyridine or imidazole, at -10 to 100°C.

Second step:

In the second step, the amino group at the 2-position of the compound [V] is converted into a halogen atom , to thereby yield a compound represented by formula [VI]:



[V] [VI]

(wherein P represents a protective group, X represents a halogen atom, and R^1 represents an ethynyl group or a cyano group).

The compound [VI] can be synthesized through the following procedure: after the amino group at the 2-position of the compound [V] is treated with a nitrite derivative, halogen atom is introduced at the 2-position of the base moiety by use of a halogen reagent; or the amino groups at

the 2- and 6-positions are treated under the same conditions, thereby forming a 2,6-dihalopurine derivative, and the halogen atom at the 6-position of the base moiety is converted into an amino group through treatment with ammonia.

Examples of reagents for substituting the amino group at the 2-position of the compound [V] by fluorine include sodium nitrite in tetrafluoroboric acid; and a nitrous acid ester (e.g., t-butyl nitrite) in hydrogen fluoride-pyridine.

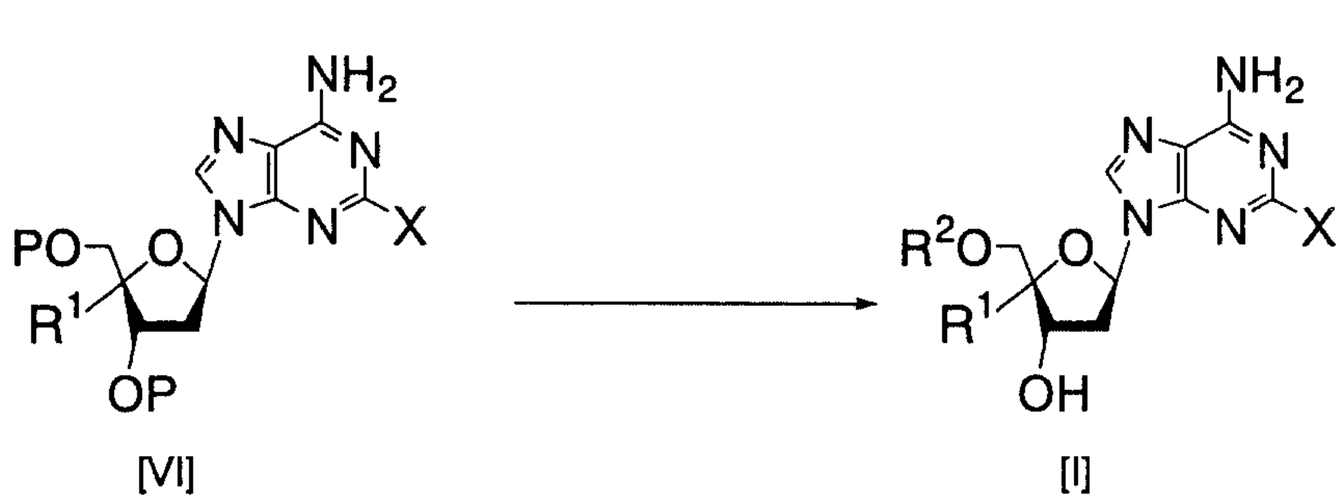
Reaction conditions vary depending on the reagent employed. For example, when t-butyl nitrite is employed in hydrogen fluoride-pyridine, t-butyl nitrite (1 to 3 mol) is added to the compound [V] in hydrogen fluoride-pyridine serving as a solvent, and the resultant mixture is allowed to react at -50°C to room temperature for about 15 minutes to about five hours. When the compound [V] is formed into a 2,6-difluoropurine derivative, the resultant derivative is treated with aqueous ammonia in an organic solvent such as dioxane or methanol.

Examples of reagents for substituting the amino group at the 2-position of the compound [V] by chlorine include a combination of antimony trichloride and t-butyl nitrite, and a combination of acetyl chloride and benzyltriethylammonium nitrite, which combinations are employed in an organic solvent such as dichloromethane.

Reaction conditions vary depending on the reagent employed. For example, when a combination of acetyl chloride and benzyltriethylammonium nitrite is employed as the reagent,

in an organic solvent such as dichloromethane, benzyltriethylammonium nitrite (1 to 5 mol) is treated with acetyl chloride (1 to 5 mol) at -50°C to room temperature for about 30 minutes to about three hours, and the resultant mixture is allowed to react with the compound [V] (1 mol) at -50°C to room temperature for one hour to a few days. When the compound [V] is formed into a 2,6-dichloropurine derivative, the resultant derivative is treated with aqueous ammonia in an organic solvent such as dioxane or methanol.

The protective groups of the thus-obtained compound [VI] are removed, to thereby yield the compound of the present invention in which R^2 is hydrogen, and if desired, the compound is phosphorylated:

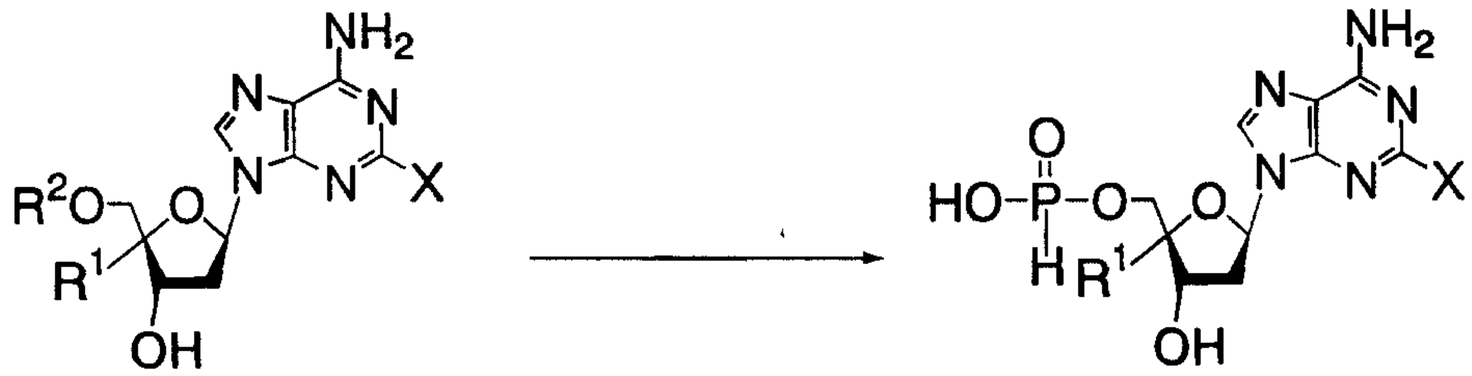


[VI] [I]

(wherein P represents a protective group, X represents a halogen atom, R^1 represents an ethynyl group or a cyano group, and R^2 represents hydrogen, a phosphate residue, or a phosphate derivative residue).

The protective groups may be removed through a technique which is appropriately selected from among typical techniques (e.g., hydrolysis under acidic conditions, hydrolysis under alkaline conditions, treatment with tetrabutylammonium fluoride, and catalytic reduction) in

accordance with the protective groups employed.



[I] [VII]

(wherein X represents a halogen atom, R^1 represents an ethynyl group or a cyano group, and R^2 represents hydrogen).

In order to produce the 5'-H-phosphonate derivative [VII] (the compound of the present invention), the compound [I] in which R^2 is hydrogen and phosphonic acid are subjected to condensation in an organic solvent by use of an appropriate condensing agent. Examples of the organic solvent which may be employed include pyridine, and dimethylformamide in the presence of a base such as triethylamine. Examples of the condensing agent which may be employed include carbodiimides such as dicyclohexyl carbodiimide, diisopropyl carbodiimide, and water-soluble carbodiimide; sulfonic acid halides such as toluenesulfonyl chloride; and phosphate chlorides such as diphenyl phosphate chloride.

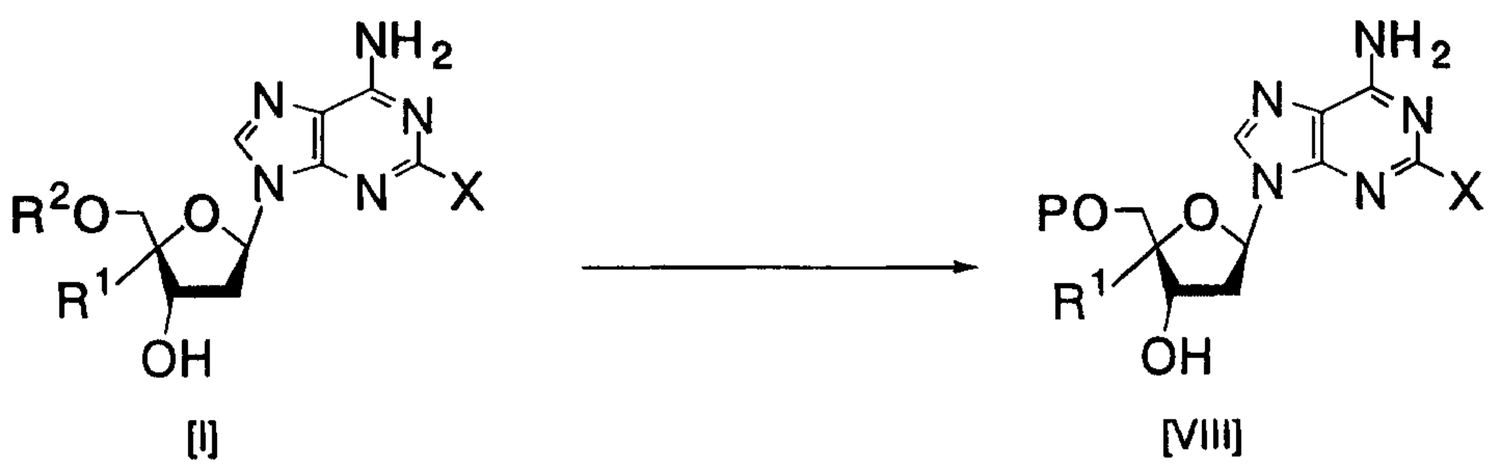
Reaction conditions vary depending on the reagent employed. For example, when dicyclohexyl carbodiimide is employed in pyridine, phosphonic acid (1 to 5 mol) and dicyclohexyl carbodiimide (1 to 10 mol) are added to 1 mol of the compound [1], and the resultant mixture is allowed to react at 0°C to 50°C for about one to about 24 hours.

When a compound in which R^2 is a monophosphate is to be produced, a compound in which R^2 is hydrogen is reacted with a phosphorylating agent; for example, phosphorus oxychloride or tetrachloropyrophosphoric acid, which selectively phosphorylates the 5'-position of a nucleoside. When a compound in which R^2 is a diphosphate or triphosphate is to be produced, the corresponding 5'-monophosphate compound is activated in the form of phosphoimidazolide, phosphomorpholidate, or anhydrous diphenylphosphate, and the thus-activated compound is reacted with phosphoric acid, pyrophosphoric acid, or a suitable salt thereof, to thereby produce a target compound in a free acid or salt form.

The compounds [II] of the present invention can be produced through the below-described steps.

First step:

In the first step, the hydroxyl group at the 5'-position of a compound represented by formula [I] in which R^2 is hydrogen is selectively protected, to thereby yield a compound represented by formula [VIII]:



[I] [VIII]

(wherein P represents a protective group, X represents a halogen atom, R^1 represents an ethynyl group or a cyano group, and R^2 represents hydrogen).

The protective group represented by P, which protects the hydroxyl group at the 5'-position, may be a protective group which is generally employed for selectively protecting a primary hydroxyl group. Specific examples of the protective group include a trimethoxytrityl group, a dimethoxytrityl group, a methoxytrityl group, a trityl group, a t-butyldimethylsilyl group, a t-butyldiphenylsilyl group, and a benzoyl group.

Introduction of the protective group can be carried out in a manner similar to that employed for the compound [V].

Second step:

In the second step, the hydroxyl group at the 3'-position of the compound [VIII] is subjected to dehydration, forming a 2',3'-carbon-carbon double bond, to thereby yield a compound represented by formula [VIV].



[VIII] [VIV]

(wherein P represents a protective group, X represents a halogen atom, and R^1 represents an ethynyl group or a cyano group).

In order to produce the compound [VIV] through dehydration of the hydroxyl group at the 3'-position of the compound [VIII], the hydroxyl group at the 3'-position of the compound [VIII] is converted into a removable functional

group such as a sulfonate group (e.g., a methanesulfonate group, a chloromethanesulfonate group, a toluenesulfonate group, or a trifluoromethanesulfonate group) or a halogen atom, and the thus-converted group is removed through treatment with a base.

Reaction conditions vary depending on the reagent employed. For example, in the case of reaction through formation of a trifluoromethanesulfonate, trifluoromethanesulfonic anhydride (1 to 5 mol) and a base (e.g., pyridine or triethylamine) (5 to 10 mol) are added to the compound [VIII] in an organic solvent such as dichloromethane or pyridine, and the resultant mixture is allowed to react at -78°C to room temperature for about one to about 24 hours.

The protective group of the thus-obtained compound [VIV] is removed, to thereby yield the compound of the present invention in which R^2 is hydrogen, and if desired, the compound is phosphorylated:



[VIV] [II]

(wherein X represents a halogen atom, P represents a protective group, R^1 represents an ethynyl group or a cyano group, and R^2 represents hydrogen, a phosphate residue, or a phosphate derivative residue).

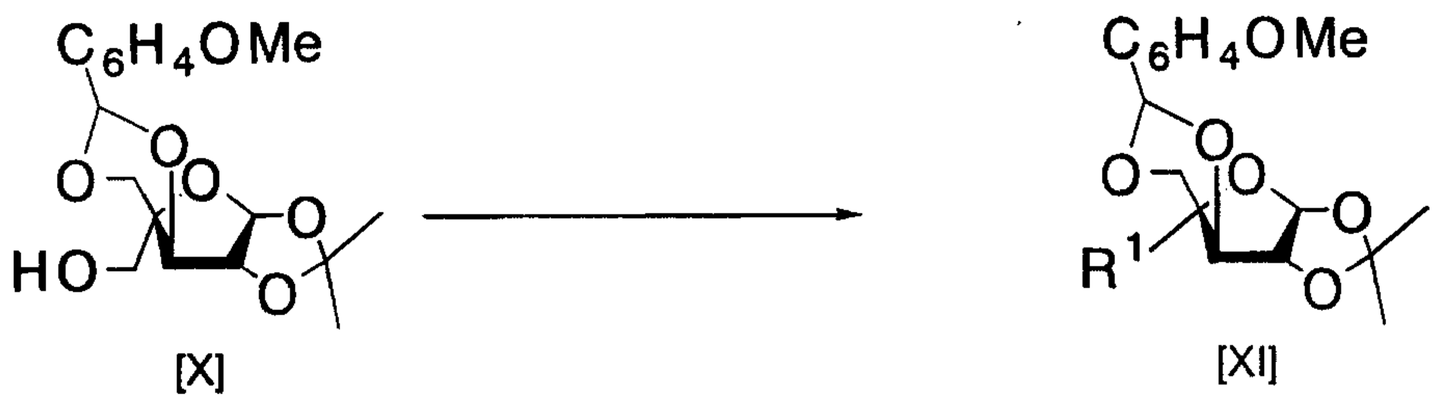
The protective group may be removed through a technique which is appropriately selected from among typical techniques (e.g., hydrolysis under acidic conditions, hydrolysis under alkaline conditions, treatment with tetrabutylammonium fluoride, and catalytic reduction) in accordance with the protective group employed.

A compound in which R^2 is a phosphate residue or a derivative thereof can be synthesized in a manner similar to that of the compound [I].

The compounds [III] of the present invention can be produced through the below-described steps.

First step:

In the first step, the hydroxymethyl group at the 4-position of a compound represented by formula [X] is oxidized to thereby form an aldehyde group, which is further converted into a triethylsilylethynyl or cyano group to thereby yield a compound represented by formula [XI]:



(wherein R^1 represents an ethynyl group, a triethylsilylethynyl group, or a cyano group).

The compound [X] (i.e., starting material) is a known compound (Biosci. Biotech. Biochem., 57, 1433-1438 (1993)). The compound [X] can be converted into a triethylsilylethynyl compound through the following procedure: the hydroxymethyl

group at the 4-position of the compound [X] is oxidized to form a formyl group, and the formyl group is converted into a dibromovinyl group, followed by removal of hydrogen bromide through treatment with a strong base.

When the hydroxymethyl group at the 4-position of the compound [X] is converted into a formyl group, an oxidizing agent is employed. Examples of the oxidizing agent which may be employed include chromium-containing oxidizing agents such as chromic anhydride-pyridine-acetic anhydride composite reagents, pyridinium chlorochromate, and pyridinium dichromate; high-valency iodine oxidizing agents such as Dess-Martin reagent; and dimethyl sulfoxide-based oxidizing agents such as a combination of dimethyl sulfoxide and acetic anhydride, oxalyl chloride, or dicyclohexyl carbodiimide.

Reaction conditions vary depending on the oxidizing agent to be employed. For example, when oxidation is carried out by use of oxalyl chloride and dimethyl sulfoxide, oxalyl chloride (1 to 5 mol) and dimethyl sulfoxide (1.5 to 6 mol) are added to 1 mol of the compound [X] in an organic solvent (e.g., dichloromethane), optionally under an inert gas atmosphere (e.g., argon or nitrogen), and the resultant mixture is allowed to react at -100°C to 0°C for about 15 minutes to about two hours. Subsequently, a base such as triethylamine is added in an amount of 2 to 10 mol to the mixture, and the resultant mixture is further allowed to react at room temperature for about 15 minutes to about two hours.

The thus-formed aldehyde can be converted into a corresponding alkyne through the following procedure: the aldehyde is subjected to carbon-increasing (i.e., C-C bond formation) reaction; the resultant compound is treated with a strong base to thereby form a metal alkynyl compound; and a protective group is introduced into the metal alkynyl compound. Carbon-increasing reaction is carried out in an organic solvent such as dichloromethane or dichloroethane, optionally under an inert gas atmosphere (e.g., argon or nitrogen). Specifically, carbon tetrabromide (1 to 5 mol) and triphenylphosphine (2 to 10 mol) are added to 1 mol of the above-formed aldehyde, and the resultant mixture is allowed to react at 0 to 50°C for about 15 minutes to about three hours.

Treatment with a strong base can be carried out in an organic solvent such as tetrahydrofuran, 1,4-dioxane, or dimethoxyethane, optionally under an inert gas atmosphere (e.g., argon or nitrogen). Specifically, a lithium compound (e.g., methyllithium, n-butyllithium, or t-butyllithium) (2 to 4 mol) is added to 1 mol of the compound obtained through carbon-increasing reaction, and the resultant mixture is allowed to react at -100 to -20°C for about five to about 60 minutes. Furthermore, when a silyl protective group is introduced into the alkynyl group of the thus-obtained compound, the aforementioned strong-base treatment is followed by addition of a silylating agent such as chlorotriethylsilane, and the resultant mixture is allowed to

react.

Meanwhile, the compound [X] can be converted into a cyano compound through the following procedure: the hydroxymethyl group at the 4-position of the compound [X] is oxidized to form a formyl group, and the formyl group is converted into an oxime group, followed by dehydration of the thus-formed oxime group.

When the hydroxymethyl group at the 4-position of the compound [X] is converted into a formyl group, an oxidizing agent is employed. Examples of the oxidizing agent which may be employed include chromium-containing oxidizing agents such as chromic anhydride-pyridine-acetic anhydride composite reagents, pyridinium chlorochromate, and pyridinium dichromate; high-valency iodine oxidizing agents such as Dess-Martin reagent; and dimethyl sulfoxide-based oxidizing agents such as a combination of dimethyl sulfoxide and acetic anhydride, oxalyl chloride, or dicyclohexyl carbodiimide.

Reaction conditions vary depending on the oxidizing agent to be employed. For example, when oxidation is carried out by use of oxalyl chloride and dimethyl sulfoxide, oxalyl chloride (1 to 5 mol) and dimethyl sulfoxide (1.5 to 6 mol) are added to 1 mol of the compound [X] in an organic solvent (e.g., dichloromethane), optionally under an inert gas atmosphere (e.g., argon or nitrogen), and the resultant mixture is allowed to react at -100°C to 0°C for about 15 minutes to about two hours. Subsequently, a base such as triethylamine is added in an amount of 2 to 10 mol to the

mixture, and the resultant mixture is further allowed to react at room temperature for about 15 minutes to about two hours.

The thus-formed aldehyde can be converted into a corresponding oxime by reacting 1 mol of the aldehyde with hydroxylamine hydrochloride (1 to 5 mol) in an organic solvent such as pyridine at room temperature to 100°C for about 30 minutes to about three hours.

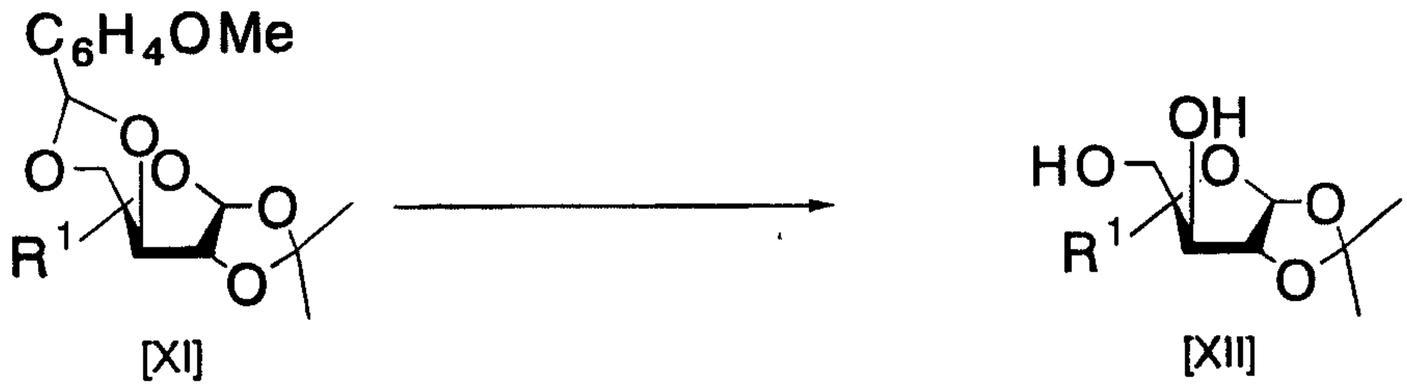
Dehydration of the thus-formed oxime can be carried out by use of a dehydrating agent (e.g., phosgene, carbonyldiimidazole, methanesulfonyl chloride, or acetic anhydride) in an organic solvent (e.g., dichloromethane, acetonitrile, or tetrahydrofuran) in the presence of a base (e.g., pyridine, triethylamine, or sodium acetate).

Dehydration conditions vary depending on the dehydrating agent to be employed. For example, when dehydration is carried out by use of methanesulfonyl chloride, in an organic solvent (such as dichloromethane, tetrahydrofuran, or pyridine), methanesulfonyl chloride (1 to 5 mol) and triethylamine (5 to 10 mol) are added to 1 mol of the oxime, and the resultant mixture is allowed to react at -50°C to room temperature for about 15 minutes to about two hours.

Second step:

In the second step, the methoxybenzylidene group which protects the hydroxyl groups at the 3- and 5-positions of the compound [XI] is removed, to thereby yield a compound

represented by formula [XII]:



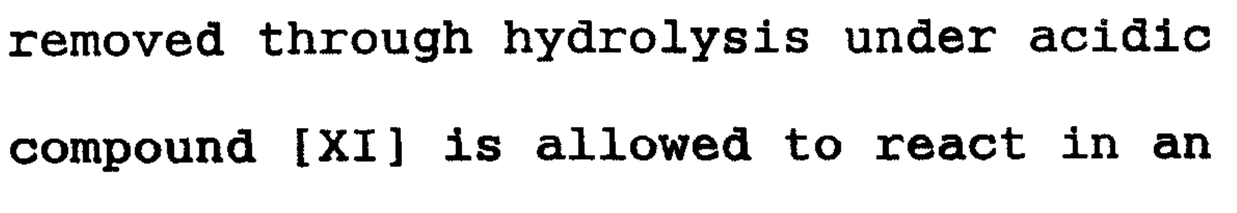
(wherein R^1 represents an ethynyl group, a triethylsilylethynyl group, or a cyano group).

The protective group may be removed through a technique which is appropriately selected from among typical techniques (e.g., hydrolysis under acidic conditions, and catalytic reduction).

Reaction conditions vary depending on the technique to be employed. For example, when the protective group is removed through hydrolysis under acidic conditions, the compound [XI] is allowed to react in an aqueous solution of an organic acid (e.g., formic acid or acetic acid) or mineral acid at 0 to 100°C for one to 24 hours.

Third step:

In the third step, the hydroxyl group at the 5-position of the compound [XII] is selectively protected, to thereby yield a compound represented by formula [XIII]:



(wherein P represents a protective group, and R^1 represents an ethynyl group, a triethylsilylethynyl group, or a cyano group).

The protective group represented by P, which protects the hydroxyl group at the 5-position, may be a protective group which is generally employed for selectively protecting a primary hydroxyl group. Specific examples of the protective group include a trimethoxytrityl group, a dimethoxytrityl group, a methoxytrityl group, a trityl group, a t-butyldimethylsilyl group, a t-butyldiphenylsilyl group, and a benzoyl group.

Introduction of the protective group can be carried out in a manner similar to that employed for the compound [V].

Fourth step:

In the fourth step, the hydroxyl group at the 3-position of the compound [XIII] is reduced, to thereby yield a compound represented by formula [XIV]:



(wherein P represents a protective group, and R^1 represents an ethynyl group, a triethylsilylethynyl group, or a cyano group).

Deoxygenation of the hydroxyl group at the 3-position can be carried out by converting the compound having the hydroxyl group into a corresponding halide (iodite, bromide or chloride), phenoxythionocarbonate, thiocarbonylimidazole, or methyldithiocarbonate, and by reducing the thus-converted compound by use of a radical reducing agent in the presence of a radical initiator.

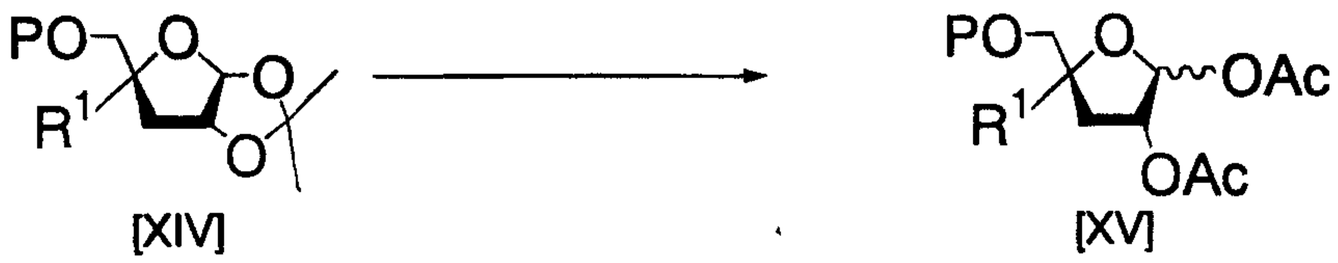
For example, when deoxygenation is carried out through formation of a phenoxythiocarbonyl compound, conversion of the hydroxyl group to a phenoxythiocarbonyl group is carried out in an organic solvent (e.g., tetrahydrofuran, acetonitrile, or dichloromethane) in the presence of a base such as dimethylaminopyridine or pyridine, optionally under an inert gas atmosphere such as argon or nitrogen. Specifically, a phenyl chlorothionoformate derivative (1 to 10 mol, preferably 1 to 2 mol) is added to 1 mol of the aforementioned compound in which only the protective group for the hydroxyl group at the 3-position has been eliminated, and the resultant mixture is allowed to react under stirring at 0 to 50°C for about 0.5 to about five hours.

Subsequently, reduction is carried out in an organic solvent (e.g., toluene or benzene) in the presence of a radical initiator such as azobisisobutyronitrile, optionally under an inert gas atmosphere such as argon or nitrogen. Specifically, a radical reducing agent such as tributyltin hydride or tris(trimethylsilyl)silane (1 to 10 mol, preferably 2 to 5 mol) is added to 1 mol of the aforementioned phenoxythiocarbonyl compound, and the resultant mixture is allowed to react under stirring at 50 to 150°C for about one to about five hours.

Fifth step:

In the fifth step, the isopropylidene group at the 1- and 2-positions of the compound [XIV] is removed, and then the thus-formed hydroxyl groups are acetylated, to thereby

yield a compound represented by formula [XV]:



(wherein P represents a protective group, and R^1 represents an ethynyl group, a triethylsilylethynyl group, or a cyano group).

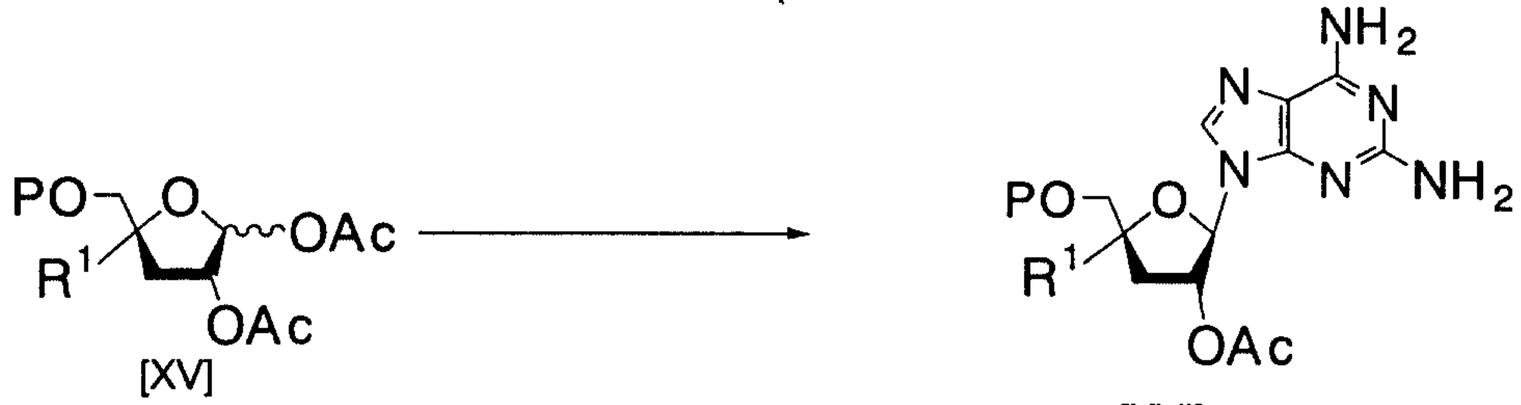
When the isopropylidene group at the 1- and 2-positions is removed through hydrolysis under acidic conditions, the compound [XIV] is allowed to react in an aqueous solution of an organic acid (e.g., formic acid or acetic acid) or mineral acid at 0 to 100°C for one to 24 hours.

Introduction of acetyl groups to the hydroxyl groups, which follows removal of the isopropylidene group, can be carried out by means of a customary technique. For example, acetyl groups are introduced to the hydroxyl groups through reaction with an acetylating agent (e.g., acetyl chloride or acetic anhydride) in an organic solvent such as pyridine, acetonitrile, or dichloromethane in the presence of a base such as pyridine or triethylamine.

For example, in the case of reaction in pyridine by use of acetic anhydride, acetic anhydride (2 to 10 mol) and, if desired, a catalytic amount of 4-dimethylaminopyridine are added to 1 mol of the compound from which the isopropylidene group has been removed, and the resultant mixture is allowed to react at 0 to 100°C for one to 24 hours.

Sixth step:

In the sixth step, the compound [XV] and 2,6-diaminopurine are subjected to condensation, to thereby yield a compound represented by formula [XVI]:



(wherein P represents a protective group, and R^1 represents an ethynyl group, a triethylsilylethynyl group, or a cyano group).

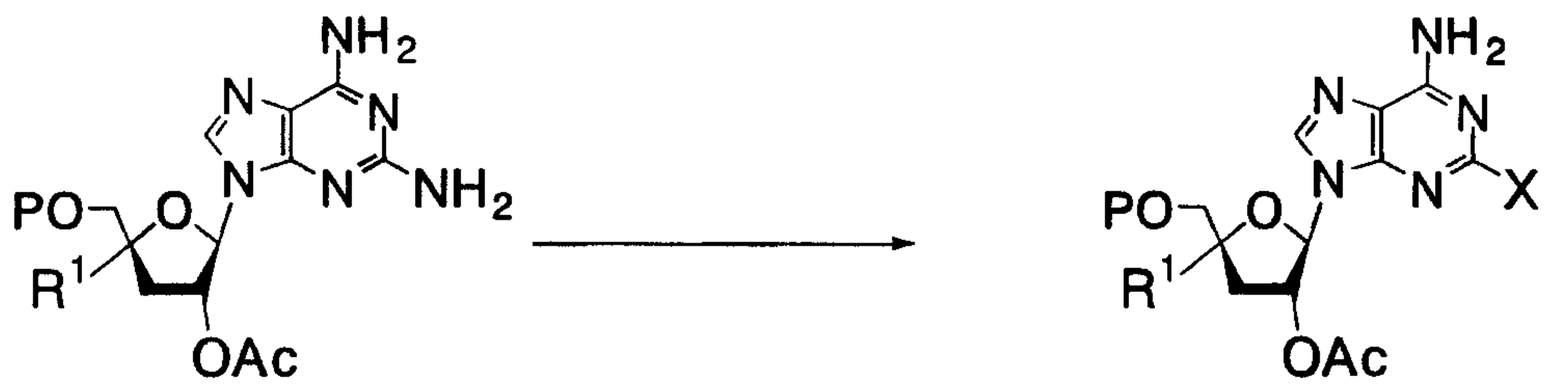
Condensation of the compound [XV] and 2,6-diaminopurine can be carried out by reacting the compound [XV] with 2,6-diaminopurine in the presence of a Lewis acid. In this case, 2,6-diaminopurine may be silylated, and such silylation of 2,6-diaminopurine may be carried out through a known technique. For example, 2,6-diaminopurine is silylated under reflux in a mixture of hexamethyldisilazane and trimethylchlorosilane, or is silylated under reflux by use of bis(trimethylsilyl)acetamide in an organic solvent such as acetonitrile or 1,2-dichloroethane. Examples of Lewis acids to be employed include trimethylsilyl trifluoromethanesulfonate, tin tetrachloride, zinc chloride, zinc iodide, and anhydrous aluminum chloride.

Condensation reaction can be carried out in an organic solvent such as dichloromethane, 1,2-dichloroethane, acetonitrile, or toluene, optionally under an inert gas

atmosphere such as argon or nitrogen. Specifically, 2,6-diaminopurine (1 to 10 mol) and a Lewis acid (0.1 to 10 mol) are added to 1 mol of the compound [XV], and the resultant mixture is allowed to react at -20 to 150°C for about 30 minutes to about 24 hours.

Seventh step:

In the seventh step, the amino group at the 2-position of the compound [XVI] is converted into halogen atom, to thereby yield a compound represented by formula [XVII]:



[XVI] [XVII]

(wherein P represents a protective group, X represents a halogen atom, and R^1 represents an ethynyl group, a triethylsilylethynyl group, or a cyano group).

The compound [XVII] can be synthesized through the following procedure: after the amino group at the 2-position of the compound [XVI] is treated with a nitrite derivative, halogen atom is introduced at the 2-position of a base moiety by use of a halogen reagent; or the amino groups at the 2- and 6-positions are treated under the same conditions, thereby forming a 2,6-dihalopurine derivative, and the halogen atom at the 6-position of base moiety is converted into an amino group through treatment with ammonia.

Examples of reagents for substituting the amino group

at the 2-position of the compound [XVI] by fluorine include sodium nitrite in tetrafluoroboric acid; and a nitrous acid ester (e.g., t-butyl nitrite) in hydrogen fluoride-pyridine.

Reaction conditions vary depending on the reagent employed. For example, when t-butyl nitrite is employed in hydrogen fluoride-pyridine, t-butyl nitrite (1 to 3 mol) is added to the compound [XVI] in hydrogen fluoride-pyridine serving as a solvent, and the resultant mixture is allowed to react at -50°C to 0°C for about 15 minutes to about five hours. When the compound [XVI] is formed into a 2,6-difluoropurine derivative, the resultant derivative is treated with aqueous ammonia in an organic solvent such as dioxane or methanol.

Examples of reagents for substituting the amino group at the 2-position of the compound [XVI] by chlorine include a combination of antimony trichloride and t-butyl nitrite, and a combination of acetyl chloride and benzyltriethylammonium nitrite, which combinations are employed in an organic solvent such as dichloromethane.

Reaction conditions vary depending on the reagent employed. For example, when a combination of acetyl chloride and benzyltriethylammonium nitrite is employed as the reagent, in an organic solvent such as dichloromethane, benzyltriethylammonium nitrite (1 to 5 mol) is treated with acetyl chloride (1 to 5 mol) at -50°C to room temperature for about 30 minutes to about three hours, and the resultant mixture is allowed to react with 1 mol of the compound [XVI]

at -50°C to room temperature for one hour to a few days.

When the compound [XVI] is formed into a 2,6-dichloropurine derivative, the resultant derivative is treated with aqueous ammonia in an organic solvent such as dioxane or methanol.

Eighth step:

In the eighth step, the acetyl group which protects the hydroxyl group at the 2'-position of the compound [XVII] is removed, to thereby yield a compound represented by formula [XVIII]:



[XVII] [XVIII]

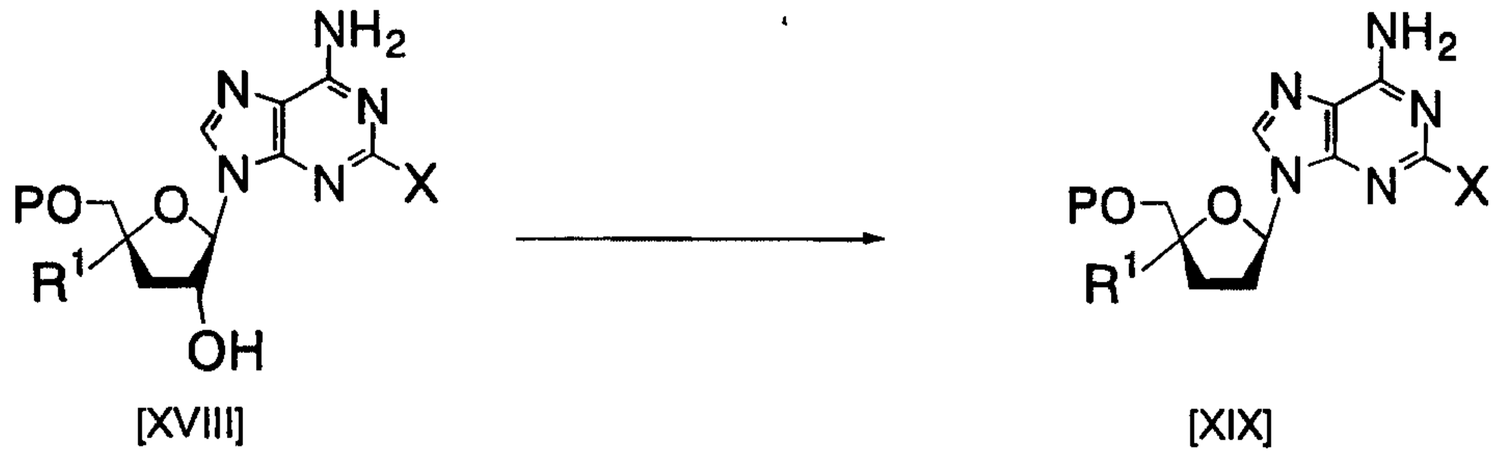
(wherein P represents a protective group, X represents a halogen atom, and R^1 represents an ethynyl group, a triethylsilylethynyl group, or a cyano group).

The acetyl group can be removed by use of an appropriate base or acid catalyst. For example, when removal of the acetyl group is carried out in a solvent mixture of water and an alcohol (e.g., ethanol), a base catalyst such as sodium hydroxide, potassium hydroxide, triethylamine, or aqueous ammonia can be employed.

For example, the acetyl group can be removed by allowing the compound [XVII] to react by use of aqueous ammonia in methanol at 0 to 100°C for one to 24 hours.

Ninth step:

In the ninth step, the hydroxyl group at the 2'-position of the compound [XVIII] is reduced, to thereby yield a compound represented by formula [XIX]:



[XVIII] [XIX]

(wherein P represents a protective group, X represents a halogen atom, and R^1 represents an ethynyl group, a triethylsilylethynyl group, or a cyano group).

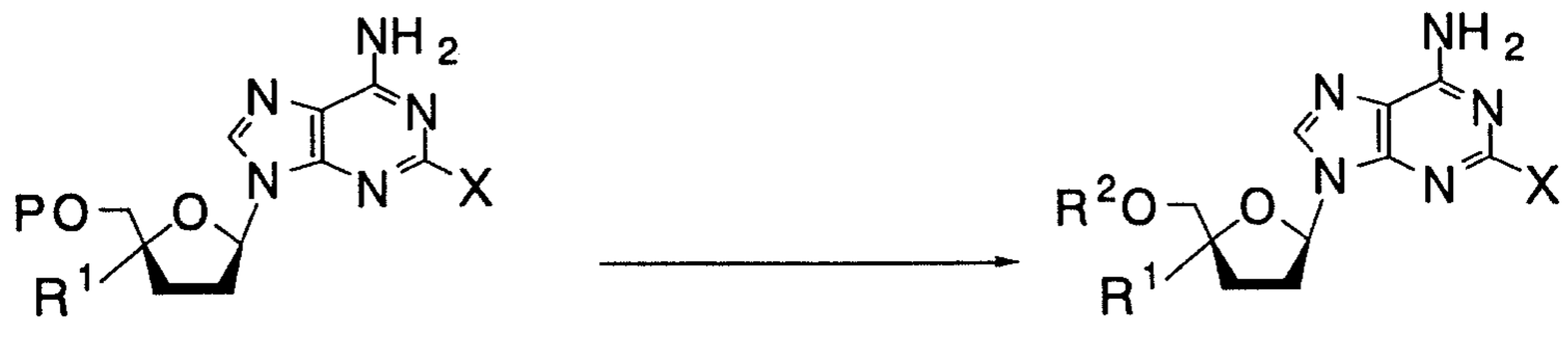
Deoxygenation of the hydroxyl group at the 3-position can be carried out by converting the compound having the hydroxyl group into the corresponding halide (iodite, bromide or chloride), phenoxythionocarbonate, thiocarbonylimidazole, or methyldithiocarbonate, and by reducing the thus-converted compound by use of a radical reducing agent in the presence of a radical initiator.

For example, when deoxygenation is carried out through formation of a phenoxythiocarbonyl compound, conversion of the hydroxyl group to a phenoxythiocarbonyl group is carried out in an organic solvent (e.g., tetrahydrofuran, acetonitrile, or dichloromethane) in the presence of a base such as dimethylaminopyridine or pyridine, optionally under an inert gas atmosphere such as argon or nitrogen. Specifically, a phenyl chlorothionoformate derivative (1 to 10 mol, preferably 1 to 2 mol) is added to 1 mol of the

aforementioned compound in which only the protective group for the hydroxyl group at the 2'-position has been eliminated, and the resultant mixture is allowed to react under stirring at 0 to 50°C for about 0.5 to about five hours.

Subsequently, reduction is carried out in an organic solvent (e.g., toluene or benzene) in the presence of a radical initiator such as azobisisobutyronitrile, optionally under an inert gas atmosphere such as argon or nitrogen. Specifically, a radical reducing agent such as tributyltin hydride or tris(trimethylsilyl)silane (1 to 10 mol, preferably 2 to 5 mol) is added to 1 mol of the aforementioned phenoxythiocarbonyl compound, and the resultant mixture is allowed to react under stirring at 50 to 150°C for about one to about five hours.

The protective group for the hydroxyl group of the thus-obtained compound [XIX] is removed, to thereby yield the compound of the present invention in which R^2 is hydrogen, and if desired, the compound is phosphorylated:



[XIX] [III]

(wherein P represents a protective group, X represents a halogen atom, R^1 represents an ethynyl group, a triethylsilylethynyl group, or a cyano group, and R^2 represents hydrogen, a phosphate residue, or a phosphate

derivative residue).

The protective group may be removed through a technique which is appropriately selected from among typical techniques (e.g., hydrolysis under acidic conditions, hydrolysis under alkaline conditions, treatment with tetrabutylammonium fluoride, and catalytic reduction) in accordance with the protective group employed.

A compound in which R^2 is a phosphate residue or a derivative thereof can be synthesized in a manner similar to that of the compound [I].

The compounds of the present invention may be isolated and purified through conventional methods, in appropriate combination, which are employed for isolating and purifying nucleosides and nucleotides; for example, recrystallization, ion-exchange column chromatography, and adsorption column chromatography. The thus-obtained compounds may further be converted to salts thereof in accordance with needs.

(3) Use

As shown in the below-described Test Examples, the compounds of the present invention exhibit excellent antiviral activity against retroviruses. Thus, compositions of the present invention containing one of the compounds of the present invention as an active ingredient find utility in the field of therapeutic drugs. Specifically, the compositions of the present invention are useful for the treatment of infectious diseases caused by a retrovirus, in particular, AIDS, which is caused by HIV infection.

The dose of the compounds of the present invention depends on and is determined in consideration of conditions such as the age, body weight, and type of disease of the patient; the severity of the disease; the drug tolerance; and the administration route. However, the daily dose is determined to fall typically within 0.00001-1,000 mg/kg, preferably 0.0001-100 mg/kg body weight. The compounds are administered in a single dose or divided doses.

Any administration route may be employed, and the compounds may be administered orally, parenterally, enterally, or topically.

When a pharmaceutical is prepared from the compounds of the present invention, the compounds are typically mixed with customarily employed additives, such as a carrier and an excipient. Examples of solid carriers include lactose, kaolin, sucrose, crystalline cellulose, corn starch, talc, agar, pectin, stearic acid, magnesium stearate, lecithin, and sodium chloride. Examples of liquid carriers include glycerin, peanut oil, polyvinylpyrrolidone, olive oil, ethanol, benzyl alcohol, propylene glycol, and water.

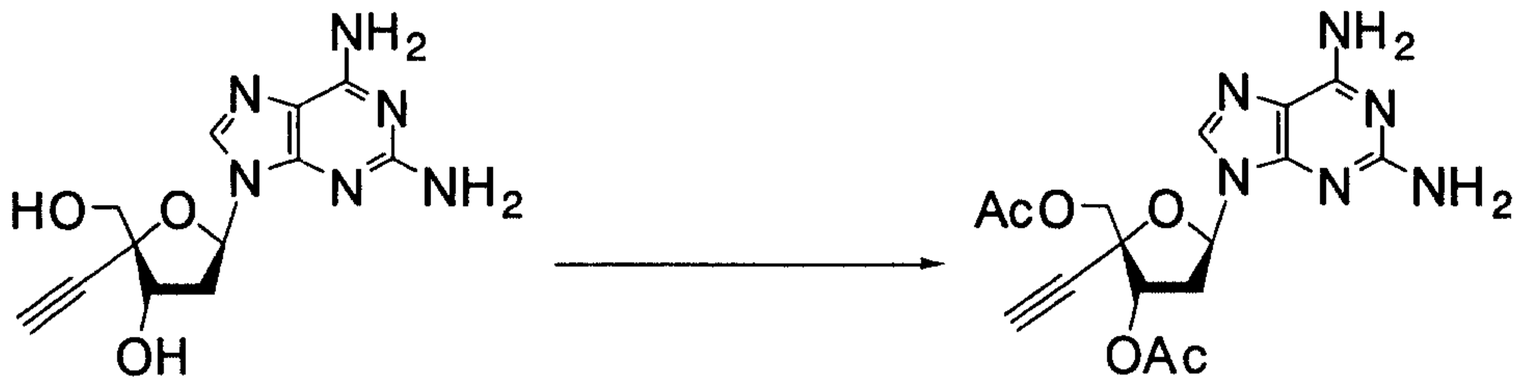
The product form is arbitrarily selected. When the carrier is solid, examples of product forms include tablets, powder, granules, capsules, suppositories, and troches, whereas when it is liquid, examples include syrup, emulsion, soft-gelatin-capsules, cream, gel, paste, spray, and injection.

EXAMPLES

The present invention will next be described in detail by way of examples including Synthesis Examples, Test Examples, and Drug Preparation Examples, which should not be construed as limiting the invention thereto.

Synthesis Example 1: Synthesis of 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine (compound 4)

(1) Synthesis of 9-(3,5-di-O-acetyl-2-deoxy-4-C-ethynyl-β-D-ribo-pentofuranosyl)-2,6-diaminopurine (compound 2)



1 2

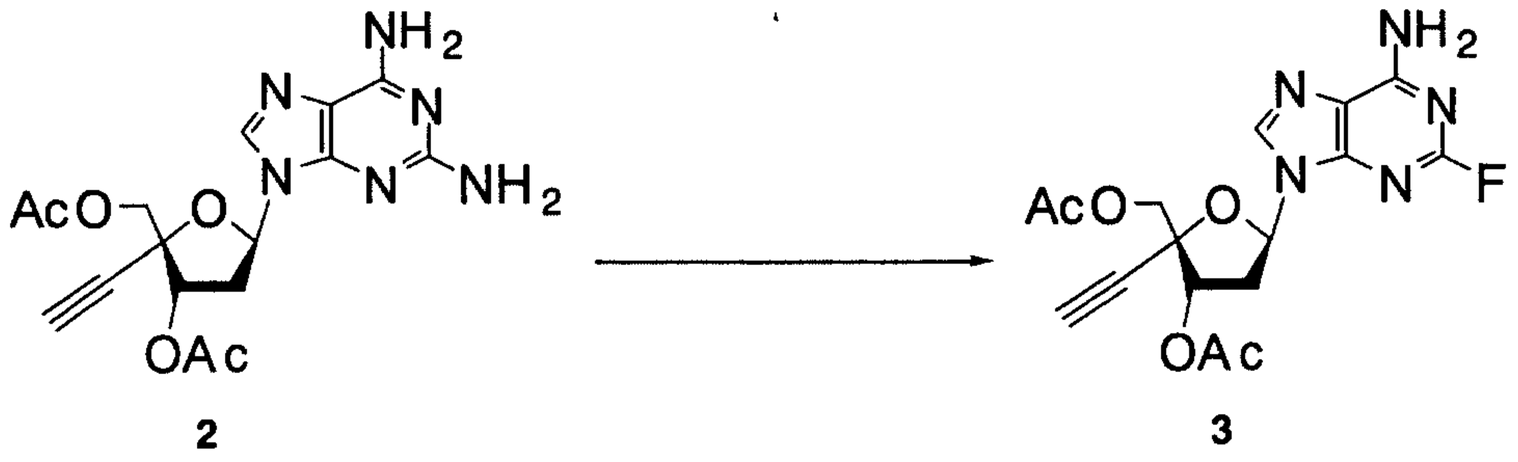
Compound 1 (0.33 g, 1.14 mmol) was suspended in acetonitrile (10.0 ml), and acetic anhydride (0.23 ml, 2.43 mmol), triethylamine (0.67 g, 4.81 mmol), and a small amount of 4-dimethylaminopyridine were added to the resultant suspension, followed by stirring at room temperature overnight.

The thus-precipitated crystals were filtered and dried, to thereby yield compound 2 (0.40 g, 1.07 mmol, 93.9%).

^{1}H-NMR(DMSO-d_6)δ7.94(1H, s, H-8), 6.76(2H, bs, NH_2), 6.27(1H, t, H-1', J=7.00), 5.84(2H, bs, NH_2), 5.60(1H, dd, H-3', J=4.00, 6.80), 4.46(1H, d, H-5'a, J=11.5), 4.21(1H, d, H-5'b, J=11.5), 3.74(1H, s, ethynyl) 3.12(1H, m, H-2'a), 2.52(1H, m,

H-2'b), 2.12, 2.03(each 3H, s, acetyl)

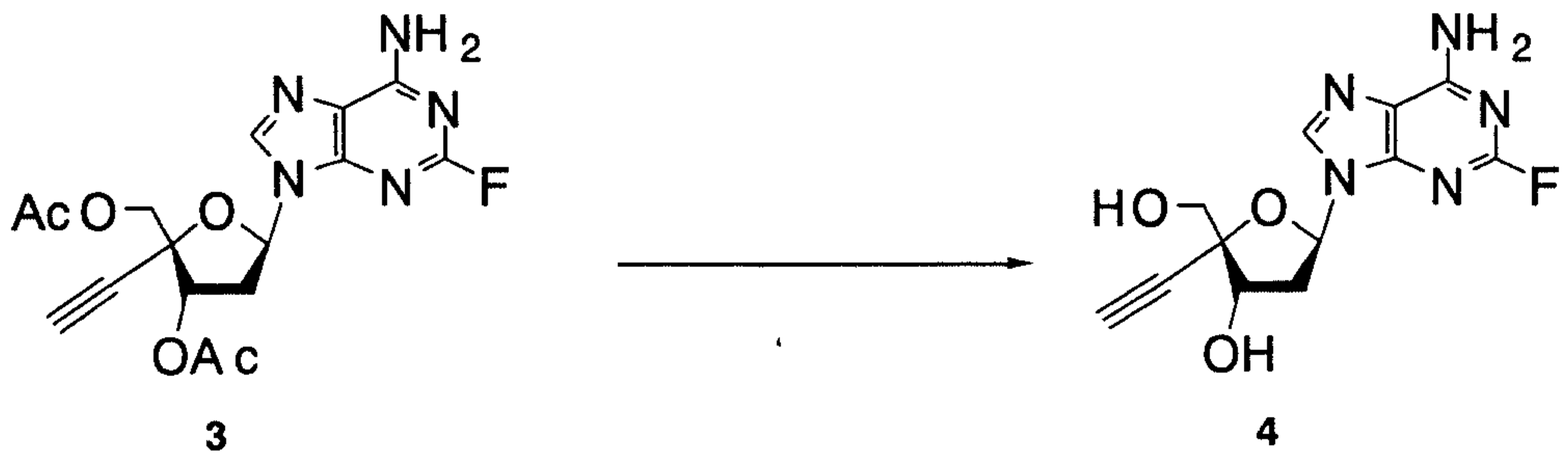
(2) Synthesis of 3',5'-di-O-acetyl-2'-deoxy-4'-C-ethynyl-2-fluoroadenosine (compound 3)



2 3

Compound 2 (450 mg, 1.20 mmol) was dissolved in 70% hydrogen fluoride-pyridine (5.00 ml), and t-butyl nitrite (0.194 ml, 1.63 mmol) was added to the resultant solution, followed by stirring at -10°C for one hour. Distilled water was added to the resultant mixture, and the resultant mixture was subjected to extraction with chloroform. The resultant organic layer was dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. A mixture of chloroform and methanol (50 : 1) was added to the resultant residue, and the thus-precipitated crystals were filtered and dried, to thereby yield compound 3 (240 mg, 0.64 mmol, 53.3%). ^{1}H-NMR(DMSO-d_6) δ8.34(1H, s, H-8), 7.94, 7.99(each 1H, bs, NH_2), 6.35(1H, t, H-1', J=6.80), 5.68(1H, dd, H-3', J=5.10, 7.05), 4.41(1H, d, H-5'a, J=11.6), 4.21(1H, d, H-5'b, J=11.6), 3.42(1H, s, ethynyl), 3.14(1H, m, H-2'a), 2.63(1H, m, H-2'b), 2.12, 2.00(each 3H, s, acetyl).

(3) Synthesis of 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine (compound 4)



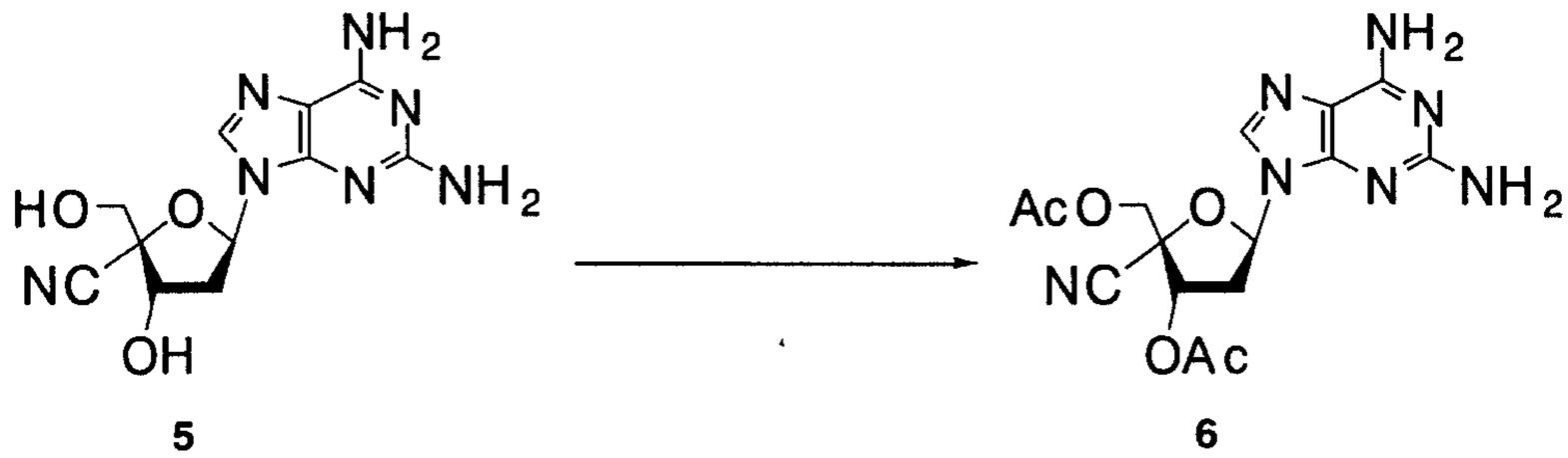
3 4

Compound 3 (200 mg, 0.53 mmol) was dissolved in methanol (7.00 ml), and 28% aqueous ammonia (5.00 ml) was added to the resultant solution, followed by stirring at room temperature for four hours. The resultant reaction mixture was concentrated under reduced pressure, and a mixture of chloroform and methanol (20 : 1) was added to the resultant residue. The thus-precipitated crystals were filtered, and then the resultant crystals were recrystallized from water, to thereby yield compound 4 (113 mg, 0.39 mmol, 73.6%).

^{1}H-NMR(DMSO-d_6) δ8.30(1H, s, H-8), 7.87, 7.84(each 1H, bs, NH_2), 6.24(1H, dd, H-1', J=5.05, 7.15), 5.57(1H, d, 3'-OH, J=5.50), 5.30(1H, t, 5'-OH, J=6.40), 4.57(1H, m, H-3'), 3.65(1H, m, H-5'a), 3.55(1H, m, H-5'b), 3.51(1H, s, ethynyl), 2.70(1H, m, H-2'a), 2.44(1H, m, H-2'b).

Synthesis Example 2: Synthesis of 4'-C-cyano-2'-deoxy-2-fluoroadenosine (compound 8)

(1) Synthesis of 9-(3,5-di-O-acetyl-4-C-cyano-2-deoxy-β-D-ribo-pentofuranosyl)-2,6-diaminopurine (compound 6)

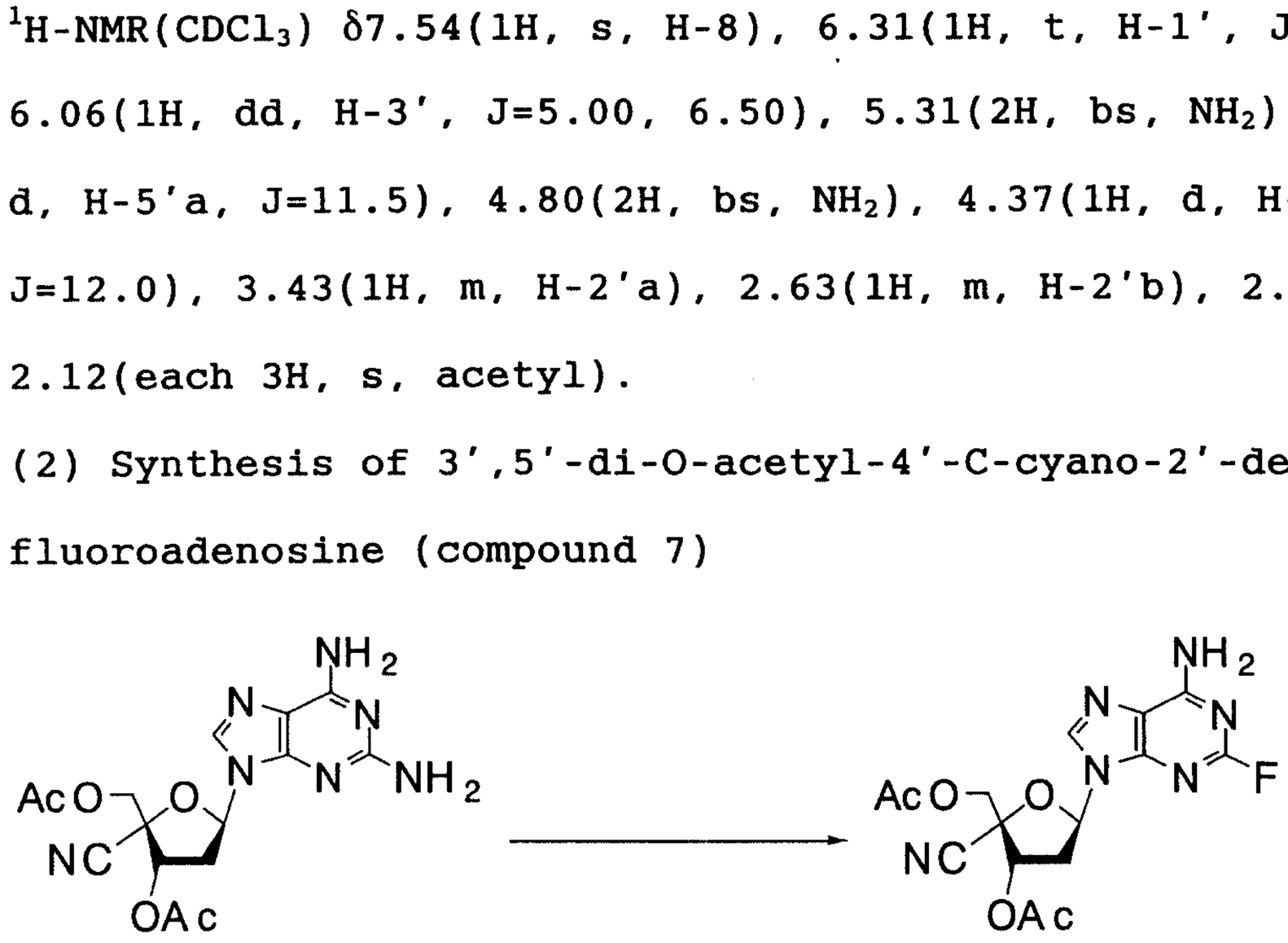


5 6

Compound 5 (122 mg, 0.418 mmol) was suspended in acetonitrile (5.00 ml), and acetic anhydride (118 μl, 1.25 mmol), triethylamine (352 μl, 2.51 mmol), and a small amount of 4-dimethylaminopyridine were added to the resultant suspension, followed by stirring at room temperature overnight. The thus-precipitated crystals were filtered and dried, to thereby yield compound 6 (128 mg, 0.341 mmol, 81.6%).

^{1}H-NMR($CDCl_3$) δ7.54(1H, s, H-8), 6.31(1H, t, H-1', J=7.00), 6.06(1H, dd, H-3', J=5.00, 6.50), 5.31(2H, bs, NH_2), 4.95(1H, d, H-5'a, J=11.5), 4.80(2H, bs, NH_2), 4.37(1H, d, H-5'b, J=12.0), 3.43(1H, m, H-2'a), 2.63(1H, m, H-2'b), 2.23, 2.12(each 3H, s, acetyl).

(2) Synthesis of 3',5'-di-O-acetyl-4'-C-cyano-2'-deoxy-2-fluoroadenosine (compound 7)



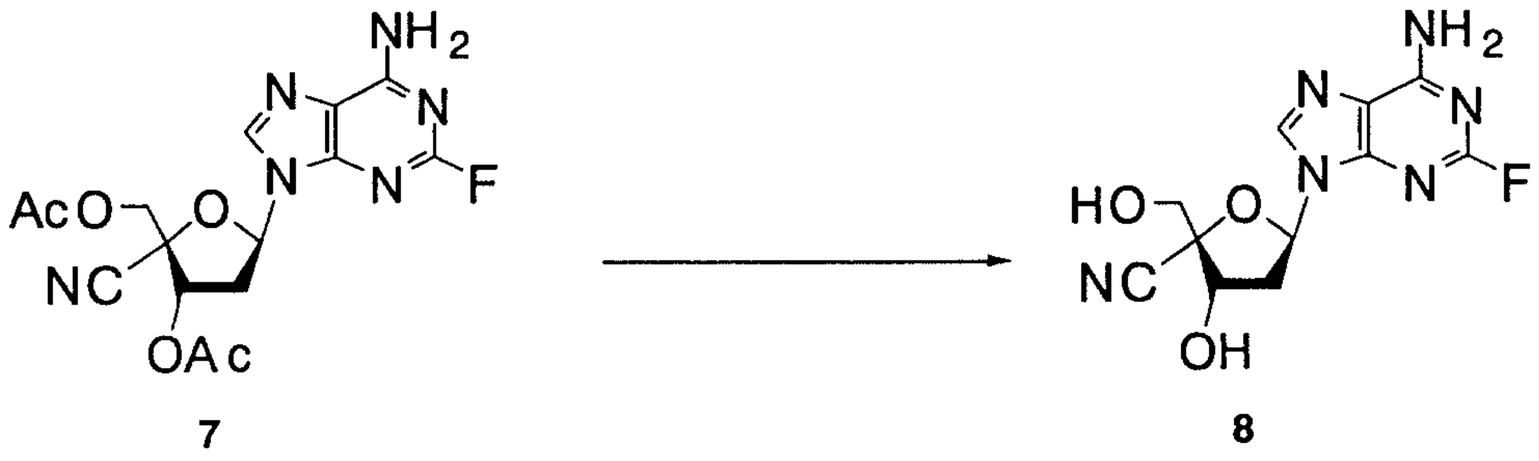
6 7

Compound 6 (118 mg, 0.314 mmol) was dissolved in 70% hydrogen fluoride-pyridine (2.30 ml), and t-butyl nitrite

(50.0 μl, 0.427 mmol) was added to the resultant solution, followed by stirring at -10°C for three hours. To the resultant mixture, t-butyl nitrite (10.0 μl, 85 μmol) was further added, and then the mixture was further stirred at -10°C for one hour. After a saturated aqueous solution of sodium bicarbonate was added to the resultant mixture, the resultant mixture was subjected to extraction with ethyl acetate, and the resultant organic layer was washed with a saturated aqueous solution of sodium chloride. The resultant organic layer was dried over magnesium sulfate, and then concentrated under reduced pressure. The resultant residue was dissolved in ethanol under heating, followed by cooling. The thus-precipitated crystals were filtered and dried, to thereby yield compound 7 (53.7 mg, 0.14 mmol, 45.2%).

^{1}H-NMR(DMSO-d_6)δ8.35(1H, s, H-8), 8.00, 7.92(each 1H, bs, NH_2), 6.54(1H, t, H-1', J=7.00), 5.83(1H, dd, H-3', J=4.00, 6.50), 4.63(1H, d, H-5'a, J=11.5), 4.44(1H, d, H-5'b, J=12.0), 3.26(1H, m, H-2'a), 2.73(1H, m, H-2'b), 2.18, 2.05(each 3H, s, acetyl).

(3) Synthesis of 4'-C-cyano-2'-deoxy-2-fluoroadenosine (compound 8)



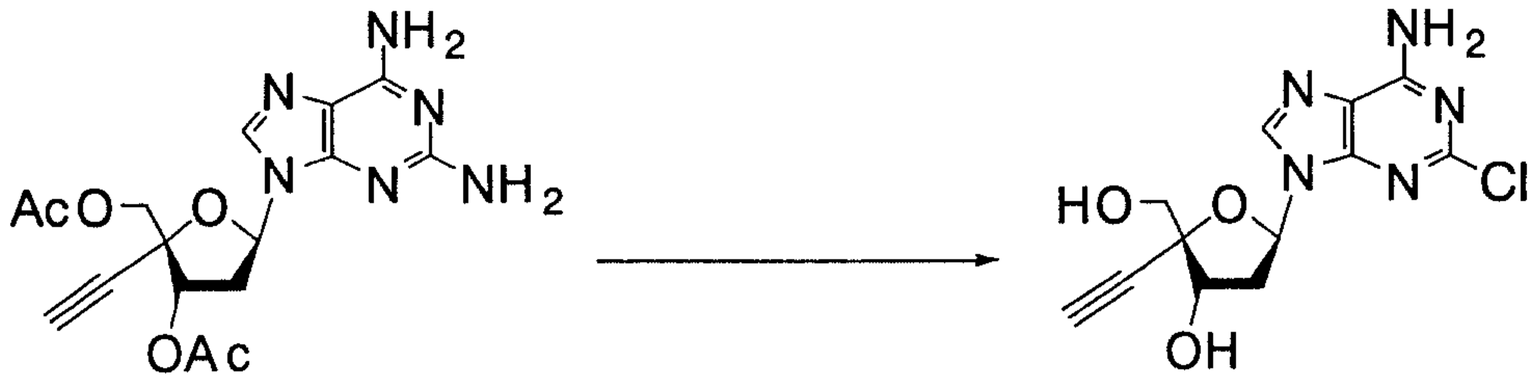
7 8

Compound 7 (53.7 mg, 0.142 mmol) was dissolved in

methanol (1.90 ml), and 28% aqueous ammonia (1.30 ml) was added to the resultant solution, followed by stirring at room temperature for 30 minutes. The resultant reaction mixture was concentrated under reduced pressure, and then the resultant residue was purified by means of silica gel column chromatography (silica gel 10 ml, hexane/ethyl acetate (5 : 1), ethyl acetate, ethyl acetate/methanol (10 : 1)), to thereby yield compound 8 (30.2 mg, 0.10 mmol, 72.3%).

^{1}H-NMR(DMSO-d_6) δ8.31(1H, s, H-8), 7.93, 7.82(each 1H, bs, NH_2), 6.43(1H, t, H-1', J=7.00), 6.36(1H, bs, 3'-OH), 5.74(1H, bs, 5'-OH), 4.70(1H, t, H-3', J=5.50), 3.80(1H, d, H-5'a, J=12.0), 3.65(1H, d, H-5'b, J=12.0), 2.93(1H, m, H-2'a), 2.47(1H, m, H-2'b).

Synthesis Example 3: Synthesis of 2-chloro-2'-deoxy-4'-C-ethynyladenosine (compound 9)

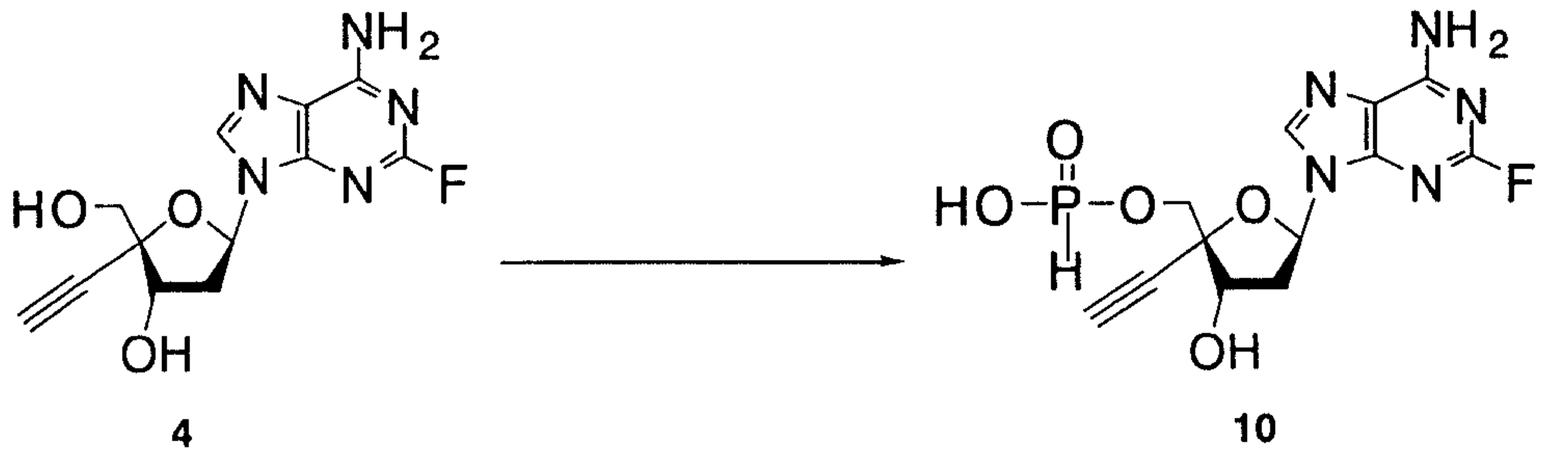


Benzyltriethylammonium nitrite (1.04 g, 4.36 mmol) was dissolved in dichloromethane (24.0 ml), and acetyl chloride (0.40 ml, 5.63 mmol) was added to the resultant solution, followed by stirring at 0°C for 30 minutes. To the resultant solution, a solution of compound 2 (340 mg, 0.91 mmol) in dichloromethane (6.00 ml) was added, and the resultant mixture was stirred at 0°C for three hours. The resultant

reaction mixture was diluted with chloroform, and subsequently the resultant organic layer was washed with water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. To the resultant residue, 28% aqueous ammonia (10.0 ml) and methanol (15.0 ml) were added, and the resultant mixture was stirred at room temperature overnight. Thereafter, the resultant reaction mixture was concentrated under reduced pressure, and the resultant residue was purified by means of silica gel column chromatography (silica gel 50 ml, chloroform : methanol = 20 : 1 to 10 : 1). The thus-purified residue was further purified by means of ODS column chromatography (ODS 50 ml, 5 to 10 to 15 to 20% acetonitrile), to thereby yield compound 9 (39.2 mg, 0.13 mmol, 14.3%).

^{1}H-NMR(DMSO-d_6) δ8.34(1H, s, H-8), 7.84(2H, bs, NH_2), 6.27(1H, dd, H-1', J=5.00, 7.00), 5.60(1H, d, 3'-OH, J=5.00), 5.33(1H, t, 5'-OH, J=6.00), 4.56(1H, m, H-3'), 3.64(1H, m, H-5'a), 3.56(1H, m, H-5'b), 3.52(1H, s, ethynyl), 2.68(1H, m, H-2'a), 2.45(1H, m, H-2'b).

Synthesis Example 4: Synthesis of 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine 5'-H-phosphonate (compound 10)



4 10

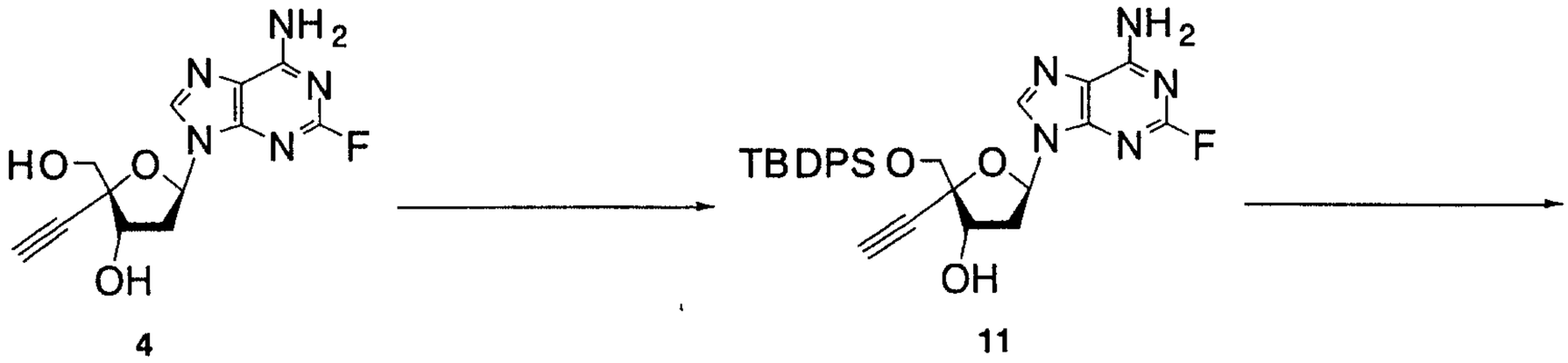
Compound 4 (50.0 mg, 0.171 mmol) was dissolved in

pyridine (2.00 ml), and phosphonic acid (21.0 mg, 0.25 mmol) and dicyclohexyl carbodiimide (106 mg, 0.51 mmol) were added to the resultant solution, followed by stirring at room temperature for five hours. The resultant precipitate was removed through filtration, and then the filtrate was concentrated under reduced pressure. The resultant residue was partitioned with water and chloroform. The resultant aqueous layer was concentrated under reduced pressure, and the thus-obtained residue was purified by means of preparative thin-layer chromatography (isopropanol : 28% aqueous ammonia : water = 7 : 1 : 2). The resultant residue was co-boiled with acetonitrile, and then treated with methanol and ether, to thereby yield a powdery compound (compound 10; 6.3 mg, 17.6 μmol, 10.3%).

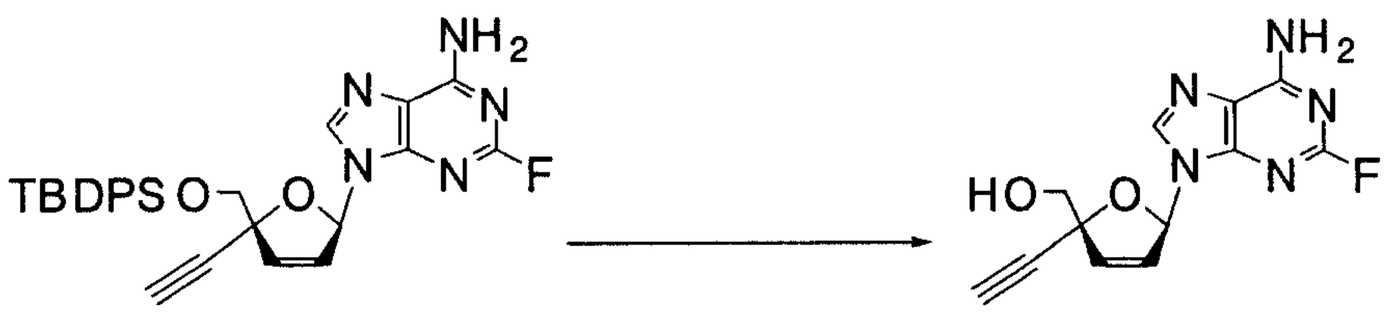
^{1}H-NMR(D_2O) δ8.13(1H, s, H-8), 6.49(1H, d, H-P, J=645), 6.25(1H, dd, H-1', J=5.00, 7.50), 3.96(2H, m, H-5'), 2.75, 2.59(each 1H, m, H-2').

^{31}P-NMR(D_2O)δ6.45.

Synthesis Example 5: Synthesis of 2',3'-didehydro-2',3'-dideoxy-4'-C-ethynyl-2-fluoroadenosine (compound 13)



4 11



12 13

Compound 4 (0.28 g, 0.95 mmol) was dissolved in dimethylformamide (7.00 ml), and t-butylchlorodiphenylsilane (0.50 ml, 1.92 mmol) and imidazole (0.26 g, 3.82 mmol) were added to the resultant solution, followed by stirring at room temperature overnight. After methanol was added to the resultant reaction mixture, the resultant mixture was concentrated under reduced pressure, and the resultant residue was partitioned with ethyl acetate and water. The resultant organic layer was dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The thus-obtained residue was purified by means of silica gel column chromatography (silica gel 100 ml, chloroform : methanol = 20 : 1), to thereby yield crude compound 11 (0.38 g).

The crude compound 11 (0.38 g) was dissolved in dichloromethane (10.0 ml), and trifluoromethanesulfonic anhydride (0.14 ml, 0.83 mmol) and pyridine (0.14 g, 1.71 mmol) were added to the resultant solution at -10°C, followed

by stirring at the same temperature for two hours. A saturated aqueous solution of sodium bicarbonate was added to the resultant reaction mixture, and then the resultant mixture was subjected to extraction with chloroform. The resultant organic layer was dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The thus-obtained crude triflate was employed in the next reaction without purification thereof.

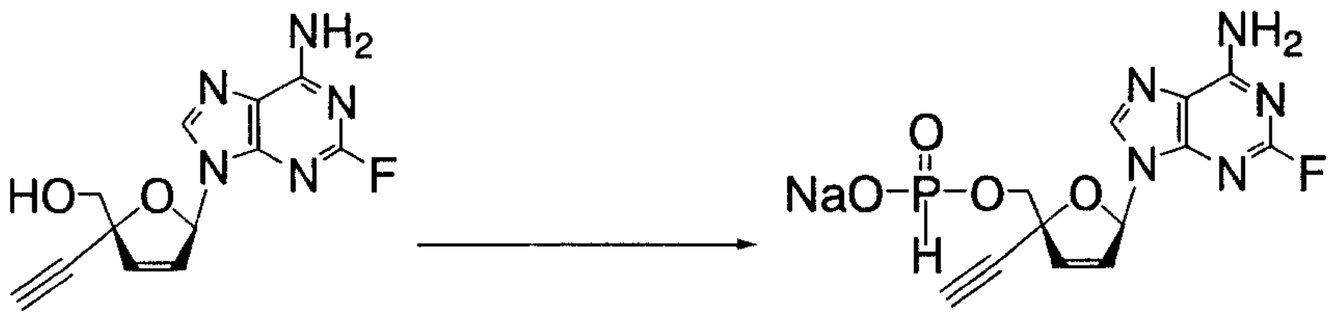
The crude triflate was dissolved in dry tetrahydrofuran (20.0 ml), and a solution of 1-M sodium hexamethyldisilazide in tetrahydrofuran (2.50 ml, 2.50 mmol) was added to the resultant solution in an argon atmosphere at -78°C, followed by stirring at the same temperature for two hours. Thereafter, the resultant reaction mixture was allowed to warm to room temperature, and then stirred overnight. Water was added to the resultant reaction mixture, and then the resultant mixture was subjected to extraction with ethyl acetate. The resultant organic layer was dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The thus-obtained residue was purified by means of silica gel column chromatography (silica gel 50 ml, chloroform : methanol = 50 : 1 to 20 : 1), to thereby yield crude compound 12 (0.20 g).

The thus-obtained crude compound 12 was dissolved in tetrahydrofuran (10.0 ml), and a solution of 1-M tetrabutylammonium fluoride in tetrahydrofuran (0.59 ml, 0.59 mmol) was added to the resultant solution, followed by

stirring at room temperature for 30 minutes. The resultant reaction mixture was concentrated under reduced pressure, and then a mixture of chloroform and methanol (10 : 1) was added to the thus-concentrated mixture. The thus-precipitated crystals were filtered, to thereby yield compound 13 (52.0 mg, 0.19 mmol, 20.0% from compound 4).

^{1}H-NMR(DMSO-d_6) δ8.08(1H, s, H-8), 7.84(2H, bs, NH_2), 6.90(1H, t, H-1', J=1.50), 6.43(1H, dd, H-3', J=2.00, 6.00), 6.27(1H, dd, H-3', J=1.00, 6.00), 5.37(1H, t, 5'-OH, J=6.00), 3.71(1H, s, ethynyl), 3.67(1H, dd, H-5'a, J=6.00, 12.0), 3.57(1H, dd, H-5'b, J=6.00, 12.0).

Synthesis Example 6: Synthesis of 2',3'-didehydro-2',3'-dideoxy-4'-C-ethynyl-2-fluoroadenosine 5'-H-phosphonate (compound 14)



13 14

Compound 13 (13.0 mg, 0.047 mmol) was dissolved in pyridine (0.7 ml), and phosphonic acid (7.7 mg, 0.094 mmol) and dicyclohexyl carbodiimide (29.2 mg, 0.14 mmol) were added to the resultant solution, followed by stirring at room temperature for one hour. The resultant reaction mixture was concentrated under reduced pressure, and the thus-obtained residue was purified by means of ODS column chromatography (ODS 10 ml, 0 to 1% acetonitrile). The resultant residue was applied to a Dowex 50Wx8 columm (Na type) and eluted. The

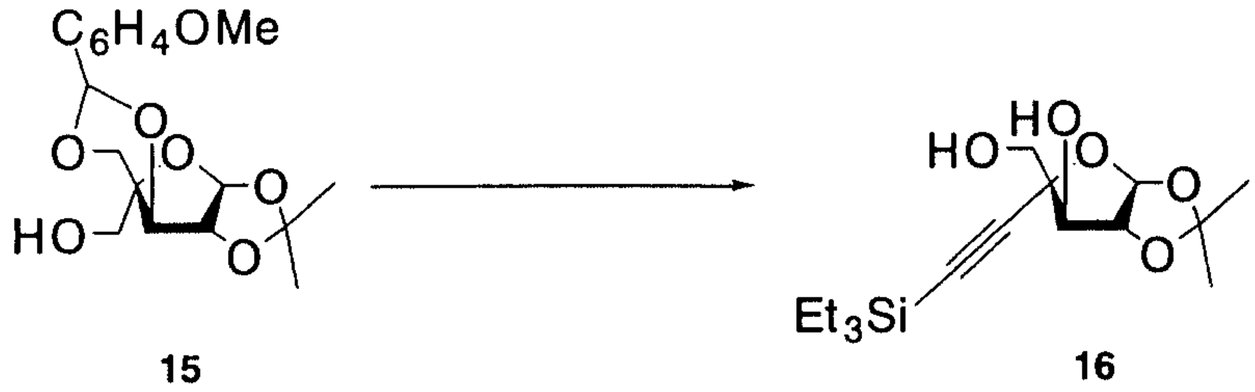
eluate was concentrated, and the resultant residue was treated with methanol and ether, to thereby yield a powdery compound (compound 14; 4.3 mg, 12 μmol, 25.5%).

^{1}H-NMR(MeOD) δ8.30(1H, s, H-8), 6.69(1H, d, H-P, J=625), 7.02(1H, bt, H-1'), 6.48(1H, dd, H-2', J=2.00, 5.50), 6.22(1H, dd, H-3', J=1.00, 5.50), 4.18(1H, dd, H-5'a, J=7.50, 11.0), 3.99(1H, dd, H-5'b, J=7.50, 11.0).

^{31}P-NMR(MeOD) δ4.11.

Synthesis Example 7: Synthesis of 2',3'-dideoxy-4'-C-ethynyl-2-fluoroadenosine (compound 23)

(1) Synthesis of 1,2-O-isopropylidene-4-C-triethylsilylethynyl-α-D-xylo-pentofuranose (compound 16)



15 16

Oxalyl chloride (0.54 ml, 6.19 mmol) was dissolved in dichloromethane (10.0 ml), and then dimethyl sulfoxide (0.90 ml, 12.7 mmol) was added dropwise to the resultant solution at -60°C, followed by stirring at the same temperature for 15 minutes. A solution of compound 15 (1.06 g, 3.13 mmol, Biosci. Biotech. Biochem., 57, 1433-1438 (1993)) in dichloromethane (15.0 ml) was added dropwise to the resultant mixture, followed by stirring at -60°C for 30 minutes. After triethylamine (1.86 ml, 13.3 mmol) was added thereto, the resultant reaction mixture was allowed to warm to room temperature, followed by stirring for 30 minutes. The

reaction mixture was diluted with chloroform, and then washed with water. The thus-obtained organic layer was dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The thus-obtained crude aldehyde was employed in the next reaction without purification thereof.

The crude aldehyde was dissolved in dichloromethane (40.0 ml), and carbon tetrabromide (2.08 g, 6.27 mmol) and triphenylphosphine (3.28 g, 12.5 mmol) were added to the resultant solution at 0°C, followed by stirring at room temperature for one hour. After triethylamine (2.60 ml, 18.7 mmol) was added to the resultant reaction mixture, the resultant mixture was diluted with chloroform, and the resultant organic layer was washed with water. The organic layer was dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The resultant residue was purified by means of silica gel column chromatography (silica gel 100 ml, hexane : ethyl acetate = 3 : 1), to thereby yield a crude dibromoethene (1.42 g).

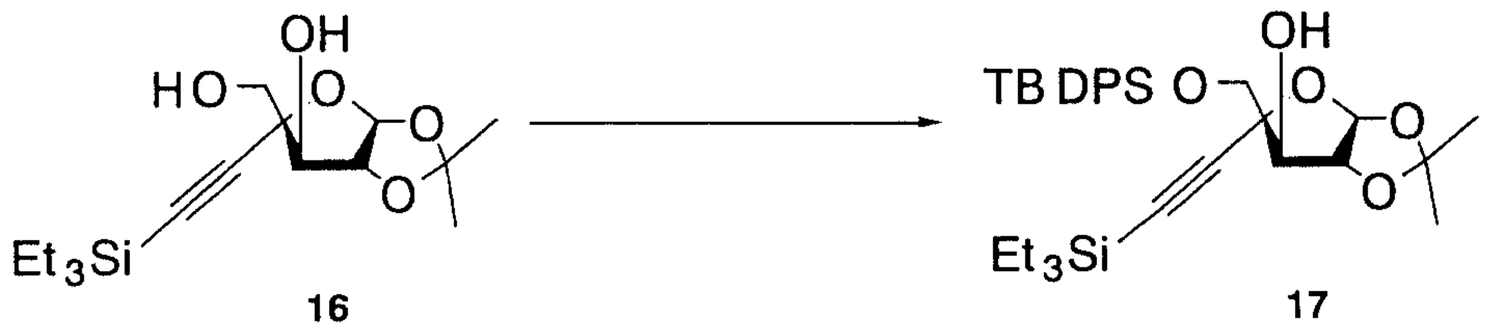
The crude dibromoethene (1.42 g, 2.89 mmol) was dissolved in dry tetrahydrofuran (20.0 ml), and a solution of 2.2-M methyllithium in ether (4.49 ml, 9.88 mmol) was added to the resultant solution in an argon atmosphere at -10°C, followed by stirring at the same temperature for five minutes. Chlorotriethylsilane (0.95 ml, 5.66 mmol) was added to the resultant mixture, and the mixture was further stirred for 30 minutes. After a saturated aqueous solution of ammonium chloride was added to the resultant reaction mixture, the

resultant mixture was stirred, and then subjected to extraction with ethyl acetate. The resultant organic layer was dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure, to thereby yield a crude alkyne.

The crude alkyne was dissolved in acetic acid (80.0 ml), and water (20.0 ml) was added to the resultant solution, followed by stirring at room temperature overnight. The resultant reaction mixture was concentrated under reduced pressure, and the resultant residue was co-boiled with toluene. The resultant residue was purified by means of silica gel column chromatography (silica gel 50 ml, hexane : ethyl acetate = 3 : 1), to thereby yield compound 16 (0.70 g, 2.13 mmol, 64.4%).

^{1}H-NMR($CDCl_3$) δ6.00(1H, d, H-1, J=3.50), 4.60(1H, d, H-2, J=4.00), 4.58(1H, d, H-3, J=5.00), 3.96-3.91(3H, m, H-5 and 3-OH), 2.50(1H, t, 5-OH), 1.64, 1.33(each 3H, s, acetonide), 0.97(9H, t, Et, J=8.00), 0.59(6H, q, Et, J=8.00).

(2) Synthesis of 5-O-t-butyldiphenylsilyl-1,2-O-isopropylidene-4-C-triethylsilylethynyl-α-D-xylo-pentofuranose (compound 17)

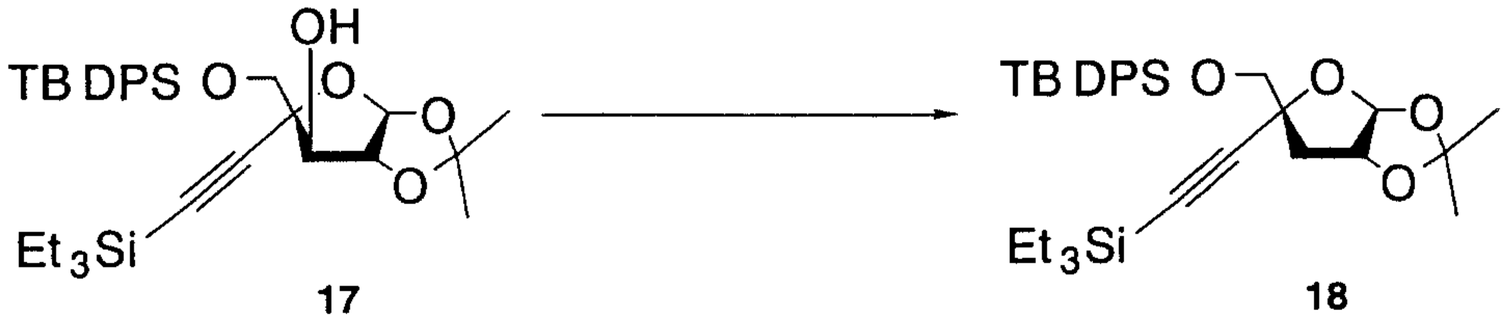


Compound 16 (0.70 g, 2.13 mmol) was dissolved in dimethylformamide (3.50 ml), and t-butylchlorodiphenylsilane

(0.66 ml, 2.54 mmol) and imidazole (0.35 g, 5.14 mmol) were added to the resultant solution, followed by stirring overnight. After methanol was added to the resultant reaction mixture, and the resultant mixture was concentrated under reduced pressure, the thus-obtained residue was dissolved in ethyl acetate. The resultant organic layer was washed with water, and then dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The resultant residue was purified by means of silica gel column chromatography (silica gel 100 ml, hexane : ethyl acetate = 5 : 1), to thereby yield compound 17 (1.10 g, 1.94 mmol, 91.1%).

^{1}H-NMR($CDCl_3$) δ7.72-7.36(10H, s, aromatic), 6.02(1H, d, H-1, J=3.50), 4.66(1H, d, H-3, J=5.50), 4.63(1H, d, H-1, J=4.00), 4.05(1H, d, H-5a, J=10.5), 3.99(1H, d, 3-OH, J=5.50), 3.93(1H, d, H-5'b, J=10.5), 1.65, 1.35(each 3H, s, acetonide), 1.06(9H, s, t-Bu), 0.93(9H, t, Et, J=8.00), 0.56(6H, q, Et, J=8.00).

(3) Synthesis of 5-O-t-butyldiphenylsilyl-3-deoxy-1,2-O-isopropylidene-4-C-triethylsilylethynyl-α-D-xylo-pentofuranose (compound 18)



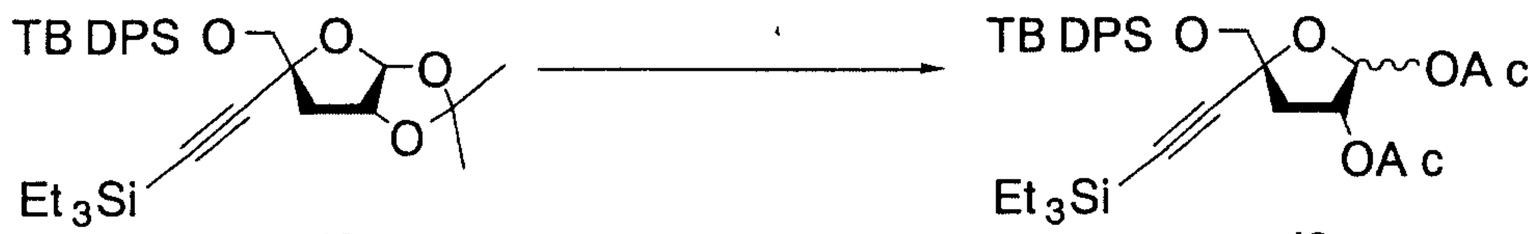
Compound 17 (1.10 g, 1.94 mmol) was dissolved in acetonitrile (20.0 ml), and phenyl chlorothionoformate (0.40 ml, 2.89 mmol) and 4-dimethylaminopyridine (0.71 g, 5.81

mmol) were added to the resultant solution, followed by stirring at room temperature for three hours. The resultant reaction mixture was diluted with ethyl acetate, and then the resultant organic layer was washed with 0.1-N hydrochloric acid and a saturated aqueous solution of sodium bicarbonate. The resultant organic layer was dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The thus-obtained crude thiocarbonate was employed in the next reaction without purification thereof.

The crude thiocarbonate was co-boiled with toluene three times, and then dissolved in toluene (30.0 ml), followed by degassing under reduced pressure. Tributyltin hydride (2.61 ml, 9.70 mmol) and a small amount of azobis(isobutyronitrile) were added to the resultant solution in an argon atmosphere at 80°C, and the resultant mixture was stirred under the same conditions for one hour. The resultant reaction mixture was concentrated under reduced pressure, and then the thus-obtained residue was purified by means of silica gel column chromatography (silica gel 100 ml, hexane : ethyl acetate = 10 : 1), to thereby yield compound 18 (1.07 g, 1.94 mmol, quant.).

^{1}H-NMR($CDCl_3$) δ7.69-7.38(10H, m, aromatic), 5.90(1H, d, H-1, J=4.00), 4.85(1H, t, H-2, J=5.00), 3.82(1H, d, H-5a, J=11.0), 3.58(1H, d, H-5b, J=10.5), 2.64(1H, dd, H-3a, J=6.00, 14.0), 2.40(1H, d, H-3b, J=14.0), 1.68, 1.36(each 3H, s, acetonide), 1.04(9H, s, t-Bu)0.92(9H, t, Et, J=8.00), 0.54(6H, q, Et, J=8.00).

(4) Synthesis of 1,2-di-O-acetyl-5-O-t-butyldiphenylsilyl-3-deoxy-4-C-triethylsilylethynyl-D-xylo-pentofuranose (compound 19)



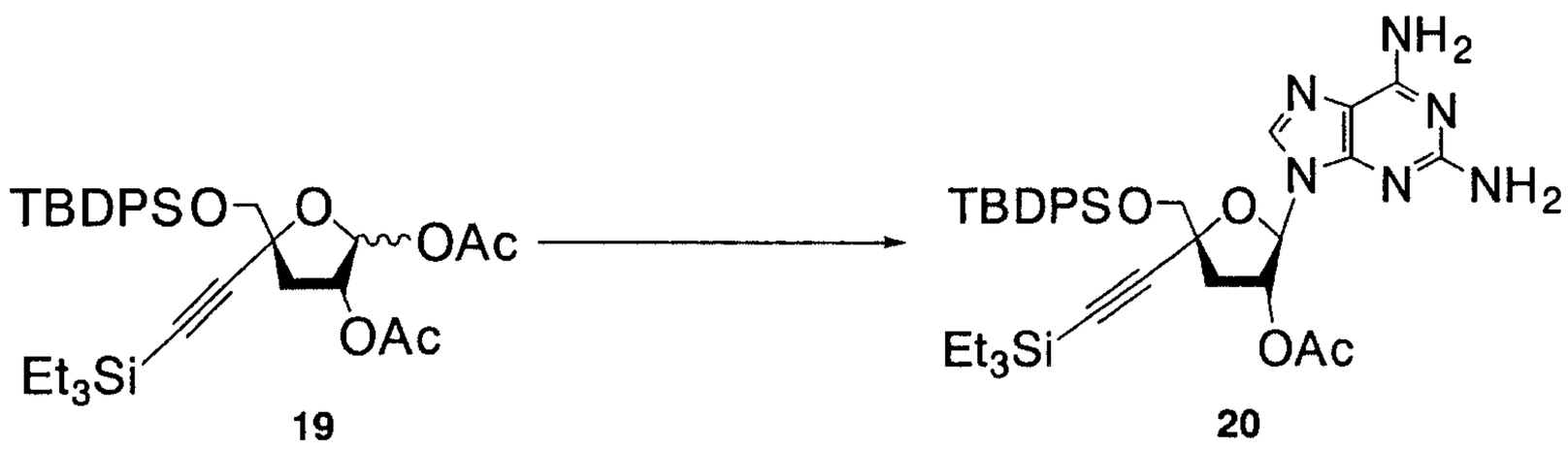
18 19

Compound 18 (1.07 g, 1.94 mmol) was dissolved in 80% acetic acid (100 ml), and trifluoroacetic acid (10.0 ml) was added to the resultant solution, followed by stirring at 40°C for three hours. The resultant reaction mixture was concentrated under reduced pressure, and then the thus-obtained residue was co-boiled with toluene. The resultant residue was purified by means of silica gel column chromatography (silica gel 100 ml, hexane : ethyl acetate = 4 : 1). The resultant residue was dissolved in pyridine (20.0 ml), and acetic anhydride (0.49 ml) was added to the resultant solution, followed by stirring at room temperature overnight. The resultant reaction mixture was concentrated under reduced pressure, and then the thus-obtained residue was co-boiled with toluene. The resultant residue was purified by means of silica gel column chromatography (silica gel 100 ml, hexane : ethyl acetate = 5 : 1), to thereby yield compound 19 (0.75 g, 1.26 mmol, 64.9%).

^{1}H-NMR($CDCl_3$) δ7.71-7.37(10H, m, aromatic), 6.44(0.3H, d, H-1-alpha, J=4.50), 6.30(0.7H, s, H-1-beta), 5.36(0.3H, m, H-2-alpha), 5.19(0.7H, d, H-2-beta, J=5.50), 3.77, 3.74(each 0.7H, d, H-5-beta, J=10.0), 3.76, 3.62(each 0.3H, d, H-5-alpha,

J=11.0), 2.86(0.3H, dd, H-3a-alpha, J=8.50, 12.5), 2.72(0.7H, dd, H-3a-beta, J=5.50, 14.0), 2.39(0.3H, dd, H-3b-alpha, J=10.0, 12.5), 2.33(0.7H, d, H-3b-beta, J=14.0), 2.11, 2.08(each 0.9H, s, acetyl-alpha), 2.10, 1.80(each 2.1H, s, acetyl-beta), 1.074(6.3H, s, t-Bu-beta), 1.067(2.7H, s, t-Bu-alpha), 0.97(6.3H, t, Et-beta, J=8.00), 0.94(2.7H, t, Et-alpha, J=8.00), 0.58(4.2H, q, Et-beta, J=8.00), 0.54(1.8H, q, Et-alpha, J=8.00).

(5) Synthesis of 9-(2-O-acetyl-5-O-t-butyldiphenylsilyl-3-deoxy-4-C-triethylsilylethynyl-β-D-xylo-pentofuranosyl)-2,6-diaminopurine (compound 20)

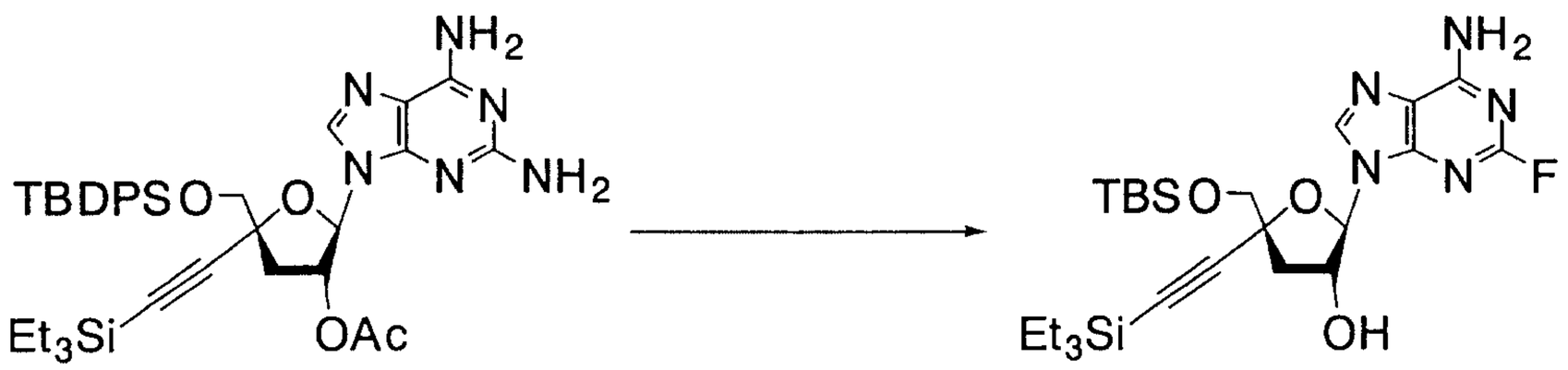


2,6-Diaminopurine (1.21 g, 8.06 mmol) was suspended in acetonitrile (24.0 ml), and N,O-bis(trimethylsilyl)acetamide (11.9 ml, 48.1 mmol) was added to the resultant suspension, followed by stirring at 80°C for three hours. The resultant solution was concentrated under reduced pressure, and then the thus-obtained residue was co-boiled with 1,2-dichloroethane three times. To the resultant residue, a solution of compound 19 (2.39 g, 4.02 mmol) in 1,2-dichloroethane (24.0 ml), and trimethylsilyl trifluoromethanesulfonate (3.05 ml, 16.9 mmol) were added, and the resultant mixture was stirred in an argon atmosphere

at 50°C for five hours and at 80°C for 10 hours. After a saturated aqueous solution of sodium bicarbonate was added to the resultant mixture, and then the mixture was stirred, the resultant solution was filtered through celite. The resultant organic layer was dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The thus-obtained residue was purified by means of silica gel column chromatography (silica gel 300 ml, chloroform : methanol = 20 : 1). The thus-purified residue was crystallized from hexane and ethyl acetate, to thereby yield compound 20 (1.60 g, 2.34 mmol, 58.2%).

^{1}H-NMR($CDCl_3$) δ7.67-7.33(10H, m, aromatic), 6.13(1H, d, H-1', J=3.50), 5.77(1H, md, H-2'), 5.28(2H, bs, NH_2), 4.49(2H, bs, NH_2), 3.67(1H, d, H-5'a, J=10.5), 3.79(1H, d, H-5'b, J=11.0), 3.18(1H, dd, H-3'a, J=7.50, 14.0), 2.37(1H, dd, H-3'b, J=3.00, 14.0), 1.06(9H, s, t-Bu), 0.99(9H, t, Et, J=8.00), 0.61(6H, q, Et, J=8.00).

(6) Synthesis of 5'-O-t-butyldimethylsilyl-3'-deoxy-4'-C-triethylsilylethynyl-2-fluoroadenosine (compound 21)



20 21

Compound 20 (100 mg, 0.146 mmol) was dissolved in pyridine (3.00 ml), and hydrogen fluoride-pyridine (7.00 ml) and t-butyl nitrite (160 μl, 1.34 mmol) were added to the

resultant solution at -15°C, followed by stirring at the same temperature for 30 minutes. After water was added to the resultant reaction mixture, the resultant mixture was subjected to extraction with ethyl acetate. The resultant organic layer was washed with a saturated aqueous solution of sodium bicarbonate, and then dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The thus-obtained residue was purified by means of silica gel column chromatography (silica gel 300 ml, chloroform : methanol = 100 : 1 to 20 : 1). The resultant residue (48.9 mg) was dissolved in dichloromethane (1.30 ml), and t-butyldimethylsilyl trifluoromethanesulfonate (36.0 µl, 0.157 mmol) and collidine (44.1 µl, 0.0331 mmol) were added to the resultant solution at 0°C, followed by stirring at the same temperature for 50 minutes. Water was added to the resultant reaction mixture, and the resultant mixture was subjected to extraction with chloroform. The resultant organic layer was washed with 0.01-N hydrochloric acid and a saturated aqueous solution of sodium bicarbonate, and then dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resultant residue was dissolved in dioxane (3.00 ml), and 28% aqueous ammonia (0.30 ml) was added to the resultant solution, followed by stirring at room temperature for 30 minutes. The resultant reaction mixture was concentrated under reduced pressure, and then the thus-obtained residue was dissolved in methanol (1.20 ml), and 28% aqueous ammonia (0.80 ml) was added to the resultant solution, followed by

stirring at room temperature for two hours. The resultant reaction mixture was concentrated under reduced pressure, and the thus-precipitated crystals were recovered through filtration, to thereby yield compound 21 (34.0 mg, 0.0652 mmol, 44.7%).

^{1}H-NMR($CDCl_3$) δ8.04(1H, s, H-8), 5.99(1H, d, H-1', J=4.00), 5.80(2H, bs, NH_2), 4.73(1H, m, H-2'), 4.23(1H, d, 2'-OH, J=5.00), 3.88(1H, d, H-5'a, J=11.0), 3.70(1H, d, H-5'b, J=11.0), 2.82(1H, dd, H-3'a, J=7.50, 13.0), 2.42(1H, dd, H-3'b, J=6.50, 13.0), 1.01(9H, t, Et, J=8.00), 0.80(9H, s, t-Bu), 0.64(6H, q, Et, J=8.00), 0.039, -0.013(each 3H, s, Me).

(7) Synthesis of 5'-O-t-butyldimethylsilyl-2',3'-dideoxy-4'-C-triethylsilylethynyl-2-fluoroadenosine (compound 22)



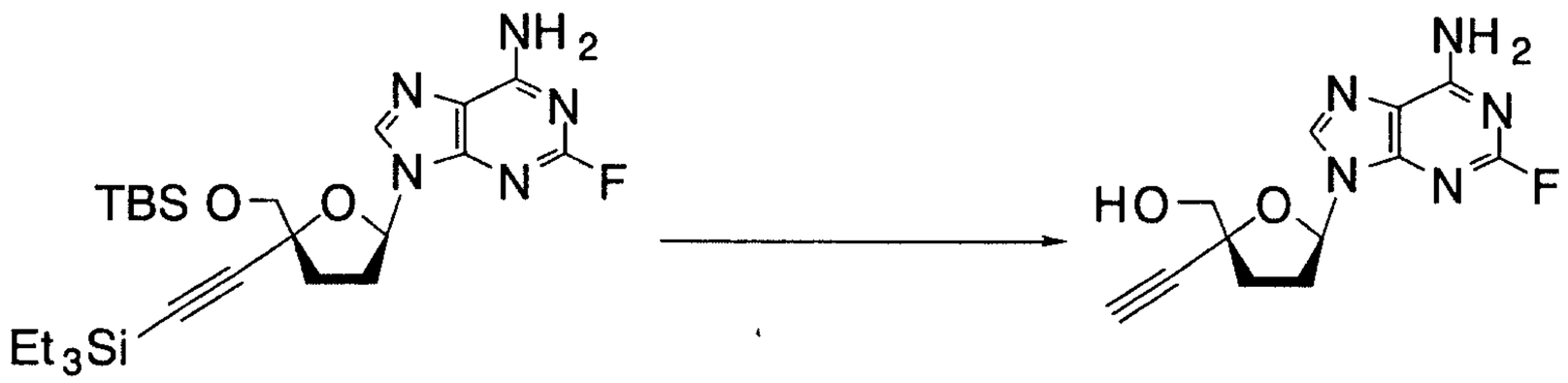
Compound 21 (32.0 mg, 0.061 mmol) was co-boiled with acetonitrile three times, and then dissolved in acetonitrile (1.00 ml). To the resultant solution, phenyl chlorothionoformate (12.7 μl, 0.092 mmol) and 4-dimethylaminopyridine (22.5 mg, 0.180 mmol) were added, and the resultant mixture was stirred at room temperature for one hour. The resultant reaction mixture was diluted with ethyl acetate, and then the thus-obtained organic layer was washed with 0.01-N hydrochloric acid and a saturated aqueous solution of sodium bicarbonate, and dried over anhydrous

magnesium sulfate. The resultant organic layer was concentrated under reduced pressure, and the thus-obtained crude thiocarbonate was employed in the next reaction without purification thereof.

The crude thiocarbonate was co-boiled with toluene three times, and then dissolved in toluene (1.00 ml), followed by degassing under reduced pressure. To the resultant solution, tris(trimethylsilyl)silane (94.6 μl, 0.306 mmol) and a small amount of azobis(isobutyronitrile) were added in an argon atmosphere at 80°C, and the resultant mixture was stirred under the same conditions for one hour. The resultant reaction mixture was concentrated under reduced pressure, and then the thus-obtained residue was purified by means of silica gel column chromatography (silica gel 10 ml, chloroform : methanol = 200 : 1 to 100 : 1), to thereby yield compound 22 (26.1 mg, 0.0516 mmol, 84.6%).

^{1}H-NMR($CDCl_3$) δ8.24(1H, s, H-8), 6.36(1H, dd, H-1', J=2.50, 7.00), 5.91(2H, bs, NH_2), 4.04(1H, d, H-5'a, J=11.0), 3.81(1H, d, H-5'b, J=11.0), 2.83(1H, m, H-2'a), 2.54(1H, m, H-3'a), 2.37(1H, m, H-2'b), 2.11(1H, m, H-3'b), 1.00(9H, t, Et, J=8.00), 0.93(9H, s, t-Bu), 0.62(6H, q, Et, J=8.00), 0.13(6H, s, Me).

(8) Synthesis of 2',3'-dideoxy-4'-C-ethynyl-2-fluoroadenosine (compound 23)

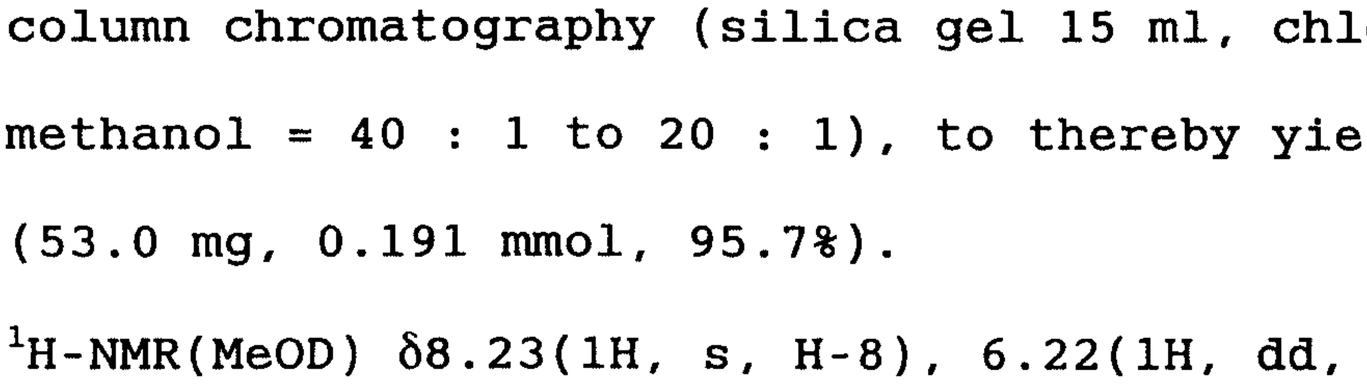


22 23

Compound 22 (101 mg, 0.200 mmol) was dissolved in tetrahydrofuran (10 ml), and a solution of 1-M tetrabutylammonium fluoride in tetrahydrofuran (0.42 ml, 0.42 mmol) was added to the resultant solution, followed by stirring at room temperature for five minutes. After acetic acid (24 μl) was added to the resultant reaction mixture, the resultant mixture was concentrated under reduced pressure. The thus-obtained residue was purified by means of silica gel column chromatography (silica gel 15 ml, chloroform : methanol = 40 : 1 to 20 : 1), to thereby yield compound 23 (53.0 mg, 0.191 mmol, 95.7%).

^{1}H-NMR(MeOD) δ8.23(1H, s, H-8), 6.22(1H, dd, H-1', J=4.00, 7.00), 3.77(1H, d, H-5'a, J=12.5), 3.61(1H, d, H-5'b, J=12.0), 2.94(1H, s, ethynyl), 2.66(1H, m, H-2'a), 2.54(1H, m, H-3'a), 2.42(1H, m, H-2'b), 2.11(1H, m, H-3'b).

Synthesis Example 8: Synthesis of 2',3'-dideoxy-4'-C-ethynyl-2-fluoroadenosine 5'-H-phosphonate (compound 24)



23 24

Compound 23 (20.0 mg, 0.07 mmol) was dissolved in

pyridine (1 ml), and phosphonic acid (11.8 mg, 0.144 mmol) and dicyclohexyl carbodiimide (44.7 mg, 0.216 mmol) were added to the resultant solution, followed by stirring at room temperature for five hours. The resultant reaction mixture was concentrated under reduced pressure, and the thus-obtained residue was purified by means of ODS column chromatography (ODS 10 ml, 0 to 1% acetonitrile). The resultant residue was applied to a Dowex 50Wx8 columm (Na type) and eluted. The eluate was concentrated, and the resultant residue was treated with methanol and ether, to thereby yield a powdery compound (compound 24; 4.7 mg, 13 μmol, 18.6%).

^{1}H-NMR(MeOD) δ8.37(1H, s, H-8), 6.77(1H, d, H-P, J=625), 6.32(1H, dd, H-1', J=4.00, 6.50), 4.09(1H, m, H-5'), 2.71(2H, m, H-2'a, H-3'a), 2.52(1H, m, H-2'b), 2.29(1H, m, H-3'b).

^{31}P-NMR(MeOD) δ4.52.

Drug Preparation Example 1: Tablets

Compound of the present invention	30.0 mg
Cellulose micropowder	25.0 mg
Lactose	39.5 mg
Starch	40.0 mg
Talc	5.0 mg
Magnesium stearate	0.5 mg

Tablets are prepared from the above composition through a customary method.

Drug Preparation Example 2: Capsules

Compound of the present invention	30.0 mg
Lactose	40.0 mg
Starch	15.0 mg
Talc	5.0 mg

Capsular drugs are prepared from the above composition through a customary method.

Drug Preparation Example 3: Injections

Compound of the present invention	30.0 mg
Glucose	100.0 mg

Injections are prepared by dissolving the above composition in purified water for preparing injections.

Test Examples will next be described. Employed in tests were the following five compounds of the present invention and four known compounds.

Invention compounds:

Compound 4: 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine;

Compound 8: 4'-C-cyano-2'-deoxy-2-fluoroadenosine;

Compound 9: 2-chloro-2'-deoxy-4'-C-ethynyladenosine;

Compound 10: 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine 5'-H-phosphonate; and

Compound 13: 2',3'-didehydro-2',3'-dideoxy-4'-C-ethynyl-2-fluoroadenosine.

Known compounds:

AZT: Azidothymidine;

EdAdo: 2'-deoxy-4'-C-ethynyladenosine;

EdDAP: 9-(4-C-ethynyl-2-deoxy-ribopentofuranosyl)-2,6-diaminopurine; and

ddAdo: 2',3'-dideoxyadenosine.

Test Example 1

<Test methods> Anti-human-immunodeficiency-virus (HIV) activity

1) MTT method using MT-4 cells

1. A test agent (100 μl) is diluted on a 96-well microplate. MT-4 cells infected with HIV-1 (IIIb strain; 100 $TCID_{50}$) and non-infected MT-4 cells are added to the microplate such that the number of cells in each well becomes 10,000. The cells are cultured at 37°C for five days.

2. MTT (20 μl, 7.5 mg/ml) is added to each well, and the cells are further cultured for 2-3 hours.

3. The cultured medium (120 μl) is sampled, and MTT terminating solution (isopropanol containing 4% Triton X-100 and 0.04N HCl) is added to the sample. The mixture is stirred to dissolve formed formazan. The absorbance at 540 nm of the solution is measured. Since the absorbance is proportional to the number of viable cells, the test agent concentration at which a half value of the absorbance is measured in a test using infected MT-4 cells represents EC_{50}, whereas the test agent concentration at which a half value of the absorbance is measured in a test using non-infected MT-4 cells represents CC_{50}.

2) MAGI assay using HeLa CD4/LTR-beta-Gal cells

1. HeLa CD4/LTR-beta-Gal cells are added to 96 wells such that the number of cells in each well is 10,000. After 12-24 hours, the culture medium is removed, and a diluted test agent (100 μl) is added.

2. A variety of HIV strains (wild strain: WT, drug-resistant strain: MDR and M184V; each being equivalent to 50 $TCID_{50}$) are added, and the cells are further cultured for 48 hours.

3. The cells are fixed for five minutes using PBS supplemented with 1% formaldehyde and 0.2% glutaraldehyde.

4. After the fixed cells are washed with PBS three times, the cells are stained with 0.4 mg/ml X-Gal for one hour, and the number of blue-stained cells of each well is counted under a transmission stereoscopic microscope. The test agent concentration at which blue-stained cells decrease to 50% in number represents EC_{50}.

<Results> Anti-human-immunodeficiency virus (HIV) activity and cytotoxicity

1) MTT method using MT-4 cells

Table 1

Drugs	MT-4 cells		
	Anti-HIV-1 activity (EC_{50}, μM)	Cytotoxicity (CC_{50}, μM)	Selectivity Index (CC_{50}/EC_{50})
Compound 4	0.000068	7.5	110000
EdDAP	0.00034	0.9	2600
EdAdo	0.0098	16	1630
AZT	0.0032	29.4	9190

2) MAGI assay using HeLa CD4/LTR-beta-Gal cells

Table 2

Drugs	HeLa CD4/LTR-beta-Gal cells		
	Anti-HIV-1_{wild} activity (EC_{50}, μM)	Anti-HIV-1_{MDR} activity (EC_{50}, μM)	Anti-HIV-1_{M184V} activity (EC_{50}, μM)
Compound 4	0.00020	0.0001448	0.003107
Compound 8	0.12	0.95	4.8
Compound 9	0.0019	0.0084	0.01
Compound 10	0.0034	0.003	0.062
Compound 13	0.80	0.15	1.8
EdAdo	0.008	0.0062	0.047
AZT	0.022	15.3	0.01

Test Example 2

<Test methods> Stability of Compound 4 against adenosine deaminase

Calf-intestine-derived adenosine deaminase (0.01 unit) was added to 0.5 ml of 0.5-mM compound 4 (50 mM Tris-HCl buffered solution (pH 7.5)), and the mixture was incubated at 25°C.

A 5-μl aliquot of the reaction mixture was removed every 15 minutes, followed by analysis by means of HPLC (high performance liquid chromatography). The peak area of a test drug at reaction time 0 was taken as 100%, and the curve was monitored over time. The HPLC analysis was performed under the following conditions.

Column: YMC-Pack ODS-A (250 × 6.0 mm)

Eluent: 15% MeCN-50mM TEAA

Flow rate: 1 mL/min.

Temperature: 30°C

Detection: 260 nm

<Results>

As shown in Fig. 1, 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine, which is Compound 4 of the present invention, was not at all deaminated, as contrasted to the case where conventional 2'-deoxy-4'-C-ethynyladenosine (EdAdo) was deaminated, proving that the compound of the present invention has resistance to adenosine deaminase.

Test Example 3

<Test methods> Stability of Compound 4 under acidic conditions

Compound 4 (2.9 mg) or 2',3'-dideoxyadenosine (ddAdo: 2.4 mg) was dissolved in 10 ml of a 37°C test solution (which had been prepared by adding 2.0 g of sodium chloride and 7.0 ml of hydrochloric acid into water to make a solution of 1,000 ml), followed by incubation at the same temperature (37°C).

A 100-µl aliquot of the reaction mixture was removed therefrom , and neutralized with aqueous 0.1-N sodium hydroxide solution, followed by analysis of 5 µl by means of HPLC. The HPLC analysis conditions are the same as those employed in Test Example 2.

<Results>

About 98% of ddAdo, which is a conventional compound, is degraded in about five minutes under the above conditions (see Fig. 3), whereas 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine, which is Compound 4 of the present invention, was degraded very slowly, proving that the compound of the present invention is relatively stable under acidic conditions (see

Fig. 2).

Test Example 4

<Test methods> *In vivo* acute toxicity test of compound 4

Groups of ICR mice (6 weeks of age, male), each group consisting of 8 mice, were given a test drug (Compound 4; dissolved or suspended in saline) via oral route or intravenous injection in amounts up to 100 mg/kg. The occurrence of death and body weight of each mouse were monitored for seven days.

<Results>

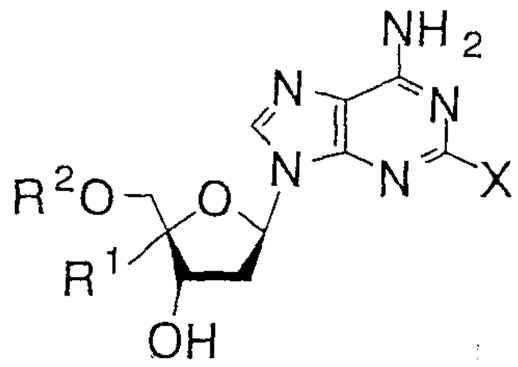
All the mice to which Compound 4 was administered up to 100 mg/kg in a single dose survived regardless of the administration route of oral or intravenous (Table 3). Also, as shown in Fig. 4, weight loss and pathological symptoms such as diarrhea were not observed. Thus, it has now been confirmed that 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine (Compound 4) of the present invention does not exhibit acute toxicity in mice.

Table 3

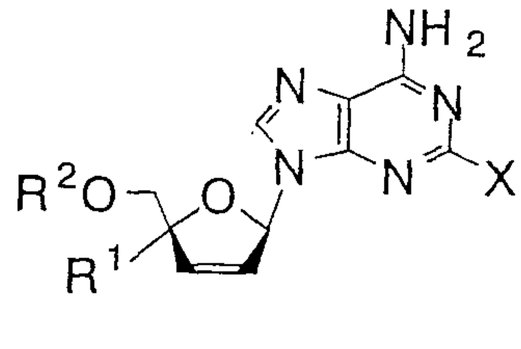
Dose (mg/kg)	Survisors/Total	
	Oral	Intravenous
Placebo	8/8	8/8
1	8/8	8/8
3	8/8	8/8
10	8/8	8/8
30	8/8	8/8
100	8/8	8/8

What is claimed is:

1. A 4'-C-substituted-2-haloadenoisine derivative represented by the following formula (1) or (II)



[I]



[II]

wherein X represents a halogen atom, R^1 represents an ethynyl group or a cyano group, and R^2 represents hydrogen,

or a phosphate residue or its derivative residue selected from a group consisting of a monophosphate, a diphosphate, a triphosphate, a phosphonate, a phosphate polyester, a phosphate amidate, a phosphorothioate, a phosphoroselenoate, or a phosphoroboranoate.

2. A 4'-C-substituted-2-haloadenosine derivative according to claim 1, wherein the halogen atom represented by X is a fluorine or chlorine atom.

3. A 4'-C-substituted-2-haioadenosine derivative according to claim 1, wherein R^1 is an ethynyl group.

4. A 4'-C-substituted-2-haloadenosine derivative according to claim 1, which is 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine.

5. A 4'-C-substituted-2-haloadenosine derivative according to claim 1, which is 4'-C-cyano-2'-deoxy-2-fluoroadenosine.

6. A 4'-C-substituted-2-haloadenosine derivative according to claim 1, which is 2-chloro-2'-deoxy-4'-C-ethynyladenosine.

7. A 4'-C-substituted-2-haloadenosine derivative according to claim 1, which is 2'-deoxy-4'-C-athynyl-2-fluoroadenosine 5'-H-phosphonate..

8. A 4'-C-substituted-2-haloadenosine derivative according to claim 1, which is 2',3'-didehydro-2',3'-dideoxy-4'-C-ethynyl-2-fluoroadenosine.

9. A 4'-C-substituted-2-haloadenosine derivative according to claim 1, which is 2',3'-didehydro-2',3'-dideoxy-4'-C-cyano-2-fluoroadenosine.

10. A 4'-C-substituted-2-haloadenosine derivative according to claim 1, which is 2',3'-didehydro-2',3'-dide-oxy-4'-C-ethynyl-2-chloroadenosine.

11. A pharmaceutical composition containing a 4'-C-substituted-2-haloadenosine derivative as recited in any one of claims 1 through 10, and a pharmaceutically acceptable carrier therefor.

12. A pharmaceutical composition according to claim 11, which is an anti-HIV drug.

13. A pharmaceutical composition according to claim 11, which is a drug for treating AIDS.

14. A use of a 4'-C-substituted-2-haloadenoisine derivative as recited in any one of claims 1 – 10, or a pharmaceutical composition containing said derivative for preparing a medicament for treating AIDS.

15. A use of a 4'-C-substituted-2-haloadenoisine derivative as recited in any one of claims 1 – 10, or a pharmaceutical composition containing said derivative for treating AIDS.

Fig. 1

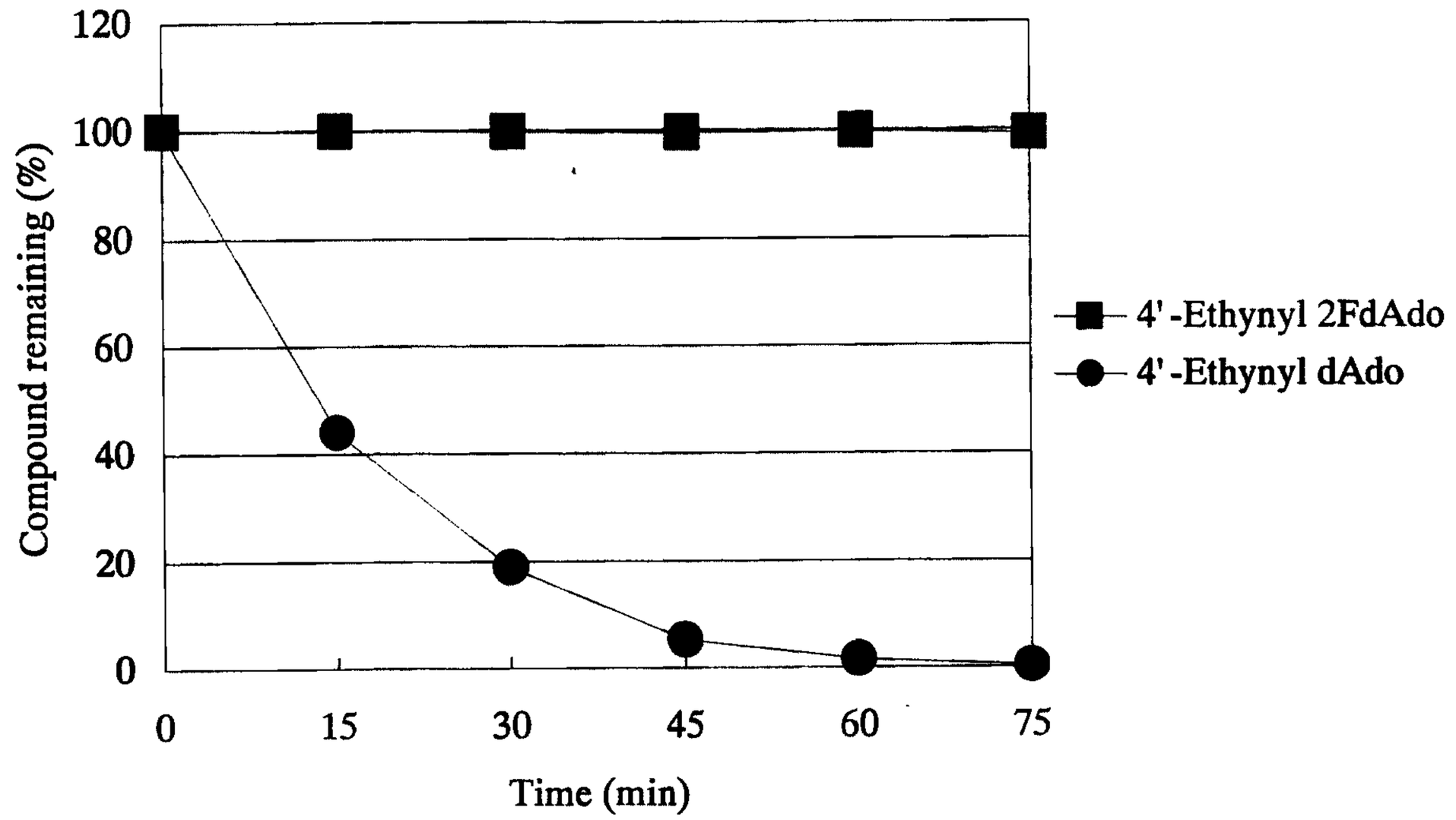

120
100
80
60
40
20
0
Compound remaining (%)
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15
30
45
60
75
Time (min)
4'-Ethynyl 2FdAdo
4'-Ethynyl dAdo

Fig. 2

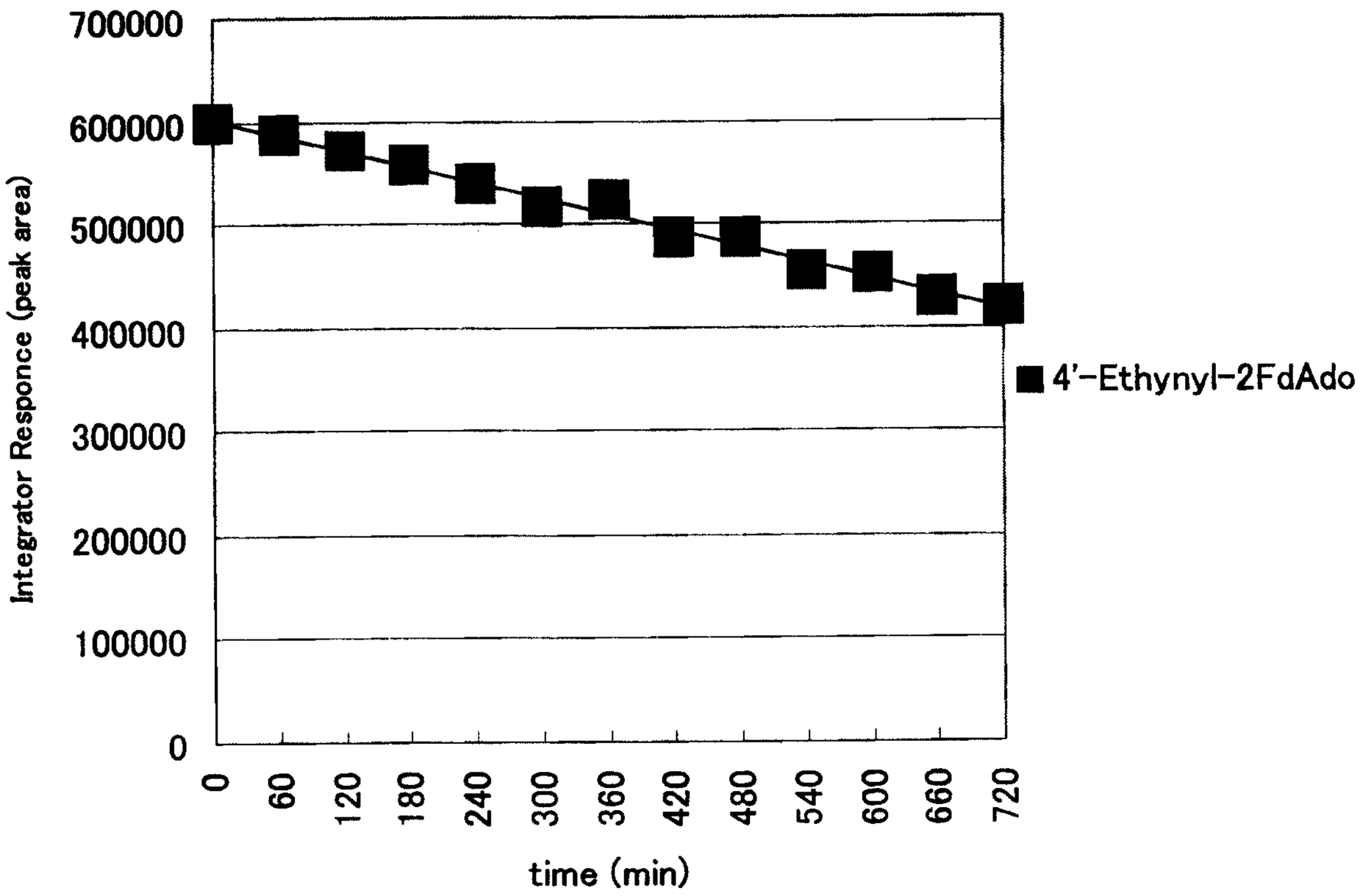

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400000
300000
200000
100000
0
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60
120
180
240
300
360
420
480
540
600
660
720
time (min)
4'-Ethynyl-2FdAdo

Fig. 3

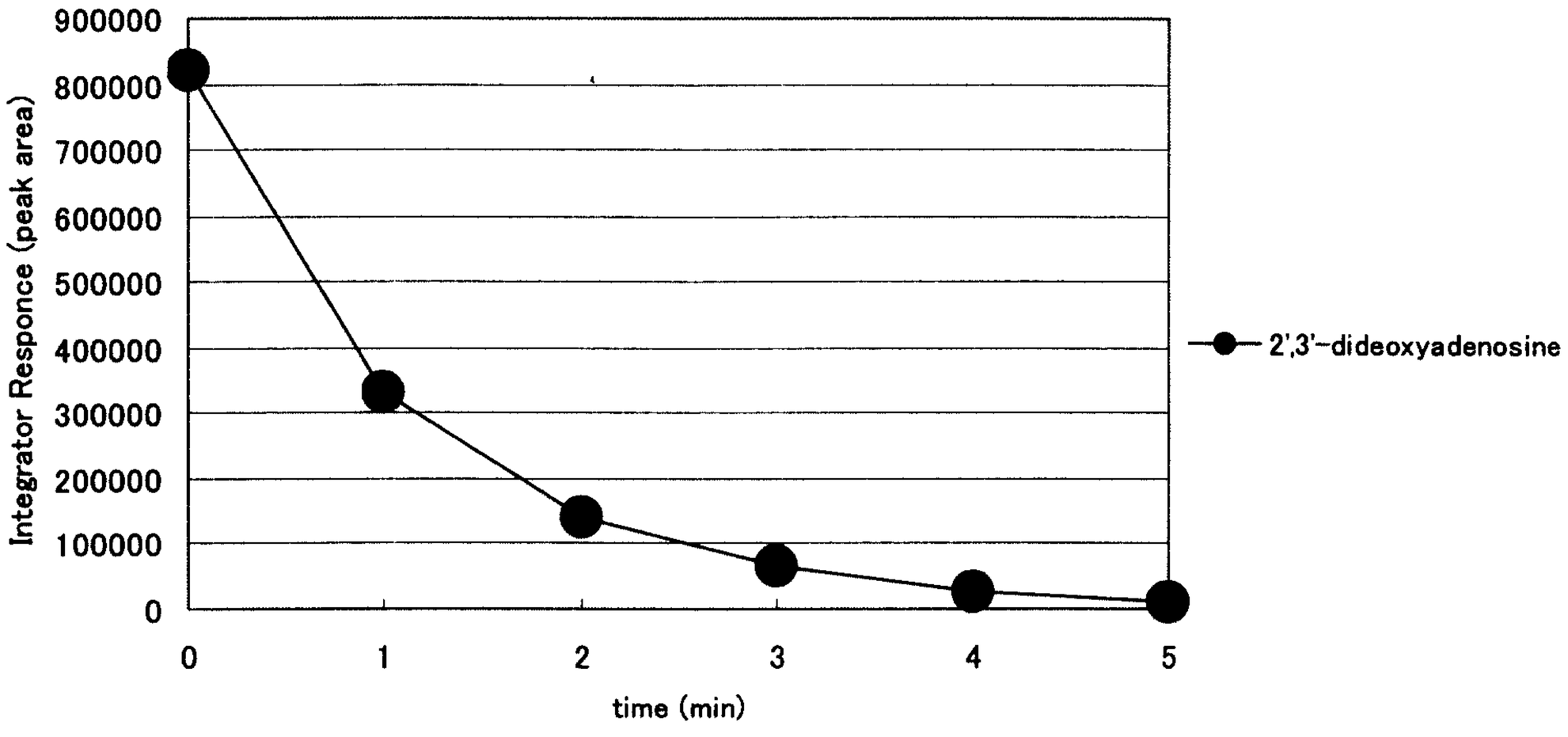
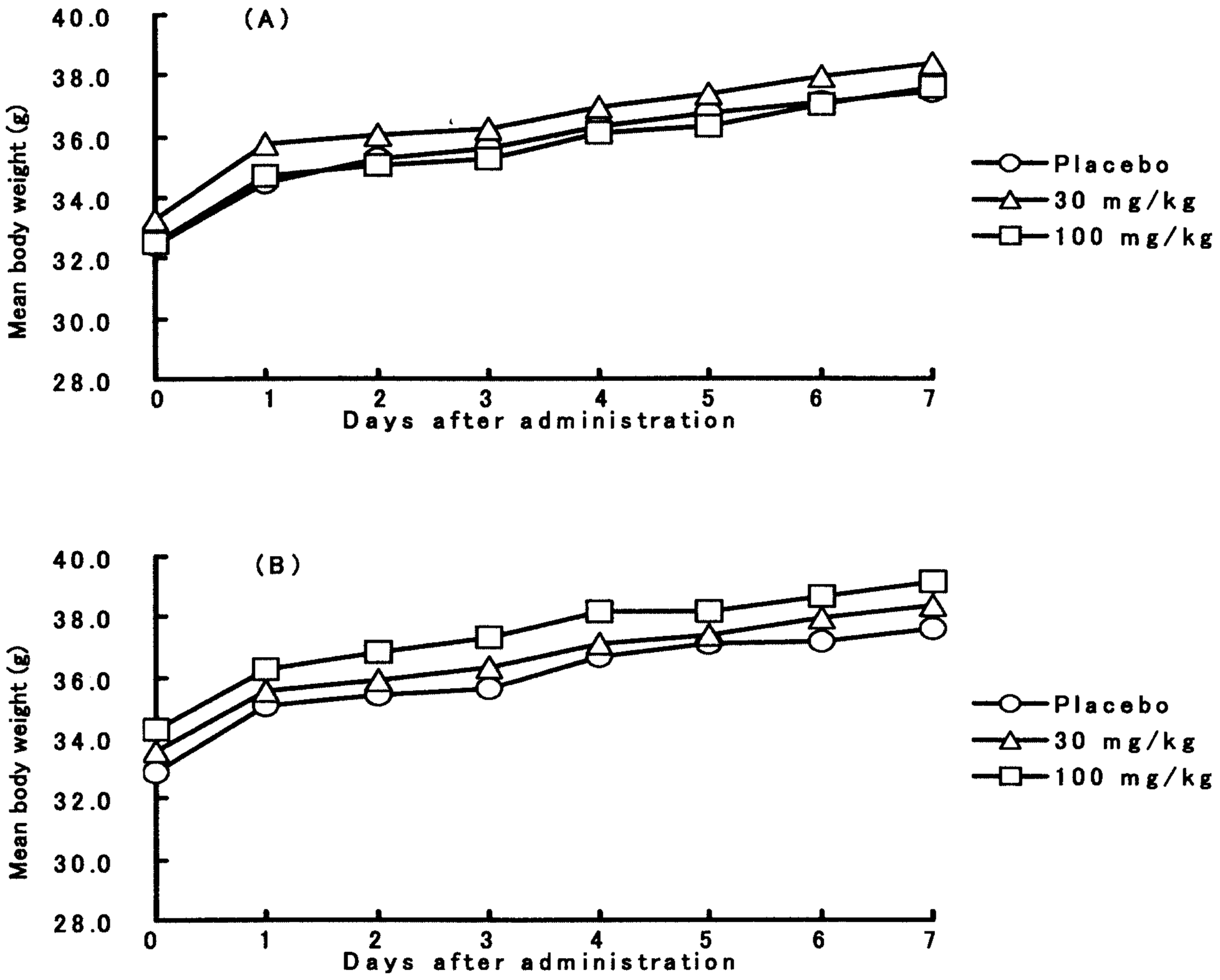
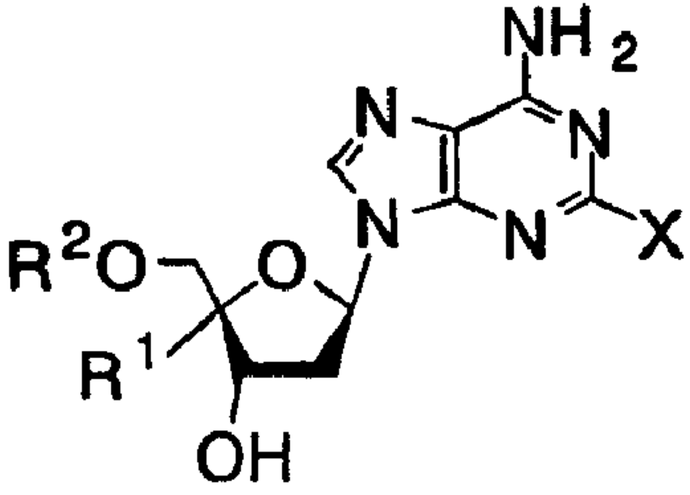
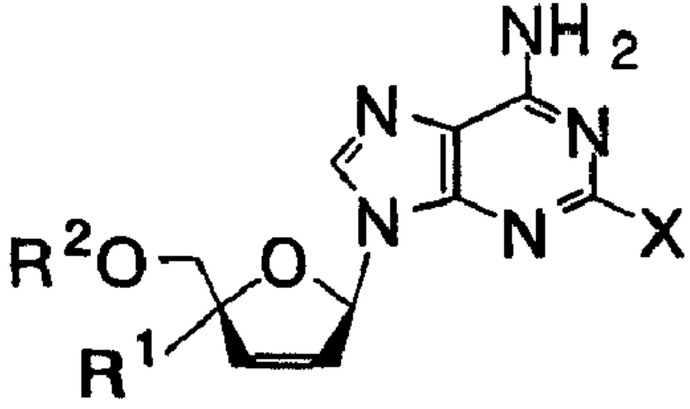

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700000
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400000
300000
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100000
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Integrator Responce (peak area)
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1
2
3
4
5
time (min)
2',3'-dideoxyadenosine

Fig. 4

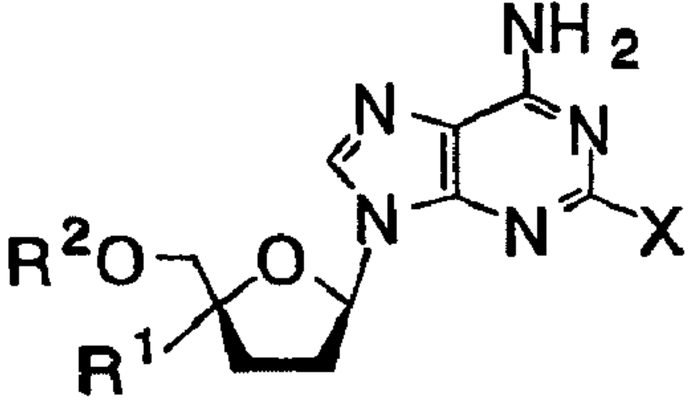

(A)
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38.0
36.0
34.0
32.0
30.0
28.0
0
1
2
3
4
5
6
7
Days after administration
Placebo
30 mg/kg
100 mg/kg
(B)
Mean body weight (g)
40.0
38.0
36.0
34.0
32.0
30.0
28.0
0
1
2
3
4
5
6
7
Days after administration
Placebo
30 mg/kg
100 mg/kg



[I]



[II]



[III]

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2′-Deoxy-4′-*C*-ethynyl-2-halo-adenosines active against drug-resistant human immunodeficiency virus type 1 variants

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ABSTRACT

One of the formidable challenges in therapy of infections by human immunodeficiency virus (HIV) is the emergence of drug-resistant variants that attenuate the efficacy of highly active antiretroviral therapy (HAART). We have recently introduced 4′-ethynyl-nucleoside analogs as nucleoside reverse transcriptase inhibitors (NRTIs) that could be developed as therapeutics for treatment of HIV infections. In this study, we present 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine (EFdA), a second generation 4′-ethynyl inhibitor that exerted highly potent activity against wild-type HIV-1 ($EC_{50} \sim 0.07$ nM). EFdA retains potency toward many HIV-1 resistant strains, including the multi-drug resistant clone HIV-$1_{A62V/V75I/F77L/F116Y/Q151M}$. The selectivity index of EFdA (cytotoxicity/inhibitory activity) is more favorable than all approved NRTIs used in HIV therapy. Furthermore, EFdA efficiently inhibited clinical isolates from patients heavily treated with multiple anti-HIV-1 drugs. EFdA appears to be primarily phosphorylated by the cellular 2′-deoxycytidine kinase (dCK) because: (a) the antiviral activity of EFdA was reduced by the addition of dC, which competes nucleosides phosphorylated by the dCK pathway, (b) the antiviral activity of EFdA was significantly reduced in dCK-deficient HT-1080/Ara-C^r cells, but restored after dCK transduction. Further, unlike other dA analogs, EFdA is completely resistant to degradation by adenosine deaminase. Moderate decrease in susceptibility to EFdA is conferred by a combination of three RT mutations (I142V, T165R, and M184V) that result in a significant decrease of viral fitness. Molecular modeling analysis suggests that the M184V/I substitutions may reduce anti-HIV activity of EFdA through steric hindrance between its 4′-ethynyl moiety and the V/I184 β-branched side chains. The present data suggest that EFdA, is a promising candidate for developing as a therapeutic agent for the treatment of individuals harboring multi-drug resistant HIV variants.

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1. Introduction

Highly active antiretroviral therapies (HAART), combining two or more reverse transcriptase inhibitors (RTIs) and/or protease inhibitors, have been successful in sig-

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nificantly reducing viral loads and bringing about clinical benefits to the treatment of patients infected with human immunodeficiency virus type 1 (HIV-1). Although HAART improves prognosis for HIV-1 infected patients (Palella et al., 1998), drug-resistant viruses emerge during prolonged therapy and some resistant viruses show intra-class cross resistance. Moreover, drug-resistant variants can be transmitted to other individuals as primary infections (Little et al., 2002). Hence, there is a great need for the development of new HIV inhibitors that retain activity against drug-resistant HIV variants.

In this regard, we have focused on the family of nucleoside reverse transcriptase inhibitors (NRTIs) and have previously reported that a series of 2′-deoxy-4′-*C*-ethynyl-nucleosides (EdNs) efficiently suppress (EC_{50}s as low as one nanomolar) various NRTI-resistant HIV strains including multi-drug resistant clinical isolates (Kodama et al., 2001). More recently, Haraguchi and others have reported that additional members of EdNs such as 2′,3′-didehydro-3′-deoxy-4′-*C*-ethynyl-thymidine (Ed4T) are also active against wild-type and drug-resistant strains (EC_{50}s ranged from 0.16 to 17 μM) and less toxic than d4T (also known as stavudine) *in vitro* (Dutschman et al., 2004; Haraguchi et al., 2003), while 4′-Ed4T is only moderately active against (−)-2′,3′-dideoxy-3′-thiacytidine (3TC or lamivudine)-resistant HIV-1_{M184V} (Nitanda et al., 2005).

To further increase the antiviral activity and reduce the cytotoxicity, we designed and synthesized a second generation of 4′-substituted adenosine analogs with halogen substitutions at their 2-position. We report here that 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine (EFdA) exhibits the highest antiviral activity than any other NRTI when assayed against wild-type or NRTI-resistant HIV clones and clinical isolates from patients treated extensively with anti-HIV agents. In addition, unlike other adenosine-based NRTIs, EFdA showed adenosine deaminase (ADA) resistance. We also show that EFdA is primarily activated through phosphorylation by cellular deoxycytidine kinase (dCK). Molecular modeling analysis has been used to rationalize the resistance profile of these analogs toward key NRTI mutations.

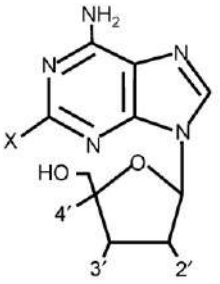
2. Materials and methods

2.1. Compounds

3′-Azido-3′-deoxythymidine (AZT, or zidovudine), 2′,3′-dideoxyinosine (ddI, or didanosine), and 2′,3′-dideoxycytidine (ddC, or zalcitabine) were purchased from Sigma (St. Louis, MO.). 3TC was kindly provided from S. Shigeta (Fukushima Medical University, Fukushima, Japan). A set of EdN analogs were designed and synthesized as described elsewhere (Ohrui, 2006). Their chemical structures are shown in Fig. 1. 2′-Deoxycoformycin (dCF) was synthesized in Yamasa Corporation (Choshi, Japan).

2.2. Cells and plasmids

MT-2 and MT-4 cells were grown in an RPMI 1640-based culture medium, and 293T cells were grown in Dulbecco's modified Eagle medium (DMEM); each of these media was



Base X	Sugar 2′	Sugar 3′	4′	Compound abbreviation
-H	-H	-OH	-C ≡ CH	*E*dA
-F	-H	-OH	-C ≡ CH	2F-*E*dA
-F	-H	-H	-C ≡ CH	2F-*E*ddA
-F	C = C		-C ≡ CH	2F-*E*d4A
-F	-H	-OH	-C ≡ N	2F-CNdA
-Cl	-H	-OH	-C ≡ CH	2Cl-*E*dA

Fig. 1. Structures of 4′-substituted adenosine analogs. All nucleoside analogs discussed here have substitutions at the 4′-position of the sugar ring.

supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, and 50 μg/ml streptomycin. HeLa-CD4-LTR/β-galactosidase (MAGI) cells were propagated in DMEM supplemented with 10% FCS, 0.2 mg/ml of hygromycin B, and 0.2 mg/ml of G418 (Kimpton and Emerman, 1992). HeLa-CD4/CCR5-LTR/β-galactosidase cells were propagated in puromycin (10 μg/ml) containing DMEM with hygromycin and G418. Peripheral blood mononuclear cells (PBMCs) were obtained from healthy HIV-1-seronegative donors by Ficoll-Hypaque gradient centrifugation and stimulated for 3 days with phytohemagglutinin M (PHA; 10 μg/ml; Sigma) and recombinant human interleukin 2 (IL-2; 10 U/ml; Shionogi & Co., Ltd., Osaka, Japan) prior to use. Human fibrosarcoma cell lines, HT-1080 and HT-1080/Ara-C^r were grown in the RPMI-based culture medium (Obata et al., 2001). To express HIV-1 receptors, we constructed a mammalian expression vector pBC-CD4/CXCR4-IH, which encodes CD4, CXCR4, and hygromycin phosphotransferase with two internal ribosome entry sites under control of cytomegalovirus promoter as described (Kajiwara et al., 2006). After the transfection into HT-1080 and HT-1080/Ara-C^r, cells were selected by 0.2 mg/ml hygromycin B. For the expression of human deoxycytidine kinase (dCK), pCI-neo (Promega, Madison, WI)-based plasmid, pCI-dCK, was transfected into HT-1080/Ara-C^r and selected with 0.2 mg/ml G418. Established cells were designated HT-1080/Ara-C^r/dCK. Puromycin resistance gene under the control of PGK promoter was inserted into pLTR-SEAP (Miyake et al., 2003), which encodes a secreted form of the placental alkaline phosphatase (SEAP) gene under control of the HIV-1 long terminal repeat (LTR) (pLTR-SEAP-puro^r). pLTR-SEAP-puro^r was transfected into the three HT-1080 cell lines and selected with 10 μg/ml puromycin.

2.3. Viruses and construction of recombinant HIV-1 clones

Two laboratory strains, HIV-1_{IIIB} and HIV-2_{EHO}, were used. Multi-drug resistant clinical HIV-1 strains, which had been exposed to over 10 anti-HIV-1 drugs for at least 3 years, were passaged in PHA-stimulated PBMCs (PHA-PBMCs) and stored at −80 °C until further use. Recombinant infectious HIV-1 clones carrying various mutations in the *pol* gene were generated using pNL101 (Jeang et al., 1993). Briefly, desired mutations were introduced into the XmaI–NheI region (759 bp) of pTZNX1, which encoded Gly-15 to Ala-267 of HIV-1 RT (strain BH 10) by a site-directed mutagenesis method (Weiner et al., 1994). The XmaI–NheI fragment was inserted into a pNL101-based plasmid, pNL-RT, generating various molecular clones with the desired mutations. To generate pNL-RT, we first introduced a silent mutation at NheI site of the pNL101, GCTAGC to GCCAGC (underlined; 7251 n.t. from the 5′-LTR) by site-directed mutagenesis. Then, the ApaI–SalI fragment of pNL101 without the NheI site was replaced with that of pSUM9 (Shirasaka et al., 1995), to introduce XmaI and NheI site in the RT coding region. The presence of intended substitutions and the absence of unintended substitutions in the molecular clones were confirmed by sequencing. Each molecular clone (2 μg/ml as DNA) was transfected into 293T cells (4×10^5 cells/6-well plate) by FuGENE 6 Transfection Reagent (Roche Diagnostics, Indianapolis, IN). After 24 h, MT-2 cells (10^6 cells/well) were added and co-cultured with 293T cells for an additional 24 h. When an extensive cytopathic effect was observed, the cell supernatants were harvested, and the virus was further propagated in MT-4 cells. The culture supernatant was harvested and stored at −80 °C until further use.

2.4. Determination of drug susceptibility

The inhibitory effect of test compounds on viral replication for 5 days was evaluated in MT-4 cells by the MTT method as described previously (Kodama et al., 2001). The sensitivity of NRTI-resistant infectious clones to test compounds was determined by the MAGI assay as described (Nameki et al., 2005). The drug susceptibility of HIV-1 clinical isolates was determined on day 7 by a commercially available p24 antigen assay (Kodama et al., 2001). Briefly, PHA-PBMCs (10^6 cells/ml) were exposed to each viral preparation at $TCID_{50}$ of 50 and cultivated in 200 μl of culture medium containing various concentration of the drug in 96-well culture plates. All assays were performed in triplicate, and the amounts of p24 antigen produced by the cells into the culture medium were determined. A 2′-deoxynucleoside competition assay was performed by the same way as the MAGI assay. An adenosine deaminase (ADA) inhibitor, dCF, was added for preventing conversion of 2′-deoxyadenosine (dA) to 2′-deoxyinosine (dI) (final concentration 0.4 μM). The effect of dCK expression on activities of test compounds was examined by measurement of SEAP activity in the supernatant. At first, the target cells (HT-1080, HT-1080/Ara-C^r, and HT-1080/Ara-C^r/dCK) were suspended in 96-well plates (5.0×10^3 cells/well). On the following day, the cells were inoculated with HIV-1_{IIIB} (500 MAGI unit/well, giving 500 blue cells in MAGI cells) in the presence of serially diluted compounds. After 48 h incubation, supernatant was collected and SEAP activity in the supernatant was measured using BD Great EscAPe SEAP chemiluminescence detection kit (BD Biosciences Clontech., Palo Alto, CA) and Wallac 1450 MicroBeta Jet Luminometer (PerkinElmer, Wellesley, MA).

2.5. The effect of ADA

The effect of ADA on EdA or EFdA was examined by high performance liquid chromatography (HPLC). ADA (0.01 U) derived from bovine intestinal tract was added into 0.5 ml of 0.5 mM EFdA in 50 mM Tris–HCl buffer (pH 7.5), and incubated at 25 °C. Samples were collected each 15 min and analyzed by HPLC.

2.6. HIV-1 replication assays

MT-2 cells (2.5×10^5 cells/5 ml) were infected with each virus preparation (500 MAGI units) for 4 h. The infected cells were then washed and cultured in a final volume of 5 ml. Culture supernatants (200 μl) were harvested from days 1 to 7 after infection, and the p24 antigen amounts were quantified (Nameki et al., 2005).

For competitive HIV-1 replication assay (CHRA), two titrated infectious clones to be examined were mixed and added to MT-2 cells (10^5 cells/3 ml) as described previously (Kosalaraksa et al., 1999; Nameki et al., 2005). To ensure that the two infectious clones being compared were of approximately equal infectivity, a fixed amount (500 MAGI units) of one infectious clone was mixed with three different amounts (250, 500 and 1000 MAGI units) of the other infectious clone. On day 1, one-third of the infected MT-2 cells were harvested, washed twice with phosphate-buffered saline, and the cellular DNA was extracted. The purified DNA was subjected to nested PCR and then direct DNA sequencing. The HIV-1 coculture which best approximated a 50:50 mixture on day 1 was further propagated. Every 4–6 days, the cell-free supernatant of the virus coculture (1 ml) was transmitted to new uninfected MT-2 cells. The cells harvested at the end of each passage were subjected to direct sequencing, and the viral population change was determined by the relative peak height in the sequencing chromatogram. The persistence of the original amino acid substitution was confirmed in all infectious clones used in this assay.

2.7. Molecular modeling studies

The programs SYBYL and O were used to prepare models of the complexes of wild-type, M184V, and insertion mutant HIV-1 RT with DNA and the triphosphates of 3TC and EFdA. The starting atomic coordinates of HIV-1 RT were from the structure described by Huang et al. (PDB code 1RTD) (Huang et al., 1998). The side-chain mutations were manually modeled using mostly conformations encountered in RT structures that carry such mutations. The local structures of mutants were optimized using energy minimization protocols in SYBYL. The triphosphates of the inhibitors were built based on the structures of dTTP in

Table 1
Antiviral activity against HIV-1 and HIV-2 strains in MT-4 cells

Compound (abbreviation)	EC_{50} (μM)[a]		Selectivity[b]	
	HIV-1_{IIIB}	HIV-2_{EHO}	CC_{50} (μM)[c]	index
2′-Deoxy-4′-*C*-ethynyl-adenosine (EdA)	0.0095 ± 0.0027[d]	0.006 ± 0.0015	104 ± 6.2	11,000
2′-Deoxy-4′-*C*-ethynyl-2-fluoroadenosine (EFdA)	0.000073 ± 0.000017	0.000098 ± 0.000022	9.8 ± 3.4	134,000
2′,3′-Dideoxy-4′-*C*-ethynyl-2-fluoroadenosine (EFddA)	1.17 ± 0.29	1.07 ± 0.23	230 ± 33	196
2′,3′-Didehydro-3′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine (EFd4A)	0.11 ± 0.033	0.089 ± 0.0007	98 ± 26	899
2′-Deoxy-4′-*C*-cyano-2-fluoroadenosine (CNFdA)	0.1 ± 0.034	0.09 ± 0.0087	>340	>3,300
2′-Deoxy-4′-*C*-ethynyl-2-chloroadenosine (ECldA)	0.00069 ± 0.00018	0.0006 ± 0.0000028	230 ± 16	339,000
2′,3′-Dideoxyinosine (ddI)	27 ± 12	24 ± 4.4	>100	>4
3′-Azido-3′-deoxythymidine (AZT)	0.0028 ± 0.00062	0.0022 ± 0.00073	30 ± 7.2	10,800

Anti-HIV activity was determined by the MTT method.
[a] EC_{50} represents the concentration that blocks HIV-1 replication by 50%.
[b] Selectivity index is calculated by the CC_{50}/EC_{50} for HIV-1_{IIIB}.
[c] CC_{50} represents the concentration that suppress the viability of HIV-1-unexposed cells by 50%.
[d] Data shown are mean values with standard deviations for at least three independent experiments.

1RTD, or of tenofovir diphosphate in the ternary complex of HIV-1 RT/DNA/TFV-DP (Tuske et al., 2004).

3. Results

3.1. Antiviral activity of 4′- and 2-substituted deoxyadenosine analogs

We evaluated the activity of 4′- and 2-substituted deoxyadenosine analogs against HIV-1 with the MTT assay using MT-4 cells. The 2′-deoxy-4′-*C*-ethynyl nucleoside with adenine as the base (EdA) exerted comparable activity to AZT (Table 1). 2-Fluoro substituted EdA, EFdA, was the most potent against HIV-1 with a sub-nanomolar EC_{50} of 0.073 nM. Selectivity of EFdA and EClda was much increased compared to parental EdA or AZT. However, EFdA was also relatively cytotoxic compared to other inhibitors of this series. The 2-chloro (Cl) substitution also provided enhanced activity but with a decreased toxicity. Further modifications of the sugar ring from 2′-deoxyribose to 2′,3′-dideoxy- or 2′, 3′-didehydro-2′,3′-dideoxy-ribose (EFddA or EFd4A) resulted in a drastic decrease of inhibitory potential. Substitution of the 4′-E group with a structurally similar 4′-cyano group also resulted in markedly decreased inhibitory activity. These results indicate that the 3′-OH and 4′-E moieties in the sugar ring are indispensable for high efficacy, and that antiviral activities are augmented by the modification with F- or Cl-moiety at the adenine 2-position. These compounds suppressed the replication of HIV-2 at comparable levels as HIV-1, consistent with the hypothesis that they act as nucleoside reverse transcriptase inhibitors (De Clercq, 1998).

3.2. Antiviral activity against HIV-1 variants resistant to NRTIs

To assess the effect of 4′- and 2-substituted adenosine analogs against drug resistant HIV-1, we generated recombinant infectious clones carrying various NRTI resistant mutations and tested them using the MAGI assay. We found that EdA, EFdA, and EClda efficiently suppressed many of the viruses resistant to approved NRTI including the multi-drug resistant (MDR) virus, although the 3TC-resistant variant HIV-1_{M184V} and the multi-drug resistant variant HIV-$1_{M41L/T69SSG/T215Y}$ (Winters et al., 1998) showed modestly increased EC_{50} values to these compounds (Table 2). Interestingly, highly active 4′-E analogs, which have 3′-OH such as EFdA or EClda, were even more effective against the dideoxy-type NRTI resistant variants K65R, L74V, and Q151M complex than they were against WT RT (Table 2). In contrast, 4′-E analogs without 3′-OH (EFddA and EFd4A) were similar or less effective with these resistant variants compared to WT, although the effect seems to be minimal. EFd4A and 2′-deoxy-4′-*C*-cyano-2-fluoroadenosine (CNFdA) were moderately active against HIV-1_{WT} and HIV-1_{MDR} (Shirasaka et al., 1995), but less active against HIV-1_{M184V}. Susceptibility of even the least active EFddA was still in the low micromolar range, but decreased against both HIV-1_{M184V} and HIV-1_{MDR}, by 84- and 13-fold, respectively.

3.3. Antiviral activity of EFdA against multi-drug resistant clinical isolates

We went on to further characterize EFdA, the most potent compound of the series, against clinical isolates from patients exposed to many anti-AIDS drugs. Five multi-drug resistant strains (HIV-1_{IVR405}, HIV-1_{IVR406}, HIV-1_{IVR412}, HIV-1_{IVR413}, and HIV-1_{A03}), which contained various drug-resistance mutations in HIV-1 genes (Table 3), were used. These clinical isolates showed high resistance to AZT, 3TC (HIV-1_{IVR406}), and ddI (HIV-1_{IVR412}). HIV-1_{IVR415} was also a drug-experienced virus but did not have NRTI-resistance mutations and showed no resistance, or less resistance to the NRTIs tested. Hence, it was used as a drug-sensitive HIV-1. Although antiviral activity of EFdA was slightly reduced against HIV-$1_{IVR405, IVR406, IVR412}$ (5.7- to 7.6-fold) compared to HIV-1_{IVR415}, the activity was high enough to suppress viral replication. It should be emphasized that EFdA was active against HIV-1_{IVR406}, which had the 3TC-resistant M184I substitution.

To evaluate antiviral activity of EFdA to M184V containing isolates in detail, two isolates harboring M184V were used. We used the MAGI assay that directly determines inhibition on a single replication cycle of HIV-1, so that we could eliminate the possible effects of multiple repli-

Table 2
Anti HIV-1 activity against drug-resistant infectious clones

	EC_{50} (μM)[a]								
	EdA	EFdA	EFddA	EFd4A	CNFdA	ECldA	ddI	AZT	3TC
WT	0.021	0.0011	1.2	0.35	0.21	0.0064	4.1	0.015	0.71
K65R	0.0082	0.00023 (×0.2)	1.56	0.2	ND[b]	0.0017 (×0.3)	ND	0.0039 (×0.3)	ND
L74V	0.01	0.00048 (×0.4)	2.52	0.54	ND	0.0015 (×0.2)	14.6	0.019 (×1.3)	ND
V75T	0.0075	0.00067 (×0.6)	9.13	0.95	ND	0.005 (×0.8)	ND	0.047 (×3.1)	ND
M41L/T215Y	0.062	0.0016 (×1.5)	6.7	1.67	ND	0.0065 (×0.1)	ND	0.12 (×8)	ND
M41L/T69SSG/T215Y[c]	0.18	0.0065 (×6)	ND	ND	ND	0.025 (×4)	21	0.20 (×13)	9.9
MDR[d]	0.011	0.00074 (×0.7)	16	0.46	0.69	0.0057 (×0.9)	40	18 (×1200)	1.1
P119S	0.018	0.00067 (×0.6)	ND	ND	ND	0.0062 (×1)	ND	0.0033 (×0.2)	0.6
T165A	0.045	0.001 (×0.9)	ND	ND	ND	0.0082 (×1.3)	ND	ND	0.66
I142V	0.077	0.001 (×0.9)	ND	ND	ND	0.0062 (×1)	ND	0.016 (×1)	0.36
T165R	0.088	0.0016 (×1.5)	ND	ND	ND	0.012 (×1.9)	ND	0.011 (×0.7)	0.28
M184V	0.088	0.0083 (×7.5)	101	6.41	1.76	0.084 (×13)	ND	0.0021 (×0.1)	>100
T165R/M184V	0.6	0.014 (×13)	ND	ND	ND	0.17 (×27)	ND	0.0053 (×0.4)	>100
I142V/T165R/M184V	0.81	0.023 (×22)	ND	ND	ND	0.41 (×65)	ND	0.0076 (×0.5)	>100
T165A/M184V[e]	0.43	0.015 (×14)	ND	ND	ND	0.16 (×25)	ND	0.0049 (×0.3)	>100
P119S/T165A/M184V[e]	0.5	0.015 (×14)	ND	ND	ND	0.20 (×32)	ND	0.0043 (×0.3)	>100

Anti-HIV activity was determined with the MAGI assay.

[a] The data shown are mean values obtained from the results of at least three independent experiments.

[b] ND: not determined.

[c] HIV-1 variant contains T69S substitution and 6-base pair insertions between codons for 69 and 70 (Ser-Gly) with AZT resistant mutations M41L/T215Y (Winters et al., 1998).

[d] Multi-dideoxynucleoside resistant HIV-1 contains mutations (AGT-GGT, SG) in the *pol* region: A62V/V75I/F77L/F116Y/Q151M (Shirasaka et al., 1995).

[e] These variants were reported by Nitanda et al. during induction of Ed4T resistant variants.

cation cycles on measured antiviral activity. In this assay, EFdA effectively suppressed both replication of HIV-1_{IVR443} and HIV-1_{IVR463}. Compared to the EC_{50} value for HIV-1_{WT} in Table 2, reduction of the activity was less than 3-fold, suggesting that EFdA suppresses relatively efficiently 3TC-resistant variants with either M184I or M184V mutations.

3.4. ADA stability of EFdA

Cellular ADA is known to convert dA to dI through deamination. Phosphorylation of the deaminated dA analogs, *e.g.*, dI, is less efficient, resulting in low conversion of the active triphosphate (TP) form. In order to assess if the activation of these compounds to their TP forms would be affected by the activity of ADA, we tested whether ADA can degrade EdA or EFdA. While EdA was almost completely deaminated after 90 min exposure to ADA, EFdA was not deaminated for up to at least 90 min (Fig. 2). These results indicate that the 2-halo-substitution in EdA confers significant resistance to degradation by ADA.

3.5. Phosphorylation of EFdA

Currently available NRTIs need to be converted to the TP form by host cellular kinases before incorporation into newly synthesized proviral DNA. It has been shown that the antiviral effect of NRTIs was reversed by the addition of their physiological counterpart 2′-deoxynucleosides (Bhalla et al., 1990; Mitsuya et al., 1985). To identify the phosphorylation pathway, we examined whether the antiviral activity of EFdA was reversed by the addition of 2′-deoxynucleosides. Surprisingly, the addition of dC decreased the antiviral activity of EFdA in a dose-dependent manner (Fig. 3). In contrast, dT and dG had no effect on the

Table 3
Antiviral activity of EFdA against clinical isolates

Clinical isolates	Amino acid substitutions in the reverse transcriptation	EC_{50} (μM)			
		AZT	ddI	3TC	EFdA
PBMCs[a]					
IVR405	M41L/E44D/D67G/V118I/Q151M/L210W/T215Y	1.76	2.45	0.55	0.0012
IVR406	M41L/E44D/D67N/V118I/M184I/L210W/T215Y/K219R	0.64	1.46	>10	0.0011
IVR412	M41L/E44D/V75L/A98S/L210W/T215F	3.97	9.11	0.83	0.0016
IVR413	M41L/E44D/D67N/V75L/A98S/V118I/L210W/T215Y/K219R	1.0	2.22	1.46	0.00021
A03	M41L/E44D/D67N/L74V/L100I/K103N/V118I/L210W/T215Y	0.53	2.15	0.49	0.0001
IVR415	None	0.0028	0.33	0.078	0.00021
MAGI cells[b]					
IVR443	I135T/Y181C/M184V	0.027	3.6	>100	0.0031
IVR463	M41L/E44D/D67N/M184V/H208Y/L210W/T215Y	0.31	7.5	>100	0.0032

All assays were performed in triplicate. AZT, ddI, and 3TC were served as a control.

[a] Antiviral activity was determined by the inhibition of p24 antigen production in the culture supernatant.

[b] HeLa-CD4/CCR5-LTR/β-gal cells was used for the MAGI assay.

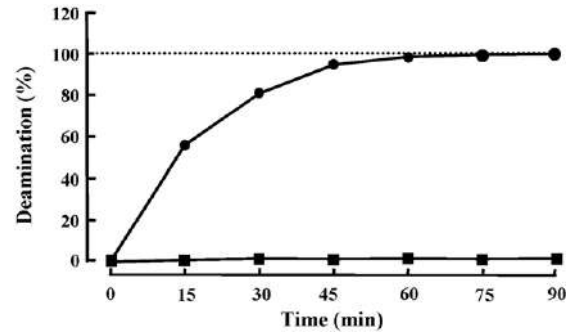


Fig. 2. Stability of EFdA following exposure to ADA. EdA or EFdA was incubated with ADA as described in Section 2. The deamination of adenine to inosine was analyzed by HPLC at indicated time points. The data represent the percent of starting compound (EdA; circle, EFdA; box) that was not deaminated by ADA.

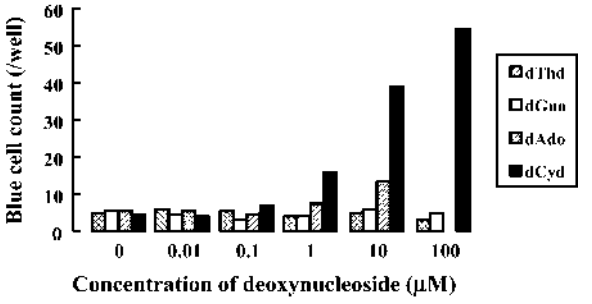


Fig. 3. Reversal of the antiviral activity of EFdA in the presence of 2′-deoxynucleosides. Each 2′-deoxynucleoside was added to the medium with serial dilution in the presence of EFdA (3.5 nM). The effect on EFdA activity was determined by the MAGI assay. An ADA inhibitor, dCF, was used during dA competition.

activity of EFdA. We could observe a slight reversal of the antiviral effect by addition of 10 μM dA with dCF. Effect of 100 μM dA could not be examined because of its cytotoxicity. It should be noted that all other tested analogs, including EFddA and EFd4A, were also reversed by the addition of dC (data not shown).

To confirm that the cellular dCK mediates the phosphorylation of EFdA, we examined the antiviral activity of EFdA in the HT-1080, dCK-deficient HT-1080/Ara-C^r (Obata et al., 2001), and dCK-transduced HT-1080/Ara-C^r cell lines. As expected, the antiviral activity of EFdA was markedly reduced in HT-1080/Ara-C^r cells (677-fold), but restored in dCK-transfected cells (Table 4). The same activity profile was observed with ddC, which is also phosphorylated by dCK (Starnes and Cheng, 1987). In contrast, AZT showed comparable activity among three cell lines, since it is known to be phophorylated mainly by thymidine kinase (Furman et al., 1986). Although dCK appears to be the main enzyme responsible for mono-phosphorylation of EFdA, other kinases, such as adenosine/deoxyadenosine kinases, may be partially involved in mono-phosphorylation of EFdA, especially since weak reduction in antiviral activity of EFdA was observed in addition of dA in high concentrations. Moreover, even in dCK-deficient HT-1080/Ara-C^r cells, EFdA exerted moderate antiviral activity. Hence, while it is possible that other kinases may be contributing to a smaller extent to the phosphorylation of EFdA, it appears that dCK is the enzyme that primarily phosphorylates this inhibitor.

3.6. Resistance to EFdA

In order to elucidate the mechanism of drug resistance to 4′-E analogs, we selected variants resistant to EdA, a parental compound of EFdA with the dose escalating methods (Nameki et al., 2005). After 58 passages in the presence of EdA, the resistant variants were obtained. Sequence analysis of the entire RT region revealed that a novel combination of mutations, I142V/T165R/M184V was introduced. Similar mutations (I119S/T165A/M184V) were observed in a Ed4T-resistant variant (Nitanda et al., 2005). Hence, we generated infectious clones containing these mutations and tested the antiviral activity of 4′-E analogs against them (Table 2). Mutation in T165, either Arg or Ala, enhanced the resistance against EdA, EFdA, and EClda in the presence of the M184V mutation. Similar resistance profiles were observed for the I142V/M184V mutations. Furthermore, the triple mutant HIV-$1_{I142V/T165R/M184V}$ had the highest resistance among all tested variants. On the other hand, I142V or T165R alone did not affect the antiviral activity of EFdA or EClda, although EdA or EFddA showed slightly decreased susceptibility. These results suggest that M184V appears to be the main mutation responsible for 4′-E analog resistance, and the addition of I142V and/or T165R augments the effect of M184V.

3.7. Replication of resistant HIV-1

For acquisition of high-level resistance to EFdA as well as EdA, three mutations, I142V, T165R, and M184V were required as described above. To examine the effect of the mutations on the viral replication kinetics we performed an assay that follows production of p24 gag antigen. All clones with M184V showed reduced replication kinetics (Fig. 4A), consistent with the reports that introduction of M184V markedly impairs replication kinetics (Wainberg et al., 1996; Yoshimura et al., 1999). Introduc-

Table 4
The effect of dCK expression on the EFdA antiviral activity[a]

Cell	EC_{50} (μM)[b]		
	AZT	ddC	EFdA
HT-1080	0.0032 ± 0.001	0.75 ± 0.22	0.00031 ± 0.0001
HT-1080/Ara-C^r	0.0027 ± 0.0005 (0.84)	84 ± 15 (112)	0.21 ± 0.03 (677)
HT-1080/Ara-C^r/dCK	0.0025 ± 0.00074 (0.78)	0.51 ± 0.16 (0.68)	0.000098 ± 0.000034 (0.32)

[a] SEAP activity in the culture supernatants were determined on day 2 after virus infection.
[b] The data shown are mean ± S.D. and fold increase in EC_{50} compared to HT-1080 is shown in parentheses.

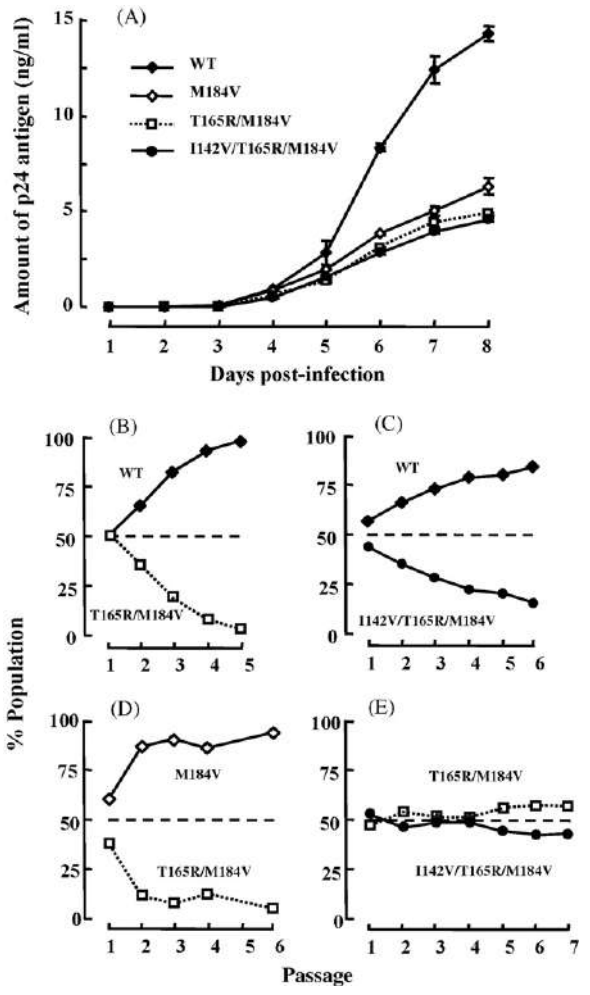


Fig. 4. Replication kinetics of HIV-1 clones with mutations. Production of p24 antigen in culture supernatant was determined with a commercially available p24 antigen kit. Profiles of replication kinetics (p24 production) of HIV-1_{WT} (closed diamonds), HIV-1_{M184V} (open diamonds), HIV-$1_{T165R/M184V}$ (open squares with broken line) and HIV-$1_{I142V/T165R/M184V}$ (open circles) were determined with MT-2 (A). Representative results from three independent triplicate determinations of p24 production with newly titrated viruses are shown. MT-2 cells were infected simultaneously with equal amounts of two HIV-1 clones to be compared. At each passage (5–6 days) the proviral sequences were determined and the percent population of each clone is reported at different passage; competition of WT and T165R/M184V (B); competition of WT with I142V/T165R/M184V (C); competition of M184V with T165R/M184V (D), and competition of T165R/M184V with I142V/T165R/M184V (E). At least two independent CHRAs were performed and are shown the representative results.

tion of T165R or I142V/T165R mutations in an M184V background (HIV-$1_{T165R/M184V}$ or HIV-$1_{I142V/T165R/M184V}$, respectively) further impaired HIV replication compared to HIV-1_{M184V}. I142V which enhanced EFdA resistance of HIV-$1_{T165R/M184V}$ (Table 2) conferred no replication rescue of HIV-$1_{T165R/M184V}$. To determine detailed replication kinetics, we performed CHRA which compares qualitatively viral replication. As shown in Fig. 4A, HIV-$1_{T165R/M184V}$ and HIV-$1_{I142V/T165R/M184V}$ showed reduced replication kinetics compared to HIV-1_{WT} (Fig. 4B and C). Replication kinetics ofHIV-$1_{I142V/T165R/M184V}$ was comparable to that of HIV-$1_{T165R/M184V}$, which showed further reduced replication kinetics compared to HIV-1_{M184V} (Fig. 4D and E). In another experiment, replication of HIV-$1_{I142V/T165R/M184V}$ was slightly decreased compared to HIV-$1_{T165R/M184V}$ (data not shown). These results suggest that introduction of three EdA mutations that also confers EFdA resistance impaired replication of HIV-1 in much greater extent compared to that of M184V.

4. Discussion

At present, HIV-1 variants containing NRTI-resistance mutations are widely observed not only in NRTI-experienced but also in NRTI-naïve patients. In such cases treatment failure is sometimes observed within short periods. The NRTI tenofovir, appears to be more effective against drug-experienced HIV-1 strains (Srinivas and Fridland, 1998). Unlike the other clinically available NRTIs, tenofovir has highly flexible acyclic ribose ring without a 3′-OH. Structural studies have suggested that the compact size of this inhibitor may contribute to the absence of highly resistant mutant strains against tenofovir (Tuske et al., 2004). Despite its unique structural profile, tenofovir is similar to other NRTIs in that it also lacks a 3′-OH group.

In contrast, the highly active 4′-E analogs such as EFdA retain the 3′-OH group of the canonical dNTP substrate. Similar to other NRTIs, they are also phosphorylated by cellular enzymes to their TP active form, which in turn serves as a substrate for HIV RT that incorporates them in an elongating primer during DNA synthesis. Following incorporation, replication is further inhibited by chain termination, although the specific mechanism of chain termination remains to be elucidated. Despite the fact that the 4′-E analogs have a 3′-OH like canonical dNTP substrates, cellular polymerases are likely to discriminate against these analogs, and not incorporate them during cellular DNA polymerization, as suggested by *in vitro* experiments with mitochondrial polymerase γ (Nakata et al., 2007). Alternatively, it is also possible that cellular proofreading systems excise the 4′-E analogs after their incorporation into cellular DNA.

The 3′-OH also plays an important role in phosphorylation of EdA analogs. Based on crystallographic results Sabini et al. reported that the interaction between 3′-OH of nucleosides and catalytic site of dCK was important for efficient nucleoside phosphorylation (Sabini et al., 2003). Alternatively, it is possible that the EFddA and EFd4A nucleosides that lack 3′-OH are poor substrates for HIV RT. The substitution at the 2-position of the purine base is also likely to contribute to highly potent *in vivo* activity of EF- or ECldA, possibly by preventing deamination of the inhibitor by ADA. ADA deaminates the adenine base into inosine, which is a poor substrate for cellular kinases. Similar ADA resistance has been reported for 2′-deoxy-2-chloroadenosine, a chemotherapeutic agent against hairy cell leukemia and chronic lymphocytic leukemia (Carson et al., 1980). ADA resistance may contribute to a longer intracellular half-life for EFdA-TP as compared to that of AZT (Nakata et al., 2007), indicating that substitution of 2-position plays an important role in sustained activity. When CEM cells were exposed to AZT or EFdA at concen-

tration of 0.1 μM, amounts of corresponding intracellular TP-forms were comparable (Nakata et al., 2007). However, inhibitory effect of EFdA in MT-4 and MAGI cells was approximately 40- and 15-fold superior compared to that of AZT (Tables 1 and 2). Taken together, HIV-1 RT appears to preferentially incorporate EFdA-TP compared to AZT-TP, although detailed enzymatic confirmation is needed. The parental EdA also seems to be a good substrate for HIV-1 RT; however, it may be subjected to deamination, resulting in comparable activity to AZT.

There are at least two mechanisms by which HIV RT can become resistant to NRTIs: first, HIV RT can acquire mutations at, or close to the dNTP-binding site, such that help it discriminate against NRTI-triphosphates, while it retains its ability to recognize the normal dNTP substrates (Huang et al., 1998). In the case of M184V/I the discrimination is based on steric conflict between a part of the inhibitor (the sulfur atom of the thioxolane ring in the case of 3TC), and the β-branched side chain of Val or Ile at the mutation site (Sarafianos et al., 1999). Mutations at other residues of the dNTP-binding site are responsible for discrimination of dideoxynucleosides from dNTPs during both the substrate-recognition (Martin et al., 1993) and the catalytic steps (Deval et al., 2002; Selmi et al., 2001). The other mechanism of NRTI resistance is based on an excision reaction (phosphorolysis) that unblocks NRTI-terminated primers using a molecule of ATP as the pyrophosphate donor (Meyer et al., 1999). The product of this reaction is a dinucleoside tetraphosphate and an unblocked primer that can continue viral DNA synthesis. In this case, the role of resistance mutations is to optimize binding of an ATP molecule that is used for the nucleophilic attack at the primer terminus. Most of AZT resistance as well as multi-NRTI resistance of RT with insertions at the fingers subdomain are thought to be based on an ATP-based unblocking mechanism. The insertions in RT destabilize the normally stable ternary complex (RT/template-primer/dNTP) and facilitate the ATP-mediated pyrophospholysis (Boyer et al., 2002). The fingers insertion mutant can excise all nucleotide analogs, with various degrees of efficiency.

Our molecular modeling studies are consistent with a mechanism of resistance to 4′-E analogs that involves steric hindrance between the 4′-E group of the inhibitors and the side chain of V184, reminiscent of the resistance mechanism to 3TC. While a single M184V mutation confers strong resistance to 3TC (>100-fold), it causes only moderate (8- to 13-fold) resistance to EFdA and ECldA (Table 2). This is consistent with our molecular modeling analysis where the bulky and rigid 4′-E moiety appears to cause some steric hindrance with the Val or Ile side chain at position 184 during incorporation of the 4′-E nucleotides by the M184V enzyme (Fig. 5). The steric interaction appears to be stronger during incorporation of 3TC-TP (Fig. 5E) than EFdA-TP (Fig. 5C). Interestingly, the M184V mutation appears to confer stronger resistance to 4′-methyl substituted nucleotides, than to the 4′-ethynyl substituted nucleotides (Kodama et al., 2001). The decreased resistance of 4′-ethynyl substituted compounds may be in part the result of compensatory favorable interactions of the longer ethynyl group with residues of the dNTP binding site, including Y115 and D185. Such interactions may moderate the effect of the steric interactions of the 4′-ethynyl with residue V184 in the M184V mutant. Resistance of M184V to dideoxy-derivatives such as EFddA was unexpectedly high (84-fold). Although we cannot explain the detailed mechanism of the difference in resistance, it is possible that the presence of a 3′-OH in the EFdA (but not in the EFddA and EFd4A) results in more stabilizing interactions with residues such as Q151 that compensate for the steric hindrance by M184V (Fig. 5D). Hence, EFddA and EFd4A may be easier to push out of the binding pocket than analogs with a 3′-OH. For stronger resistance to 4′-E-2-halo-dAs, other mutations at positions 142 and 165 in addition to the M184V are required (Table 2) to substantially decrease inhibitor binding. It should be noted that the T165R/M184V mutations were also observed during induction of resistant variants to parental compound EdA. Nitanda et al. also reported that resistant variants for 4′-Ed4T contain M184V with T165A (Nitanda et al., 2005). As shown in Fig. 5A, the effect of the T165R mutation seems to be through the loss of a hydrogen bond between the side chain of Q182 and the side chain OH moiety of T165. Instead, there may be a hydrogen bond between C=O of the main chain of 184 and Q182 in the case of T165R/A, which would affect the positioning of the residue in position 184. Interestingly, HIV-2 has an Arg residue at position 165, whereas HIV-1 has Thr. We could not find decreased susceptibility in HIV-1_{T165R} or HIV-2, indicating that R165 becomes relevant only when Val is at the 184 position. When this residue is Met (T165R in M184 background), resistance is not affected substantially (1.5- to 2-fold resistance, Table 2) because of the flexibility of the Met side chain. However, when the 184 residue is Val (T165R/M184V), the position of 184 may be affected in a way that exacerbates the steric interactions between the ethynyl group of the incoming EFdA and the side chain of V184, resulting in resistance to EFdA and the other 4′-E analogs (13–27-fold resistance, Table 2). At this point it is not clear why the I142V mutation further augmented the effects of M184V and T165R. Finally, as shown in Fig. 5B, there are no apparent substantial steric problems for binding of EFdA to HIV-$1_{M41L/T69SSG/T215Y}$ RT, and the enzyme–inhibitor interactions are likely to be similar to those with dNTP and consistent with the relatively low resistance observed with this variant (Table 2) that is known to cause strong excision-based NRTI resistance. Crystallographic studies with the RT resistant variants complexed with the inhibitors should provide more insights into the mechanism of resistance.

The M184V, one of three mutations associated with EFdA resistance, develops rapidly under therapy with 3TC and has been reported to alter several profiles of RT function, including decreased RT processivity (Back et al., 1996), reduced nucleotide-dependent primer unblocking (Gotte et al., 2000), and increased fidelity (Wainberg et al., 1996). These profiles result in impaired viral fitness, hypersensitivity to other NRTIs, especially AZT (Larder et al., 1995), and delayed appearance of mutations, respectively. Our results show that modest resistance to EdA comes at a significant cost for the virus: The I142V and T165R mutations reduced even further viral replication kinetics of M184V-containing virus. Furthermore, the virus containing these mutations retains the AZT hyper-susceptibility which is induced by

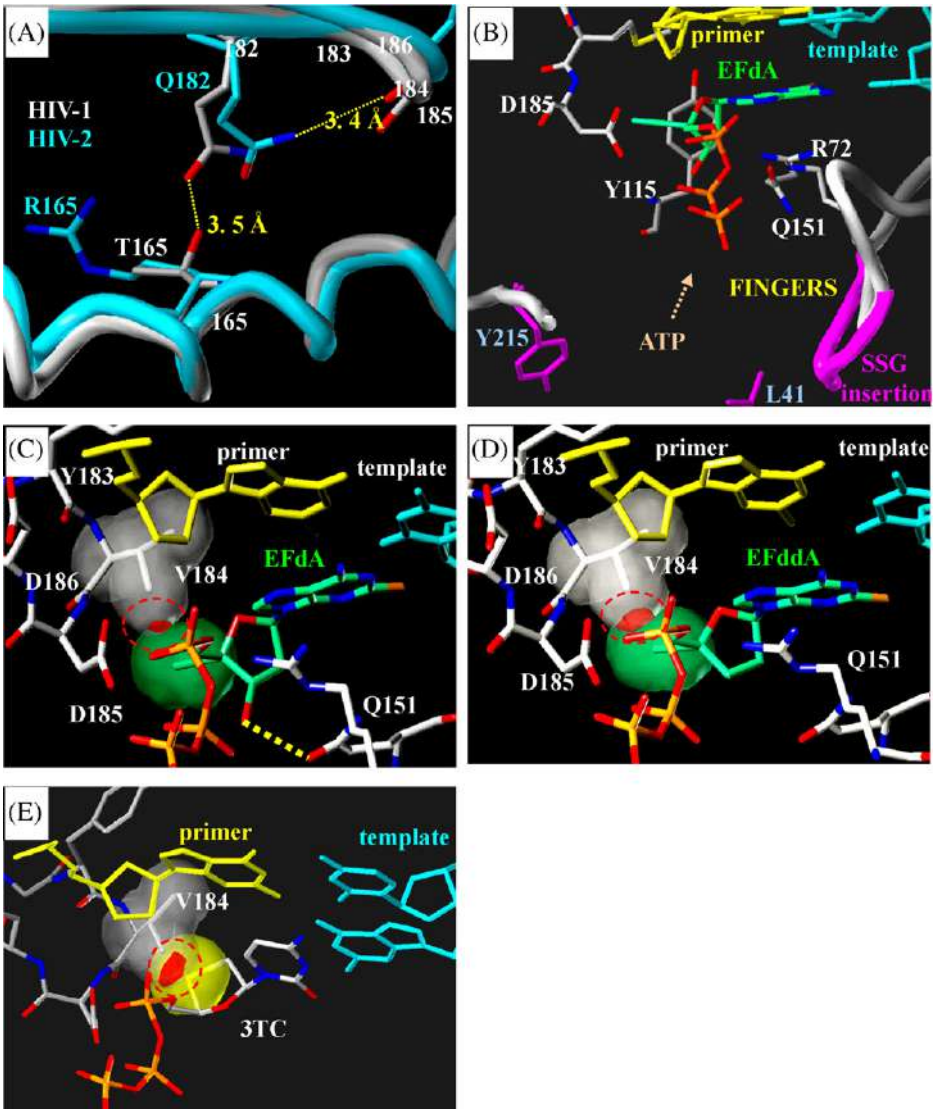


Fig. 5. Structural modeling of reverse transcriptase and compounds. (A) Superposition of the polymerase active sites of HIV-1 (gray) and HIV-2 (magenta) reverse transcriptases. Q182 makes a hydrogen bond with T165 in HIV-1. In the T165R mutant of HIV-1, the arginine side chain is expected to have a conformation similar to the one observed for R165 in HIV-2. In this context, Arg does not make a hydrogen bond with the side chain of residue 182 that may now interact with the main chain carbonyl of M184, which is at the immediate vicinity of the inhibitor-binding site. Such interaction may explain how the T165R mutation exacerbates the role of the M184V mutation in resistance to EFdA. (B) Proposed interactions of EFdA-triphosphate (TP) at the polymerase active site of the "fingers-insertion" NRTI-resistant HIV-1 RT, carrying the M41L/T69SSG/T215Y mutations. Possible steric interactions between the 4′-E group of EFdA-TP (C) or EFddA-TP (D) and the sulfur (S) of the pseudosugar ring of 3TC-TP (E). Van der Waals surfaces of 4′-E group (C and D) and S at sugar ring (E) are indicated in green and yellow. Possible steric interactions are shown as overalap of Van der Waal volumes of interacting atoms (in red).

M184V (Table 2), although further experiments are needed. These results suggest that the I142V and T165R mutations simply enhance resistance of M184V RT to EdA rather than optimize the viral fitness of the M184V virus. The increased cost for the virus to overcome inhibition pressure by EdA may have significant clinical benefits in the treatment of HIV infections.

Since EFdA is initially phosphorylated mainly by dCK and its activity was attenuated by addition of dC (data not shown), it is likely that dC analogs, such as 3TC and emtricitabine (FTC) that are mainly phosphorylated by dCK would act as a competitor of EFdA phosphorylation. However, one of dC analogs, apricitabine (ATC) showed little competition for the intracellular phosphorylation of 3TC and FTC (Bethell et al., 2007). Thus, interaction of NRTIs using identical phosphorylation enzymes should be carefully examined.

In conclusion, we have shown that the 2-halogen substituted EdAs have exceptionally potent subnanomolar antiviral activities. The 2-F substituted analog exhibited the highest potency and had a selectivity index significantly improved over that of approved NRTIs. In fact, results from our parallel studies with mice show no toxicity of EFdA (data not shown). The earlier studies also showed that a

parental nucleoside, EdA was not toxic in mice (Kohgo et al., 2004). The half-life of the intracellular TP form of EFdA is substantially extended (~17 h) compared to that of AZT (~3 h) (Nakata et al., 2007), suggesting that it may be possible to administer these inhibitors once a day. Further investigation may lead to their development as potential therapeutics against HIV infections.

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Antiviral Properties of Deazaadenine Nucleoside Derivatives

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Abstract: Viral infections have menaced human beings since time immemorial, and even today new viral strains that cause lethal diseases are being discovered with alarming frequency. One major example is HIV, the etiological agent of AIDS, which spread up in the last two decades. Very recently, other virus based diseases such as avian flu have spread fear around the world, and hemorrhagic fevers from central Africa serious threaten human health because of their very deadly effects. New antiviral agents are still greatly needed to counter these menaces. Many scientists are involved in this field of research, and many of the recently discovered effective antiviral compounds are nucleoside analogues. Among those derivatives, deazapurine nucleoside analogues have demonstrated potent inhibitory effect of viral replication.

This review reports on recently generated data from preparing and testing deazapurine nucleoside derivatives as inhibitors in virus replication systems. Although most of the reported data have been produced in antiHIV, antiHCMV, and antiHSV biological testing, very recently other new important fields of application have been discovered, all in topical subjects of strong interest. In fact, deazapurine nucleosides have been found to be active as chemotherapeutics for some veterinary systemic viral infections, for which no antiviral drugs are licensed yet. Furthermore, they demonstrated efficacy in the inhibition of Hepatitis C virus replication. Finally, these compounds showed high potency as virucides against Ebola Virus, curing Ebola infected mice with a single dose administration.

Keywords: deazaadenine nucleosides, antiviral agents, veterinary viral infections, Hepatitis C chemotherapy, Ebola Virus chemotherapy.

INTRODUCTION

Since the introduction of the concept and term of "nucleoside" [1], medicinal chemists have dedicated considerable effort to copying and modifying natural nucleosides, with the purpose of discovering nucleoside based drugs to fight various diseases, such as viral infections. A great number of drugs structurally related to nucleosides have been approved for clinical use and are consumed by millions of patients around the world [2-5]. Many of the nucleoside antiviral drugs that have been approved as therapeutic agents are formulated with compounds belonging to the family of purine nucleoside analogues.

Although the long history of nucleoside-based drugs started many decades ago, new derivatives are still greatly needed, especially as antiviral agents. This need is pushed, among other reasons, by the slow development of new antiviral vaccines. In fact, to date there are no licensed vaccines, for example, for Respiratory Syncitial Virus (RSV), Human Papilloma Virus (HPV), Herpes Simplex Virus types 1 and 2 (HSV-1, HSV-2), Varicella-Zoster Virus (VZV), Epstein-Barr Virus (EBV), Human CytoMegaloVirus (HCMV), and Hepatitis C Virus (HCV).

Although more than 25 drugs, many of which are structurally related to nucleosides (Table **1**), are used in the treatment of HIV infections, new drugs are nonetheless needed because existing ones have severe side effects and because of the emergence of viral resistance.

Another unmet medical need in antiviral research is an antiviral agent with a broad spectrum of action, which would be of great importance since often there are strong difficulties in quickly and accurately diagnosing a viral infection. This is dramatically true in AIDS patients, who are commonly superinfected with other viruses, a fact that highlights the importance of a broad spectrum antiviral drug as medical tool. Researchers from academia and from pharmaceutical industry are engaged in many paths of enquiry in the search for new antiviral nucleosides [6-9].

Among the many different approaches to discovering new antiviral purine nucleosides, attention has been dedicated mostly to modification of the heterocycle and to the presence of hydroxyl groups in the sugar moiety. In particular, deaza analogues of natural purine nucleosides have been thoroughly investigated from both the synthetic and the biological point of view. In fact, many papers have been published on the preparation of 1-deazapurine [6, 10-25], 3-deazapurine [6, 20, 26-32], 7-deazapurine [6, 33-41], 1,3-dideazapurine [6, 42-45], 1,7-dideazapurine [46, 47], and 3,7-dideazapurine [48-52] nucleosides.

Synthesized compounds have shown to possess biological activity not only as inhibitors of viral replication, but also as adenosine receptor ligands [6, 10, 22, 23, 25, 53-56], antiproliferative agents [11, 14, 27, 32, 57-62], and inhibitors of different classes of enzymes [21, 23, 27, 38, 63-66].

In the following sections, the antiviral properties of deazapurine nucleosides are reviewed in detail, with each section dedicated to a specific deazapurine ring, i. e. 1-deaza, 3-deaza, etc.

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Table 1. Drugs Used in the Treatment of HIV Infection [4, 5]

Brand Name	Generic Name	Manufacturer Name	Approval Date
Agenerase	amprenavir	GlaxoSmithKline	15-Apr-99
Aptivus	tipranavir	Boehringer Ingelheim	22-Jun-05
Combivir	lamivudine and zidovudine	GlaxoSmithKline	27-Sep-97
Crixivan	indinavir, IDV, MK-639	Merck	13-Mar-96
Emtriva	FTC, emtricitabine	Gilead Sciences	02-Jul-03
Epivir	lamivudine, 3TC	GlaxoSmithKline	17-Nov-95
Epzicom	abacavir/ lamivudine	GlaxoSmithKline	02-Aug-04
Fortovase	saquinavir	Hoffmann-La Roche	7-Nov-97
Fuzeon	enfuvirtide, T-20	Hoffmann-La Roche & Trimeris	13-Mar-03
Hivid	zalcitabine, ddC, dideoxycytidine	Hoffmann-La Roche	19-Jun-92
Invirase	saquinavir mesylate, SQV	Hoffmann-La Roche	6-Dec-95
Kaletra	lopinavir and ritonavir	Abbott Laboratories	15-Sep-00
Lexiva	Fosamprenavir Calcium	GlaxoSmithKline	20-Oct-03
Norvir	ritonavir, ABT-538	Abbott Laboratories	1-Mar-96
Rescriptor	delavirdine, DLV	Pfizer	4-Apr-97
Retrovir	zidovudine, AZT, azidothymidine, ZDV	GlaxoSmithKline	19-Mar-87
Reyataz	atazanavir sulfate	Bristol Myers-Squibb	20-Jun-03
Sustiva	efavirenz	Bristol Myers-Squibb	17-Sep-98
Trizivir	abacavir, zidovudine, and lamivudine	GlaxoSmithKline	14-Nov-00
Truvada	tenofovir disoproxil/emtricitabine	Gilead Sciences, Inc.	02-Aug-04
Videx EC	enteric coated didanosine	Bristol Myers-Squibb	31-Oct-00
Videx	didanosine, ddI, dideoxyinosine	Bristol Myers-Squibb	9-Oct-91
Viracept	nelfinavir mesylate, NFV	Agouron Pharmaceuticals	14-Mar-97
Viramune	nevirapine, BI-RG-587	Boehringer Ingelheim	21-Jun-96
Viread	tenofovir disoproxil fumarate	Gilead	26-Oct-01
Zerit	stavudine, d4T	Bristol Myers-Squibb	24-Jun-94
Ziagen	abacavir	GlaxoSmithKline	17-Dec-98

1-DEAZAPURINE NUCLEOSIDE ANALOGUES

1-Deazapurine nucleosides have been thoroughly studied, from both the synthetic and the biological point of view. In spite of that, only few recent papers have dealt with antiviral properties of 1-deazaadenosine derivatives.

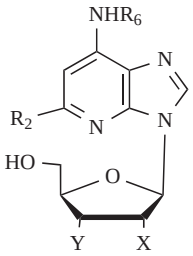
Compounds reported in Table **2** were tested for their ability to inhibit HIV-1 and HSV-1 replication in C-8166 and Vero cells, respectively [18]. In the latter test, only a few nucleosides (**2**, **25**, **28**, **29**) showed an appreciable antireplicative activity (in the range 5-15 microM), but no selectivity could be demonstrated. Most compounds showed a not negligible cytotoxicity for Vero cells.

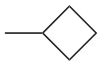
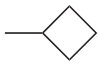
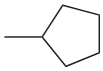
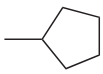
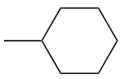
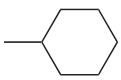
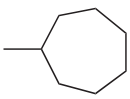
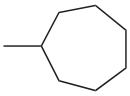
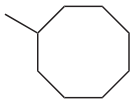
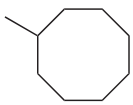
In the anti-HIV assay, results showed that N^6-unsubstituted (**1**, **2**, **17**, **18**, **33**, **34**) and N^6-methyl (**3**, **4**, **19**, **20**, **35**, **36**) derivatives were inactive, whereas nucleosides bearing cycloalkyl substituents possessed moderate to good anti-HIV-1 activity, compared to the reference compound 2',3'-dideoxyadenosine (ddA). The pattern of activity is dependent on the structure of the sugar moiety. In particular, the presence of a higher sized cycloalkyl substituent (C7 and C8) brought about the highest activity in every series, with the 2'-deoxy derivatives being the most potent compounds. For purine derivatives, in a series of 6-alkoxypurine nucleosides, it has been reported that antiviral activity increased with the length of the alkoxy chain [67]. However, the most active in the series were as active as the parent compound ddI. In the deaza series, the parent N^6 unsubstituted adenine derivatives are completely inactive.

2',3'-Dideoxy nucleosides were more effective than the corresponding ribose nucleosides, with the highest activity once again shown by compounds bearing larger ring size substituents. Worth to note it is the fact that compounds **45**, **47**, and **48** were slightly more potent, although less selective, than ddA itself.

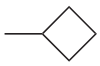
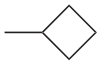
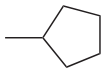
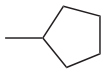
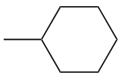
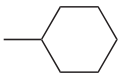
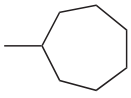
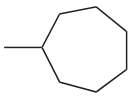
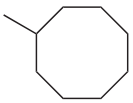
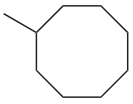
Furthermore, a chlorine atom in 2 position improved both activity and therapeutic index in all series. This result points

Table 2. Antiviral Activity of 1-Deazaadenine (Deoxy)Ribofuranoside Analogues (μM) [18]

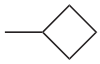
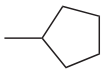
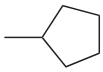
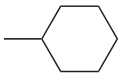
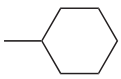
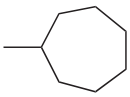
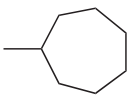
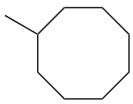
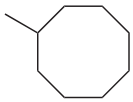


Cp	R_2	R_6	X	Y	HIV[a]	TC_{50}[b]
1	Cl	H	OH	OH	>100	>100
2	H	H	OH	OH	>100	2
3	Cl	CH_3	OH	OH	>100	>100
4	H	CH_3	OH	OH	>100	>100
5	Cl		OH	OH	30	>100
6	H		OH	OH	>100	>100
7	Cl		OH	OH	100	>100
8	H		OH	OH	>100	>100
9	Cl		OH	OH	10	100
10	H		OH	OH	16	>100
11	Cl		OH	OH	20	50
12	H		OH	OH	100	400
13	Cl		OH	OH	8	50
14	H		OH	OH	20	100
15	Cl		OH	OH	2	40
16	H		OH	OH	4	50
17	Cl	H	H	OH	>100	>100

(Table 2) contd…..

Cp	R_2	R_6	X	Y	HIV[a]	TC_{50}[b]
18	H	H	H	OH	>100	>100
19	Cl	CH_3	H	OH	>100	>100
20	H	CH_3	H	OH	>100	>100
21	Cl		H	OH	8	>100
22	H		H	OH	>100	>100
23	Cl		H	OH	4	>100
24	H		H	OH	20	>100
25	Cl		H	OH	0.8	7
26	H		H	OH	40	>100
27	Cl		H	OH	4	50
28	H		H	OH	8	100
29	Cl		H	OH	0.2	5
30	H		H	OH	30	80
31	Cl		H	OH	2	40
32	H		H	OH	4	40
33	Cl	H	H	H	>100	>100
34	H	H	H	H	>100	>100
35	Cl	CH_3	H	H	>100	>100
36	H	CH_3	H	H	50	100
37	Cl		H	H	>100	>100
38	H		H	H	100	>100
39	Cl		H	H	4	>100

(Table 2) contd…..

Cp	R_2	R_6	X	Y	HIV[a]	TC_{50}[b]
40	H		H	H	50	>100
41	Cl		H	H	20	50
42	H		H	H	80	>100
43	Cl		H	H	4	20
44	H		H	H	40	50
45	Cl		H	H	0.8	8
46	H		H	H	8	40
47	Cl		H	H	0.4	10
48	H		H	H	0.8	10
ddA[c]					2	>100

[a]Concentration of compound able to lower virus effects by 50%; [b]Concentration of compound which reduced cell growth by 50%; [c]2',3'-dideoxyadenosine.

out an additional difference between the purine and the 1-deazapurine systems, since it has been reported that a chlorine atom in 2 position of both ddA and dideoxydidehydroadenosine (ddeA) decreased antiretroviral activity and increased host cell toxicity [68].

Some of the 1-deazanucleosides reported in Table **2** have also been tested for their ability to inhibit the replication of two animal Lentivirus, with the purpose of finding a pharmaceutical therapy for infections that represent a not negligible economic loss for farming industry, and an animal system for testing potential human antiviral drugs [69].

The two chosen virus systems were:

- The Maedi Visna Virus (MVV), responsible for serious illnesses in sheep, which shows striking similarity in biochemical and genomic terms with Human Immunodeficiency Virus (HIV) [70, 71];
- The Bovine HerpesVirus 1 (BHV-1), one of the most widespread pathogenic agents in cattle, and very similar to human Herpes Virus [72].

Results, reported in Table **3**, demonstrated that all tested 1-deazaderivatives were inactive against BHV-1, whereas most of them showed efficacy against MVV higher than the reference compound 3TC; in general, in the MVV antireplicative assay, a chlorine atom in 2 position and a cycloalkyl substituent in N^6 brought about an increase in antireplicative potency, while maintaining cytotoxicity to a low level [70].

3-DEAZAPURINE NUCLEOSIDE ANALOGUES

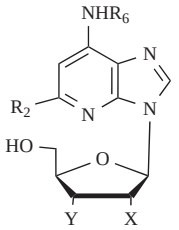
More than two decades ago, Montgomery and coworkers showed that carbocyclic 3-deazaadenosine (**53**) behave as an inhibitor of S-adenosylhomocysteine hydrolase (AdoHcy hydrolase) and possesses antiviral activity against HSV-1, Vaccinia Virus, and HL-23 C-type virus [73]. A few years lather, it was shown that sugar "dissected" analogs of carbocyclic 3-deazaadenosine were still efficacious on the same biological systems, although with lower figures [26]. The most active of reported compounds (Table **4**) was **55**, with an inhibitory potency on AdoHcy hydrolase similar to that of parent compound carbocyclic 3-deazaadenosine and an interesting antireplicative efficacy on Vaccinia Virus. It is evident from data that, for the enzyme inhibitory activity, crucial is the presence of both the 2' and 3' hydroxyl groups (**54** and **57** *vs* **55**).

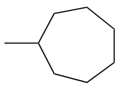
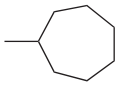
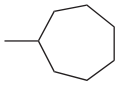
Few years later, Daelemans and coworker reported that some 3-deazapurine nucleosides were inhibitors of HIV Type 1 replication [74]. In detail (Table **5**), a close correlation was found among the inhibitory effect of the compounds on AdoHcy hydrolase activity, on HIV-1 replication in HeLa CD4+ LTR-LacZ cells, and on the HIV-1 Tat-dependent and –indipendent transactivation of the long terminal repeat, whereas no inhibitory effect was seen on HIV-1 Reverse Trancriptase or on a Tat-indipendent cytomegalovirus promoter [74]. The results suggest that AdoHcy hydrolase and the associated S-adenosylmethionine-dependent methylation mechanism play a role in the HIV replication. Worth to note it is the fact that 3-deazaadeenine derivatives **58** and **59** showed about 5 times higher efficacy than the corresponding purine counterparts [74]. The lack of hydroxymethyl group in 4' position brought about a diminution of 10-fold in enzyme inhibitory activity (**59** *vs* **53**), whereas an opposite effect is evident (25-fold increase) on virus inhibition.

In more recent years, Bray *et al.* have reported that C-c^3Ado and c^3-Neplanocin A are able to completely protect from death immunocompetent mice infected with the lethal Ebola Zaire (EBO-Z) virus (Table **6**) [75]. In particular, c^3-Neplanocin A administration caused 100% of survival (5 surviving out of 5 treated) at a dose as low as 0. 25 mg/kg. Treatment of EBO-Z infected Severe Combined ImmunoDeficient (SCID) mice brought about an evident increase of survival time, although without elimination of infecting agents. The research demonstrated also that an early administration (days 0, 1 or 2 after infection) is necessary for the efficacy of treatment [75]. As shown in Table **6**, c^3-Neplanocin A is active at much lower doses than C-c^3Ado.

Liu and coworkers have reported a number of halogen substituted 3-deazapurine nucleoside analogues (Table **7**), which were tested for their antireplicative activity on HBV and HIV-IIIB [31]. Two compounds of the series showed some antireplicative efficacy on HBV, whereas no appreciable activity against HIV was found [31].

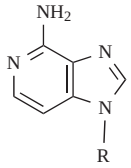
Table 3. Antiviral Activity of 1-Deazaadenosine Analogues Against Animal Virus Systems [70]

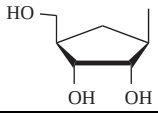
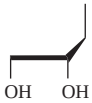
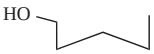
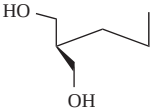


Cp	R_2	R_6	X	Y	BHV-1[a,b]	TC_{50}[c]		MVV[a,d]	TC_{50}[c]
9	Cl		OH	OH	>50	180		0.50	260
17	Cl	H	H	OH	>50	105		15	>400
21	Cl		H	OH	>50	185		2.7	236
29	Cl		H	OH	>50	120		2.7	236
49	Cl	H	OH	H	>50	140		0.15	>400
50	H	H	OH	H	>50	260		4.1	>400
51	Cl		OH	H	>50	191		0.15	145
52	H		OH	H	>50	293		0.50	210
3TC[e]								5	>300

[a]Concentration of compound able to lower virus effects by 50%; [b]BHV-1 refers to Bovine Herpes Virus type 1; [c]Concentration of compound which reduced cell growth by 50%; [d]MVV refers to Maedi Visna Virus, a retrovirus similar to HIV; [e]2',3'-dideoxy-3'-thiacytidine.

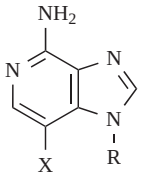
Table 4. Biological Properties of 3-Deazaadenosine Analogues [26, 73]

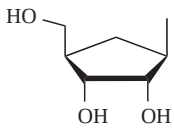
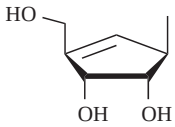
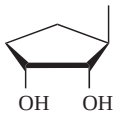
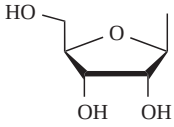
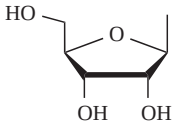


Cp	R	% enzyme inhibition[a]	% virus inhibition[b]
53 (C-c^3Ado)		100	95
54		12	33
55		84	53
56		11	51
57		12	27

[a]AdoHcy hydrolase inhibition efficacy at 0.2 mM concentration of tested compounds; [b]Vaccinia Virus antireplicative efficacy of 10 microM concentration of tested compounds.

Table 5. Biological Properties of 3-Deazaadenosine Analogues; nt = Not Tested (μM) [74]



Cp	R	X	Enzyme inhib. (IC_{50}, μM)[a]	Virus inhib. (IC_{50}, μM)[b]
53 (C-c^{3}Ado)		H	0.018	7.4
58 (c^{3}Neplanocin A)		H	0.031	0.41
59 (c^{3}DHCaA)		H	0.21	0.29
60 (NKO-6-16-3)		F	13	nt
61 (NOM-14-6-1)		Cl	21.7	nt

[a]Concentration of the inhibitor required for 50% inhibition of AdoHcy hydrolase activity; [b]Concentration of the inhibitor required for 50% inhibition of β-Gal Expression driven by the HIV-1 LTR in HIV-1-infected CD4$^+$ Hela Cells.

Table 6. Effect of Various Doses of C-c^{3}Ado and c^{3}Neplanocin A on Survival of Adult, Immunocompetent BALB/c Mice Infected with EBO-Z (300 LD_{50}) and Treated on Day 1 Postinfection; For Structure of Compounds 53 and 58 See Table 5 [75]

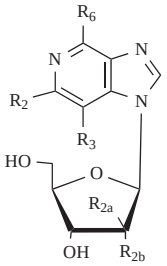
Drug	Dose (mg/kg)	Survivors/ total	Percent surviving
53 (C-c^{3}Ado)	80	5/5	100
	40	4/5	80
	20	4/5	80
	10	0/5	0
58 (c^{3}Neplanocin A)	1	5/5	100
	0.5	5/5	100
	0.25	5/5	100
	0.125	2/5	40
Placebo	-	0/5	0
None	-	0/5	0

Quite recently, Gordon and colleagues reported anti-HIV-1 efficacy of six 3-deazaadenosine analogues, comparing their activity with that of two well established adenine counterparts (Table **8**) [76]. The anti-HIV-1 effect was determined quantifying the inhibition of HIV-1 p24 antigen production in Peripheral Blood Mononuclear Cells (PBMC) infected with HIV-1 strains A012 or A018. On both strains, **58** and Neplanocin A were the most potent inhibitors of antigen production, with practically identical figures, and efficacy data of tested compounds were almost superimposable. 3-DeazaAdo (**73**) and carbocyclic 3-DeazaAdo (**53**) were also potent inhibitors, with IC_{50} in the submicromolar range. Dideoxyderivatives were at least 10-fold less potent than the ribose counterparts (**75** *vs* **73** and **76** *vs* **53**). AdoHcy hydrolase inhibition showed a potency rank order very close to the HIV antireplicative efficacy. The coefficient of correlation (r^2) between enzyme inhibition and antireplicative effect on either HIV strains was 0.9, when compound **74** was excluded from the analysis [76].

7-DEAZAPURINE NUCLEOSIDE ANALOGUES

In the field of 7-deazapurine derivatives, Townsend and coworkers have invested considerable effort, and found a number of analogues and derivatives of the naturally occurring 7-deazapurine antibiotic tubercidin, toyocamycin, and sangivamycin.

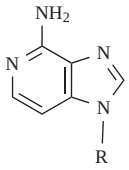
Table 7. Anti-HBV and Anti-HIV Activity of 3-Deazaadenosine Analogues [31]



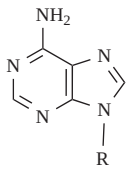
Cp	R_2	R_3	R_6	R_{2a}	R_{2b}	HBV inhibition (EC_{50}, μM)[a]	HIV inhibition (EC_{50}, μM)[a]
62	F	H	NH_2	H	OH	>10	>100
63	F	H	$NHCH_3$	H	OH	>10	>100
64	F	H	NH_2	OH	H	>10	>100
65	H	F	NH_2	H	OH	>10	>100
66	H	Cl	NH_2	H	OH	>10	>100
67	H	F	NH_2	H	OH	9.2	>100
68	H	Cl	NH_2	H	OH	>10	>100
69	F	F	NH_2	H	OH	>10	>100
70	F	Cl	NH_2	H	OH	7.5	>100
71	NH_2	Cl	OH	H	OH	>10	>100
72	NH_2	Br	OH	H	OH	>10	>100

[a]Concentration of compound able to lower virus effects by 50%.

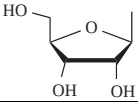
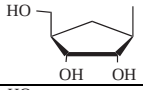
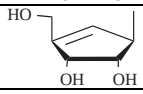
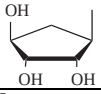
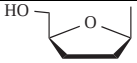
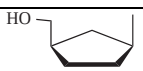
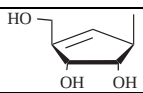
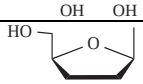
Table 8. Biological Properties of 3-Deazaadenosine Analogues [76]



53, 58, 73-76

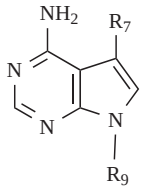


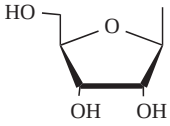
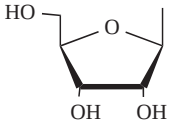
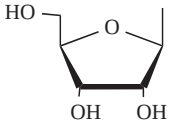
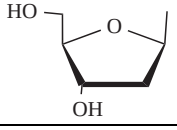
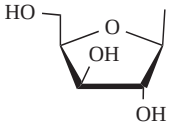
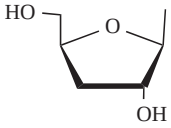
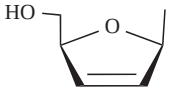
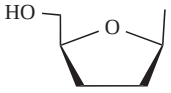
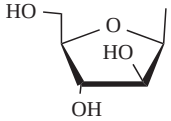
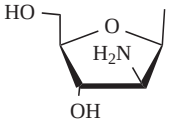
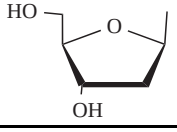
Neplanocin A, 2',3'-dideoxyAdo

Cp	R	Enzyme inhib. (IC_{50}, μM)[a]	Virus inhib. (IC_{50}, μM)[a]	
			A012 strain	A018 strain
73 (c^3Ado)		3.9	0.15	0.20
53 (C-c^3Ado)		0.24	0.14	0.22
58 (c^3Neplanocin A)		0.023	0.010	0.016
74 (c^3Noraristeromycin)		0.83	3.48	2.84
75 (2',3'-Dideoxyc^3Ado)		30.1	4.8	2.5
76 (C-2',3'-Dideoxyc^3Ado)		50.5	3.7	2.0
Neplanocin A		0.007	0.011	0.018
2',3'-DideoxyAdo		28.0	6.3	4.8

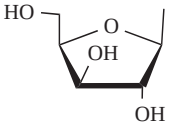
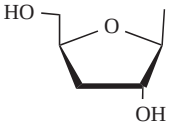
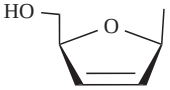
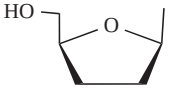
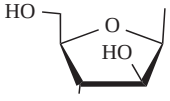
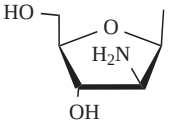
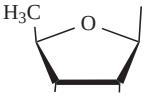
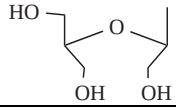
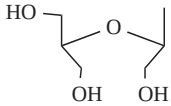
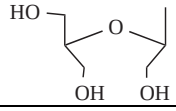
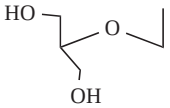
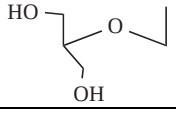
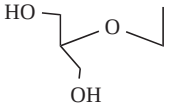
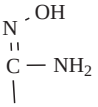
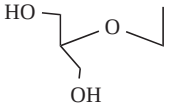
[a]Concentration of the inhibitor required for 50% inhibition of AdoHcy hydrolase activity.

Table 9. Biological Properties of 7-Deazaadenosine Analogues; nt = Not Tested [77-79]

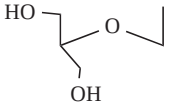
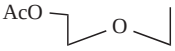
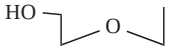
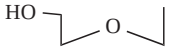
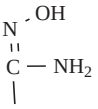
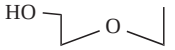
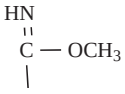
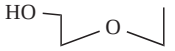
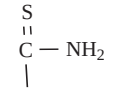
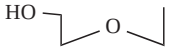
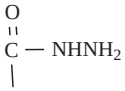
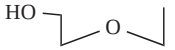


Cp	R_7	R_9	Virus inhib. (IC_{50}, μM)[a]		Ref.
			HCMV	HSV-1	
77 (Tubercidin)	H		0.5	nt	77
78 (Toyocamycin)	CN		0.05	nt	77
79 (Sangivamycin)			0.3	nt	77
80	CN		35	4.5[b]	78
81	CN		0.3	0.4[b]	78
82	CN		0.4	2.6[b]	78
83	CN		5.1	41[b]	78
84	CN		19	21[b]	78
85	CN		1.7	29[b]	78
86	CN		14	320[b]	78
87			5.7	8.2[b]	78

(Table 9) contd…..

Cp	R_7	R_9	Virus inhib. (IC_{50}, μM)[a]		Ref.
			HCMV	HSV-1	
88			0.6	1.9[b]	78
89			0.4	2.6[b]	78
90			2.7	14[b]	78
91			19	21[b]	78
92			1.7	29[b]	78
93			14	320[b]	78
94			3.1	2.5[b]	78
95	H		>100	>100	77
96	CN		>100	>100	77
97			>100	>100	77
98	H		>100	>100	77
99	CN		>320	>100	77
100			144	>100	77
101			>100	>100	77

(Table 9) contd…..

Cp	R_7	R_9	Virus inhib. (IC_{50}, μM)[a]		Ref.
			HCMV	HSV-1	
102			8	>100	77
103	CN		>100	>100	79
104	CN		>100	>100	79
105			>320	>100	79
106			>100	>100	79
107			>100	>100	79
108			11	100	79
109			>100	>100	79

[a]Concentration of the inhibitor required for 50% inhibition of virus plaque formation; [b]All compounds were assayed by ELISA in quadruplicate.

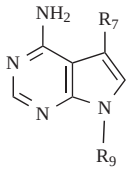
Derivatives closely related to tubercidin, toyocamycin, and sangivamycin were prepared and tested on the same antiviral assays [77-79]. Parent compounds **77**, **78**, and **79** were very active in inhibiting replication of Human CytoMegalo Virus (HCMV), but were also very cytotoxic [77] (Table **9**). Among compounds **80**-**94**, tiosangivamycin analogues were on the whole more effective than corresponding toyocamycin derivatives [78] (Table **9**). Both in toyocamycin and sangivamycin series the hydroxyl group in 2' seems to be crucial: in fact, compounds **81** and **82**, which conserved the OH in 2' with the correct orientation as the parent toyocamycin, are the most active derivatives in the series. A similar result is evident also in the tiosangivamycin series (**88** and **89**). Seco-ribose (**95**-**97**) and (dihydroxypropoxy)methyl derivatives (**98**-**102**) were inactive, except for compound **102**, which was as active as ganciclovir and showed no appreciable cytotoxicity [77]. Also, 7[(2-hydroxyethoxy)methyl] derivatives (**103**-**109**) showed no antiviral efficacy, except for compound **108**, which possessed some activity along with no cytotoxicity [79] (Table **9**).

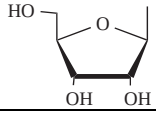
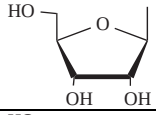
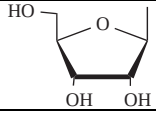
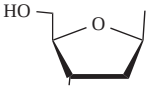
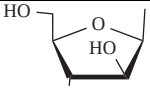
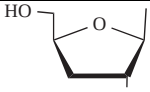
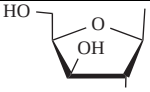
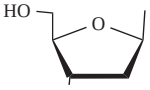
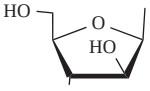
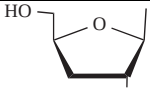
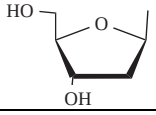
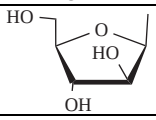
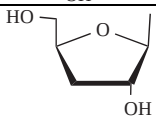
Previously, De Clerq and coworkers had tested tubercidin (**77**), toyocamycin (**78**), sangivamycin (**79**) and some sugar-modified analogues (**80**, **82**, **85**, **110**-**116**) on a wide panel of replicating viruses [80, 81] (Table **10**). Overall, sugar modified derivatives were much less toxic on cells used for virus replication, although they were also less effective as antiviral drugs. The only sugar modified nucleoside that maintains efficacy similar to the parent compound on some virus strains is the xylotubercidin (**113**) [80, 81] (Table **10**).

Derivatives with small substituents in 7 position were also prepared and tested against HCMV and HSV-1 [82, 83] (Table **11**). Compounds **117**, **118**, **120**-**122**, and **128**-**130** proved active against HCMV and HSV-1. The 6-aminoderivatives (**117**, **119**-**123**) showed higher efficacy than 6-hydroxylamino counterparts (**118**, **127**-**130**), whereas the 6-alkylamino analogues (**124**-**126**) were devoid of any activity. Among substituents in 7 position, bromo (**118**, **121**, **124**, **126**, **129**) was the most efficacious for antireplicative activity against both HCMV and HSV-1 [82, 83] (Table **11**).

Ara-toyocamycin, ara-sangivamycin, and some derivatives (**85, 115, 132**-**147**) were also prepared and tested as antivirals [84-86] (Table **12**). Once again, tested nucleosides were more active on HCMV than on HSV-1; in particular, parent compounds **85** and **115** showed a potency 20-fold higher in inhibiting HCMV than HSV replication. Quite peculiar is the fact that compounds **143** and **145**, α anomers, possess high efficacy against HCMV replication. In particular, **143** is surprisingly 4 times more active than the β counterpart **142** [84-86] (Table **12**).

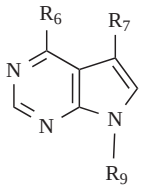
Table 10. Biological Properties of 7-Deazaadenosine Analogues [80, 81]

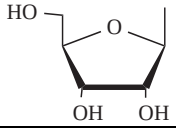
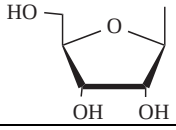
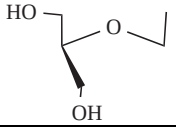
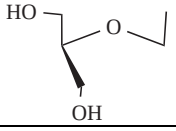
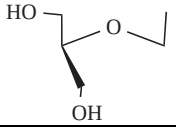
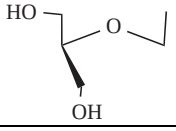
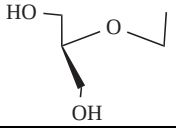
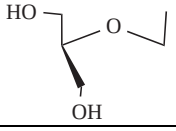
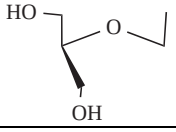
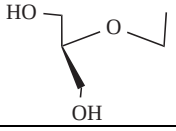
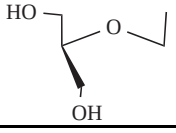


Cp	R_7	R_9	Virus inhib. (MIC_{50}, μg/ml)[a]					
			HSV-1[b]	HSV-2[b]	VV[b]	VSV[b]	B-4[c]	polio-1[c]
77 (Tubercidin)	H		0.1	0.1	0.07	0.02	0.07	0.02
78 (Toyocamycin)	CN		>0.04	>0.04	≥0.04	>0.04	≥0.4	≥0.4
79 (Sangivamycin)			0.07	0.07	0.02	0.02	>0.4	0.07
110 (2'-De-tubercidin)	H		50	40	10	125	>400	150
111 (Ara-tubercidin)	H		125	40	40	>400	>400	>400
112 (3'-De-tubercidin)	H		>10	>10	1.5	>10	2	2
113 (Xylo-tubercidin)	H		0.2	0.07	0.3	>10	≥4	≥4
80 (2'-De-toyocamycin)	CN		≥1	0.3	0.1	0.5	>400	150
85 (Ara-toyocamycin)	CN		7	2	1	≥10	≥10	≥10
82 (3'-De-toyocamycin)	CN		≥0.4	≥0.4	0.1	>1	0.7	≥1
114 (2'-De-sangivamycin)			2	1	0.4	2	>10	>10
115 (Ara-sangivamycin)			4	1	0.2	3	>10	>10
116 (3'-De-sangivamycin)			>10	>10	2	>10	≥40	≥40

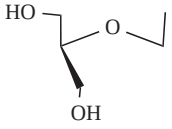
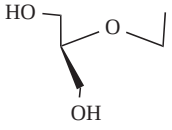
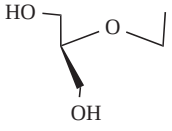
[a]Minimum inhibitory concentration required to reduce virus-induced cytopathogenicity by 50%; when the MIC_{50} is higher than the Minimum Toxic Concentration on cells used for replication it is shown as ">"; [b]Virus replicating on Primary Rabbit Kidney Cells; [c]Virus replicating on HeLa Cells.

Table 11. Biological Properties of 7-Deazaadenosine Analogues; nt = Not Tested [82, 83]



Cp	R_6	R_7	R_9	Virus inhib. (IC_{50}, μM)[a]	
				HCMV	HSV-1
117 (Br-Tubercidin)	NH_2	Br		0.5	nt
118	NHOH	Br		5.6	nt
119	NH_2	H		>100	>100
120	NH_2	Cl		9.8	16
121	NH_2	Br		1.6	2
122	NH_2	I		3.1	4
123	NH_2	CH_3		>100	>100
124	$NHCH_3$	Br		>100	>100
125	NHC_2H_5	Cl		>100	>100
126	NHC_2H_5	Br		>100	>100
127	NHOH	H		>100	nt

(Table 11) contd…..

Cp	R_6	R_7	R_9	Virus inhib. (IC_{50}, μM)[a]	
				HCMV	HSV-1
129	NHOH	Br		1.2	2.9
130	NHOH	I		18	30
131	H	H		>100	nt

[a]Concentration of the inhibitor required for 50% inhibition of virus plaque formation.

Very recently, Olsen and collaborators have identified the 7-deaza-2'-C-methyladenosine (**148**) as a very promising agent for the treatment of Hepatitis C [87-89] (Fig. **1**). In fact, **148** possess an IC_{50} on HCV replication of 108 nM, and undetectable cytotoxicity up to 1 mM, with a consequent therapeutic index of at least 10,000. Furthermore, **148** showed high ability in cell penetration, and subsequent phosphorylation. It was also tested for its efficacy in inhibiting a number of viruses encoding philogenetically-related polymerases; also metabolism, pharmacokinetic properties, and bio-availability of **148** were studied in detail [87, 88] (Fig. **1**).

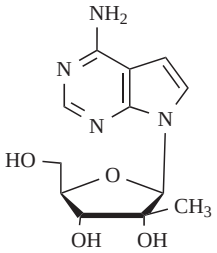


Fig. (1). 7-Deaza-2'-C-methyladenosine (**148**).

1,3-DIDEAZAPURINE NUCLEOSIDE ANALOGUES

In the field of 1,3-dideazapurine (benzimidazole) nucleosides, most of the work done on their properties as potential antiviral drugs comes from efforts by Townsend and coworkers, who have reported a number of substituted 5,6-dichloro benzimidazole nucleosides [90, 91] (Table **13**). Compounds bearing a chloro or bromo substituent in 4 position (**172**, **174**), also in the acetylated form (**171**, **173**), showed high efficacy against HCMV. Increasing polarity (**172** *vs* **176**) or steric hindrance (**174** *vs* **161**) brought about an evident loss of activity.

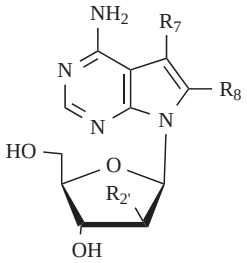
In a more detailed search for antiviral drugs among benzimidazole ribonucleosides, Zou and coworkers prepared a number of nucleosides variously substituted in positions 2, 4, 5, and 6 [92-94] (Table **14**). Results showed that anti-HSV-1 efficacy was very low, if any, whereas inhibition of HCMV replication was favored by the presence of halogen substituents (**172**, **174**, **187**, **188**, **195**, **198**, **199**), except for fluorine (**197**). The presence of substituents other than halogen, like amino (**178**, **179**, **190**), thio (**183-185**, **191-193**), azido (**189**), or methyl (**200**) brought about a dramatic loss of efficacy [92-94] (Table **14**).

Lyxose benzimidazole nucleosides were also assayed as antireplicative drugs on HCMV and HSV-1 (Table **15**) [95]. Overall, 5'-deoxylyxose nucleosides (**217-222**) were more active than the corresponding lyxose analogues (**201-216**). 2-Halo derivatives (**201**, **202**, **205**, **206**, **217-220**) showed higher efficacy in both series, as compared with alkylamino (**203**, **204**, **207-210**, **221**, **222**) or thio (**211-216**) counterparts. Among thio derivatives, only the S-Benzyl group (**213**, **214**) brought about some antireplicative activity, demonstrating once again that in 2 position only apolar substituents are tolerated (Table **15**) [95].

Other 5,6-dichlorobenzimidazole derivatives, substituted in 2 and/or 4 position, bearing variously modified sugar (deoxy-, fluoro-, acetyl-, trityl-, benzyl-derivatives) were prepared and tested on the above mentioned viral systems (Table **16**) [96-98]. Results showed that the presence of small protecting groups on the oxygen of the sugar did not affect efficacy (**224** *vs* **225** and **226** *vs* **227**), whereas larger groups produced nucleosides with much lower antireplicative activity (**235** and **236** *vs* **172**). Removal of the hydroxymethyl group in 4' brought about some increase in efficacy (**225** *vs* **172** and **227** *vs* **174**), whereas substitution of a hydroxyl group of the sugar moiety with a fluorine atom provoked an evident loss in activity (**237-242**) (Table **16**) [96-98].

Very recently, Sinha and coworkers tested some benzimidazole nucleosides for their antireplicative efficacy against a large number of viral strains (Table **17**) [99]. Tested compounds showed no efficacy in inhibiting viral replication.

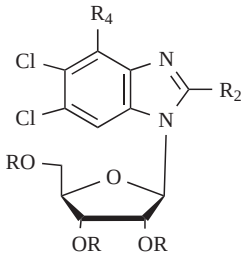
Table 12. Biological Properties of 7-Deazaadenosine Analogues; nt = Not Tested [83-85]

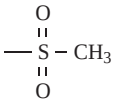
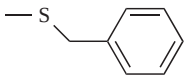
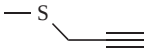
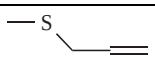
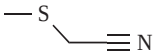
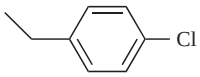
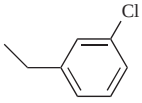
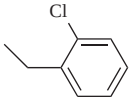
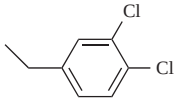
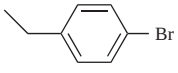
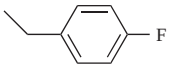
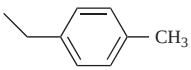
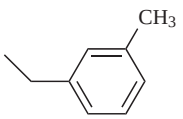


Cp	R_7	R_8	$R_{2'}$	Virus inhib. (IC_{50}, μM)[a]			Ref.
				HCMV	HSV-1[b]	HIV-1[c]	
85	CN	H	OH	1.7	29	nt	84
132	CN	H	N_3	34	>100	nt	85
133	CN	H	NH_2	14	>100	nt	85
134	CN	H	Cl	21	>100	nt	85
135	CN	H	Br	162	>100	nt	85
136	CN	H	I	40	>100	nt	85
115		H	OH	0.6	13	nt	84
137		H	N_3	28	>100	nt	85
138		H	NH_2	>100	>100	nt	85
139		H	Cl	>100	>100	nt	85
140		H	Br	>100	>100	nt	85
141		H	I	>100	>100	nt	85
142	CN	Br	F	12	>100	12	86
143 (α anomer)	CN	Br	F	3.2	>100	32	86
144	CN	H	F	1.0	46	12	86
145 (α anomer)	CN	H	F	16	6.5	>100	86
146		H	F	0.025	0.47	0.02	86
147		H	F	0.22	8.7	0.07	86

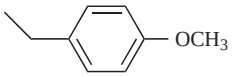
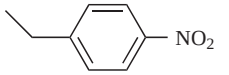
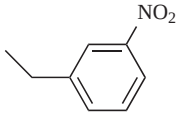
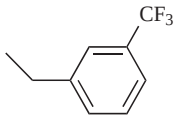
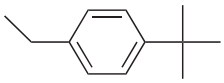
[a]Concentration of the inhibitor required for 50% inhibition of virus plaque formation. [b]All compounds were assayed by ELISA in quadruplicate. [c]Assay for HIV-1 was performed by measurement of RT activity.

Table 13. Biological Properties of 1,3-Dideazapurine Derivatives; nt = Not Tested [90, 91]



Cp	R	R_2	R_4	Virus inhib. (IC_{50}, μM) [a]			Cytotoxicity (IC_{50}, μM)
				HCMV plaque[a]	HCMV titer[b]	HSV-1 ELISA[c]	
149	H	H	H	42	19	30	24
150	H	SH	H	>100	nt	nt	>100
151	H	SCH_3	H	>100	nt	nt	>100
152	H		H	10	nt	nt	>100
153	H		H	22	7	25	100
154	H		H	25	nt	100	100
155	H		H	>100	nt	>100	24
156	H		H	30	10	>100	100
157	H		H	>100	nt	>30	100
158	H		H	25	nt	70	21
159	H		H	40	nt	60	32
160	H		H	28	nt	>30	32
161	H		H	30	nt	30	32
162	H		H	30	nt	>30	21
163	H		H	30	nt	>30	32
164	H		H	40	nt	38	32

(Table 13) contd…..

Cp	R	R_2	R_4	Virus inhib. (IC_{50}, μM)[a]			Cytotoxicity (IC_{50}, μM)
				HCMV plaque[a]	HCMV titer[b]	HSV-1 ELISA[c]	
165	H		H	40	nt	>100	66
166	H		H	40	nt	40	32
167	H		H	40	nt	50	32
168	H		H	40	nt	40	32
169	H		H	20	nt	20	32
170	H	NH_2	H	40	8	50	32
171	Ac	Cl	H	2.0	0.2	nt	100
172	H	Cl	H	2.9	1.4	102	238
173	Ac	Br	H	1.5	0.2	>100	100
174	H	Br	H	0.7	0.2	130	118
175	H	I	H	30	26	21	118
176	H	OH	NO_2	>100	nt	>100	100
177	H	OH	H	>100	nt	nt	100
Acyclovir				86	85	1.7	>100

[a]Concentration of the inhibitor required for 50% inhibition of virus plaque formation. [b]Titer reduction assay on Human Foreskin Fibroblast (HFF) cells. [c]All compounds were assayed by ELISA technique.

1,3,7-TRIDEAZAPURINE NUCLEOSIDE ANALOGUES

Chen and coworkers have prepared few indole nucleosides and tested them for their efficacy against HCMV and HIV-1 virus replication (Table **18**) [100]. The three newly synthesized compounds showed some anti-HCMV efficacy, but all of them were less active and more toxic with respect to the reference compound **172**, causing the author to conclude that these values "preclude their further development as antiviral drugs".

CONCLUSIONS

A large number of deazapurine nucleoside derivatives have been widely studied as inhibitors of virus replication systems. Studies reported in literature regard mainly anti-HIV, antiHCMV, and antiHSV biological testings, and some general consideration can be drawn.

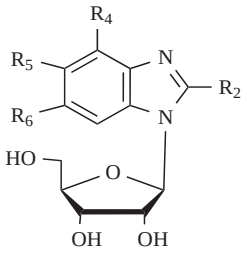
Against HIV, the most active 1-deazapurine analogues possess a large, non polar substituent on N^6, and preferably a chlorine atom in 2 position, with the sugar moiety being preferably either 2'-deoxy or 2',3'-dideoxy. So, there is a close pattern between lipophilicity and efficacy, and it can also be noted that 2'- and 3'-hydroxyl groups are not essential for activity. Regarding 3-deazapurine nucleosides, tested compounds are endowed with interesting activity only when a carbocyclic sugar is present.

As antiHCMV, both 7-deaza- and 1,3-dideaza-purine derivatives are active. In the former case, an intact tetrahydrofuran ring in the structure of the sugar is crucial for efficacy, whereas substituents in 7 can modulate activity. In the latter series, 2,5,6-trihalobenzimidazole nucleosides are endowed with the highest efficacy. In general, an increase in lipophilicity is accompanied by an increase in activity.

AntiHSV efficacy of 7-deaza- and 1,3-dideaza-derivatives shows a pattern similar to that of HCMV, except for the fact that trihalo-1,3-dideazapurine nucleosides are endowed with lower activity.

Deazapurine nucleoside analogues are also active in new exciting fields of application. For example, 1-deazapurine nucleosides show antireplicative efficacy in some veterinary systemic viral infections, for which no antiviral drugs are licensed yet.

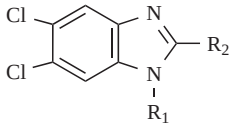
Table 14. Biological Properties of 1,3-Dideazapurine Derivatives; nt = Not Tested [92-94]

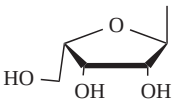
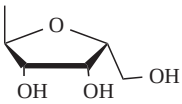
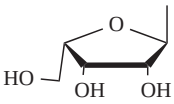
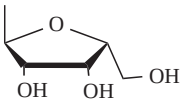
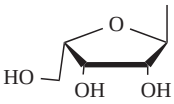
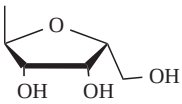
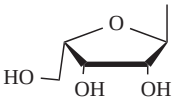
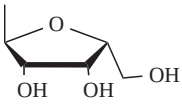
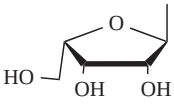
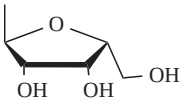
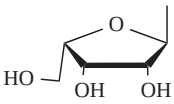
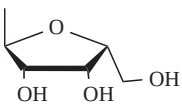


Cp	R_2	R_4	R_5	R_6	Virus inhib. (IC_{50}, μM) [a]			Cytotoxicity (IC_{50}, μM)
					HCMV plaque[a]	HCMV titer [b]	HSV-1 ELISA[c]	
172	Cl	H	Cl	Cl	2.8	1.3	102	238
174	Br	H	Cl	Cl	0.7	0.2	130	118
178	Cl	H	NH_2	H	>300	nt	>100	>300
179	Cl	H	H	NH_2	>100	nt	>100	>300
180	Cl	H	Cl	H	150	100	>100	>100
181	Cl	H	H	Cl	58	52	>100	>100
182	H	H	Cl	Cl	42	19	30	24
183	SH	H	H	Cl	>100	nt	>100	>100
184	SCH_3	H	H	Cl	>100	nt	>100	>100
185	SCH_2Ph	H	H	Cl	>100	nt	>100	>100
186	OCH_3	H	H	Cl	>100	nt	>100	>100
187	Cl	Cl	H	Cl	10	4.5	>300	77
188	Br	Cl	H	Cl	1.6	2.1	>300	127
189	N_3	Cl	H	Cl	110	41	nt	>100
190	NH_2	Cl	H	Cl	>100	>100	>300	>100
191	SH	Cl	H	Cl	>100	nt	>300	>100
192	SCH_3	Cl	H	Cl	110	nt	>300	150
193	SCH_2Ph	Cl	H	Cl	41	nt	140	32
194	Cl	Cl	Cl	H	16	6.0	>100	>100
195	Br	Cl	Cl	H	2.4	1.0	>100	161
196	Cl	H	H	H	>100	>100	nt	>100
197	Cl	H	F	F	>100	75	>100	>100
198	Cl	H	Br	Br	3.8	1.3	>100	44
199	Cl	H	I	I	2.5	2.2	57	13
200	Cl	H	CH_3	CH_3	>100	nt	>100	>100

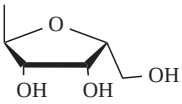
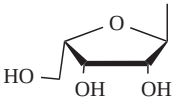
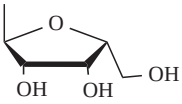
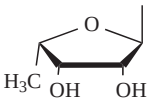
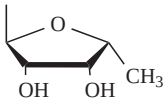
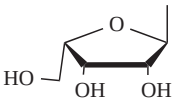
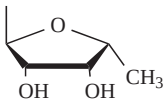
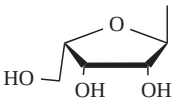
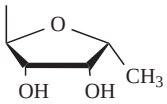
[a]Concentration of the inhibitor required for 50% inhibition of virus plaque formation. [b]Titer reduction assay on Human Foreskin Fibroblast (HFF) cells. [c]All compounds were assayed by ELISA technique.

Table 15. Biological Properties of 1,3-Dideazapurine Derivatives; nt = Not Tested [95]



Cp	R_1	R_2	Isomer	Virus inhib. (IC_{50}, μM)[a]			Cytotoxicity (IC_{50}, μM)
				HCMV plaque[a]	HCMV titer[b]	HSV-1 ELISA[c]	
201		Br	L	2.8	0.8	>100	>100
202		Br	D	6	65	50	>100
203		$NHCH_3$	L	>100	nt	>100	>100
204		$NHCH_3$	D	>100	>100	>100	>100
205		Cl	L	18	3.4	>100	190
206		Cl	D	18	>100	90	>100
207		NHC_3H_7	L	>100	19	>100	>100
208		NHC_3H_7	D	60	17	>100	>100
209		$NHcC_3H_5$	L	>100	35	>100	>100
210		$NHcC_3H_5$	D	>100	70	>100	>100
211		SH	L	>100	>100	>100	>100
212		SH	D	>100	>100	>100	>100

(Table 15) contd…..

Cp	R_1	R_2	Isomer	Virus inhib. (IC_{50}, μM)[a]			Cytotoxicity (IC_{50}, μM)
				HCMV plaque[a]	HCMV titer[b]	HSV-1 ELISA[c]	
214		SCH_2Ph	D	32	15	55	32
215		SCH_3	L	>100	nt	>100	>100
216		SCH_3	D	>100	>100	>100	>100
217		Br	L	0.4	2.0	>100	32
218		Br	D	32	12	50	100
219		Cl	L	0.2	0.2	20	32
220		Cl	D	32	8.0	60	45
221		NHC_3H_7	L	32	2.8	>100	100
222		NHC_3H_7	D	10	20	nt	>100

[a]Concentration of the inhibitor required for 50% inhibition of virus plaque formation. [b]Titer reduction assay on Human Foreskin Fibroblast (HFF) cells. [c]All compounds were assayed by ELISA technique.

Furthermore, one 7-deazapurine nucleoside analogue (7-deaza-2'-C-methyladenosine) is active in inhibiting Hepatitis C viruses, with a very low cytotoxicity.

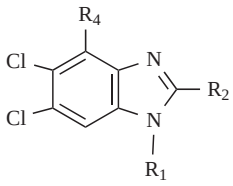
Finally, some 3-deazapurine derivatives show high potency against Ebola Virus, producing 100% survival of infected mice at a dose as low as 0. 25 mg/kg.

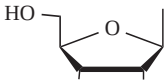
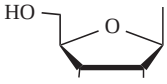
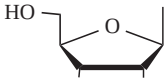
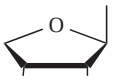
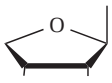
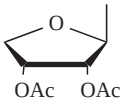
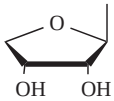
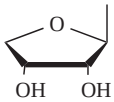
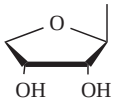
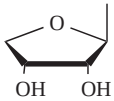
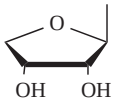
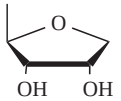
In conclusion, although to date there are many licensed antiviral agents, several reasons push the needs for new effective inhibitors of viral replication. Deazapurine analogs, with their wide field of antiviral efficacy, can be an excellent starting point for new, very effective, antiviral drugs.

ACKNOWLEDGMENTS

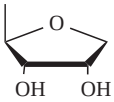
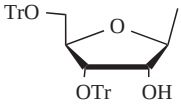
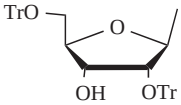
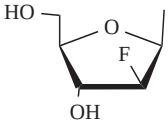
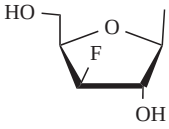
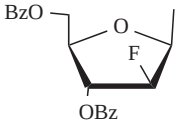
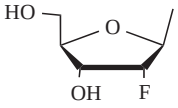
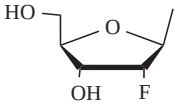
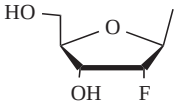
Supported by grants from the Ministry of Research (FIRB 2001 and FIRB 2003) and by the University of Camerino (Fondo di Ateneo per la Ricerca).

Table 16. Biological Properties of 1,3-Dideazapurine Derivatives; nt = Not Tested [96-98]



Cp	R_1	R_2	R_4	Virus inhib. (IC_{50}, μM)[a]			Cytotoxicity (IC_{50}, μM)
				HCMV plaque[a]	HCMV titer[b]	HSV-1 ELISA[c]	
172		Cl	H	2.8	1.3	102	238
174		Br	H	0.70	0.20	130	118
223		NH*i*C_3H_7	H	0.45	0.60	>100	>100
224		Cl	H	0.25	0.10	>100	41
225		Cl	H	0.24	0.12	>100	32
226		Br	H	0.22	0.05	50	59
227		Br	H	0.24	0.06	>100	32
228		NH*i*C_3H_7	H	7.9	5.0	>100	>100
229		NH*c*C_3H_5	H	19	6.8	60	>100
230		SCH_2Ph	H	32	nt	65	32
231		NH*i*C_3H_7	Br	1.0[d]	nt	nt	13
232		NH*i*C_3H_7	H	2.3[d]	nt	nt	15

(Table 16) contd…..

Cp	R_1	R_2	R_4	Virus inhib. (IC_{50}, μM)[a]			Cytotoxicity (IC_{50}, μM)
				HCMV plaque[a]	HCMV titer[b]	HSV-1 ELISA[c]	
234		$NHiC_3H_7$	Cl	0.8[d]	nt	nt	nt
235		Cl	H	>10	nt	>100	>100
236		Cl	H	>10	nt	>100	>100
237		Cl	H	8	8	50	32
238		Cl	H	9.4	13	>100	40
239		Cl	H	23	nt	>100	>100
240		H	H	>100	nt	>100	>100
241		Br	H	17	nt	>100	100
242		$NHiC_3H_7$	H	>100	nt	>100	>100
Gancyclovir				7.4	1.6	3.5	>100

[a]Concentration of the inhibitor required for 50% inhibition of virus plaque formation. [b]Titer reduction assay on Human Foreskin Fibroblast (HFF) cells. [c]All compounds were assayed by ELISA technique. [d]Compounds **231**-**234** were assayed for their ability to reduce microscopically visable cytophatic effect of virus replication.

ABBREVIATIONS

HIV = Human Immunodeficiency Virus

TC_{50} = Toxic Concentration 50

BHV-1 = Bovine Herpes Virus type 1

MVV = Maedi Visna Virus

AdoHcy hydrolase = Adenosine Homocysteine hydrolase

IC_{50} = Inhibitory Concentration 50

EBO-Z = Ebola Zaire Virus

EC_{50} = Effective Concentration 50

HCMV = Human CytoMegalo Virus

HSV-1 = Herpes Simplex Virus type 1

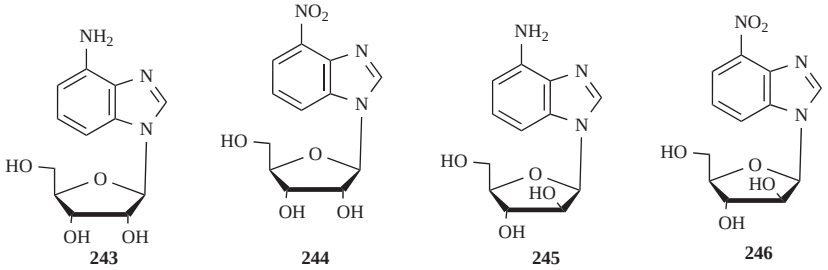
VV = Vaccinia Virus

VSV = Vesicular Stomatitis Virus

B-4 = Coxsackie virus type B-4

polio-1 = Polio virus type 1

Table 17. Biological Properties of 1,3-Dideazapurine Derivatives [99]

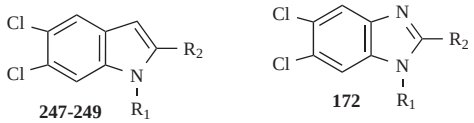


243 **244** **245** **246**

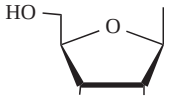
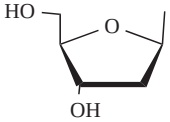
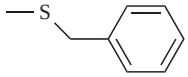
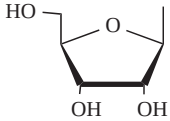
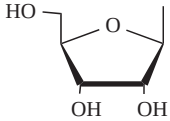
Virus/cell	IC_{50} (μg/ml)			
	243	**244**	**245**	**246**
HIV-1(III_B)/MT-4 cells	>125	>125	>125	79.4
Vesicular Stomatitis Virus/HeLa cells	>40	>40	>40	>80
Coxsakie Virus B4/HeLa cells	>40	>40	>40	>80
Respiratory Syncytial Virus/HeLa cells	>40	>120	>40	>80
Parainfluenza-3 Virus/Vero cells	>200	>200	>40	>400
Reovirus-1/Vero cells	>200	>200	>40	>400
Sindbis Virus/Vero cells	>200	>200	>40	>400
Coxsakie Virus B4/Vero cells	>200	>200	>40	>400
Punta Toro Virus/Vero cells	>200	>200	>40	>400
Herpes Simplex Virus-1 (KOS)/HEL cells	>200	>200	>200	>400
Herpes Simplex Virus-2/HEL cells	>200	>200	>200	>400
Vaccinia Virus/HEL cells	>200	>200	>200	>400
Vesicular Stomatitis Virus/HEL cells	>200	>200	>200	>400
Herpes Simplex Virus-1 TK^-KOS ACV^r/HEL cells	>200	>200	>200	>400

Reported compounds were tested for their antireplicative properties on the indicated virus/cell systems.

Table 18. Biological Properties of 1,3,7-Trideazapurine Derivatives; nt = Not Tested [100]



247-249 **172**

Cp	R_1	R_2	Virus inhib. (IC_{50}, μM)[a]			Cytotoxicity (IC_{50}, μM)
			HCMV plaque[a]	HCMV titer[b]	HSV-1 ELISA[c]	
172		Cl	2.8	1.3	102	238
247			32	nt	40	32
248		Cl	42	50	>100	>100
249		Br	18	42	>100	100

[a]Concentration of the inhibitor required for 50% inhibition of virus plaque formation. [b]Titer reduction assay on Human Foreskin Fibroblast (HFF) cells. [c]All compounds were assayed by ELISA technique.

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Synthesis and Antiproliferative and Antiviral Activity of 2′-Deoxy-2′-fluoroarabinofuranosyl Analogs of the Nucleoside Antibiotics Toyocamycin and Sangivamycin

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The glycosylation of 3,4-dicyano-2-[(ethoxymethylene)amino]pyrrole (**7**) with 2-deoxy-2-fluoro-α-D-*erythro*-pentofuranosyl bromide (**2**) furnished an anomeric mixture of nucleosides (**8a,b**). This mixture was separated, and the individual anomers were treated with methanolic ammonia to effect a concomitant deblocking and ring closure. This furnished both anomers of 2′-deoxy-2′-fluoro-ara-toyocamycin (**9a,b**). The cyano moiety of **9b** was converted to the carboxamide moiety to furnish 2′-deoxy-2′-fluoro-ara-sangivamycin (**10**) and to the thiocarboxamide moiety to furnish 2′-deoxy-2′-fluoro-ara-thiosangivamycin (**11**). The target compounds **10** and **11** showed similar antiproliferative activity against L1210 cells *in vitro*, with IC_{50}'s of 3 and 5 μM. Antiviral evaluation revealed a somewhat different pattern of activity. All analogs, both α and β anomers, were active against human cytomegalovirus (HCMV), albeit the β anomers were most active. The β anomers also were active against herpes simplex virus type 1 (HSV-1) and human immunodeficiency virus (HIV). Compound **10** was most active in the series, ca. 10-fold more potent than **11**; IC_{50}'s for **10** ranged from 4 to 25 nM for HCMV, HIV, and varicella zoster virus (VZV) and from 30 to 500 nM for HSV-1. Even though compound **10** was cytotoxic, which will probably preclude its use as an antiviral drug (IC_{50}'s = 0.2–5.5 μM), the difference between cytotoxicity and activity against HCMV, HIV, and VZV was sufficient to indicate specific activity against a viral target.

Recent work in our laboratory on pyrrolo[2,3-*d*]-pyrimidine nucleosides has concentrated on the synthesis of sugar-modified analogs of the naturally occurring nucleoside antibiotics toyocamycin and sangivamycin as potential antiviral drugs.[1–6] We,[1,7] as well as others,[8–12] have previously synthesized the 2′-deoxy and the arabinofuranosyl analogs of sangivamycin and reported the inhibitory properties of these analogs on the replication of human cytomegalovirus *in vitro*[1] and *in vivo*.[12] This work prompted us to synthesize other closely related 2′-deoxy-2′-substituted arabinofuranosyl analogs of toyocamycin and sangivamycin for evaluation of their antiviral activities. We have successfully synthesized[4] the 2′-deoxy-2′-chloro-, 2′-deoxy-2′-bromo-, and 2′-deoxy-2′-iodoarabinofuranosyl analogs of sangivamycin and toyocamycin, using routes previously described[13–15] for the synthesis of similar arabinofuranosyl derivatives of adenosine and neoplanocin A. The antiviral evaluations of these compounds showed that the 2′-deoxy and arabinofuranosyl derivatives of sangivamycin were the most active against human cytomegalovirus. This activity generated considerable interest for us to synthesize and evaluate for antiviral activity the 2′-deoxy-2′-fluoro derivative of sangivamycin. However, the synthetic routes described above were not amenable[16,17,29] to the introduction of a fluoro substituent into the 2′ position of the sugar moiety. This prompted us to develop an alternative method for the synthesis of the 2′-deoxy-2′-fluoroarabinofuranosyl analog of sangivamycin. We report herein the synthesis of this sangivamycin derivative and several analogs along with their antiproliferative and antiviral activities.

Results and Discussion

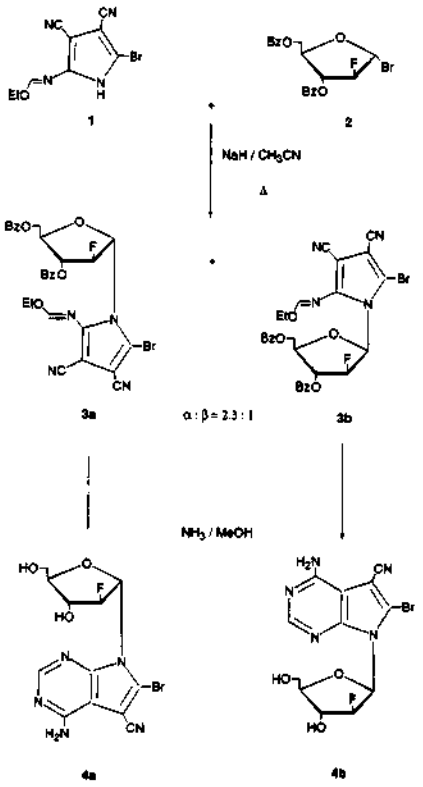
Chemistry. The major impediment in the application of the methodology which we had previously used for the introduction of substituents in the 2′ position of preformed nucleoside derivatives was that the 5′ and 3′ positions, which required protection, were most conveniently blocked by means of a bifunctional silylating agent.[18] After blocking the 5′ and 3′ position of the free nucleoside, the 2′-hydroxyl group could be expediently sulfonylated to afford a leaving group which would be displaced with the desired nucleophile. In the case where the silyl ether bonds of the protecting group are fairly stable to the nucleophilic reagent, the reaction proceeds smoothly. On the other hand, when the nucleophile employed is fluoride ion, side reactions seriously impede the course of the reaction.

Thus, we chose to synthesize the fluoroarabinosides by the coupling of a fluorine-containing pentose moiety with a suitable heterocycle followed by an elaboration of this intermediate into the target compounds. The requisite sugar had been reported[19] as an intermediate in the synthesis of 9-(2-deoxy-2-fluoroarabinofuranosyl)-adenine and 9-(2-deoxy-2-fluoroarabinofuranosyl)guanine.[20] However, this reported synthesis[21] was long and

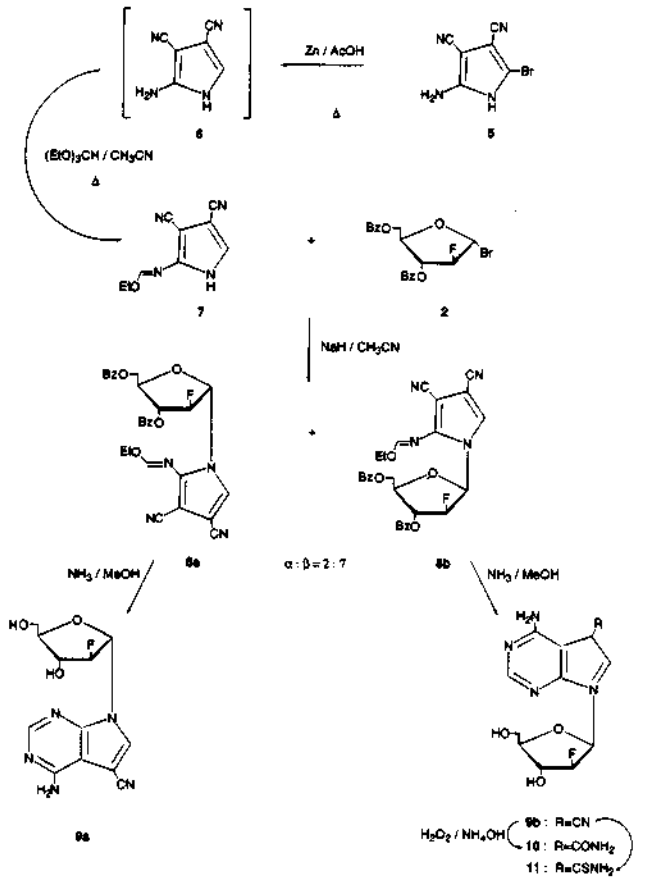
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Scheme 1



Scheme 2



tedious. Recently, a very facile and straightforward approach, to a closely related sugar (**2**), has been reported,[22] and we chose this route for the synthesis of the pentose moiety. With the bromo sugar **2** in hand, we first tried to glycosylate the aglycone of toyocamycin using the sodium salt method. This method had been used previously to prepare acyclic analogs of toyocamycin and sangivamycin.[3] Unfortunately, this aglycone was very insoluble in acetonitrile, and the sodium salt could only be prepared in dimethylformamide. The glycosylation of this sodium salt with **2** in dimethylformamide did not proceed smoothly, and this method was abandoned. The next approach we investigated involved a glycosylation of the sodium salt of the pyrrole **1**, which had been previously reported[10,11] to be a useful intermediate in the synthesis of sugar-modified analogs of toyocamycin and sangivamycin (Scheme 1). Treatment of the sodium salt of **1** with **2** in acetonitrile, at reflux temperature, afforded a 2.3:1 anomeric mixture of nucleosides (**3a,b**) which was readily separated by silica gel chromatography. Treatment of each nucleoside with methanolic ammonia effected a simultaneous deblocking and ring closure to yield two compounds whose ^{1}H NMR spectra were very different yet whose UV spectra and elemental analyses were identical. From the analytical data, their structures were assigned as the α and β anomers of the 6-bromo derivatives **4a,b**.

The anomeric configuration could not be unequivocally assigned from the coupling constants of the 1' proton. Thus, the anomeric configuration of **4a,b** was assigned using the method reported for assigning the anomers of 9-(2-deoxy-2-fluoroarabinofuranosyl)adenine,[19] i.e., by forming the 5'-tosylate of each of the anomers and then observing the UV spectrum of a refluxing solution of each of the 5'-tosylates in *p*-dioxane. Each of the anomers (**4a,b**) was treated with 2 equiv of *p*-toluenesulfonyl chloride in pyridine, and the reaction was followed by TLC. Only one more mobile product was formed in either case, and after 5 h, the new products were isolated. The UV spectrum of each of these products exhibited the same absorption maximum (285 nm) as the starting materials. These compounds were dissolved separately in *p*-dioxane, and the individual solutions were heated at reflux. Whereas the UV spectrum of the tosylate solution derived from nucleoside **4a** remained unchanged after 24 h at reflux, the absorption maximum displayed in the UV spectrum of the solution with the tosylate derived from nucleoside **4b** shifted from 285 to 294 nm, indicative of the formation of a cyclonucleoside. TLC analyses also showed that no starting material was left, and the product did not migrate on the TLC plate. Thus, evidently the minor nucleoside **4b** could be assigned the β configuration, and the major nucleoside **4a** possessed the α configuration.

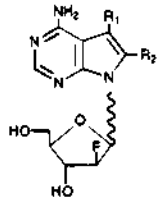
The poor yield of the desired β anomer prompted us to investigate an alternative route for the preparation of the desired β compound. We elected to remove the bromine substituent from the pyrrole[23] **1** on the premise that removal of the bromine moiety residing at the 2 position would decrease the acidity of the N1 proton, as well as reduce the steric hindrance toward alkylation at N1. This should lead to an increase in the rate and stereospecificity of the glycosylation reaction. We treated the pyrrole **5** with zinc dust in acetic acid at reflux to yield the intermediate desbromopyrrole **6** (Scheme 2). Without isolation, compound **6** was treated with triethyl orthoformate in acetonitrile, and the product was isolated by silica gel chromatography to yield the desired pyrrole aglycone **7** in a modest 22% yield. In contrast to the reaction of the sodium salt of **1** with the bromo

sugar **2**, a reaction of the sodium salt of **7** with **2** was rapid even at room temperature. This reaction yielded two glycosylated pyrroles (**8a,b**) in a 2:7 ratio. The anomeric mixture was resolved, and each anomer was separately treated with methanolic ammonia to yield the desired 2′-deoxy-2′-fluoro-ara-toyocamycin derivatives (**9a,b**). During the initial deblocking procedure, we noticed the formation of a second slower running product on TLC, which gave us a mixture of products. This slower moving product was assumed to be the 5-methylformimidate, which was formed by the addition of methanol to the cyano moiety.[10] It was subsequently found that by dissolving the crude product in water and treating the solution with a small amount of triethylamine, the methylformimidate could be converted back into the desired product.[24] This eliminated the need for a chromatographic separation of a reaction mixture and afforded a pure product.

The anomeric configurations of these isomers (**9a,b**) were assigned by comparing their ^{1}H NMR spectra with those of the 6-bromo derivatives **4a,b** which had been obtained previously, as well as to the spectra of the previously reported 2′-deoxy-2′-fluoroadenosine analogs. In the case of the 6-bromo derivatives, the coupling constants between H-1′, H-2′ and H-1′, F-2′ were smaller and greater, respectively, in the α anomer **4a** when compared to the corresponding coupling constants of the β isomer **4b**. On the basis of the assumption that this pattern would hold in the case of the anomers **9a,b**, we made a preliminary assignment of the anomeric configuration of **9b** as β and that of **9a** as α. Further support for this assignment was made by the observation that in the case of the 6-bromo derivatives **4a,b**, the 4′ and 2′ protons of the α anomer each appeared more than 0.6 ppm downfield when compared with the positions of the corresponding protons of the β anomer. The 4′ and 2′ protons of the nucleoside **9a**, which had been tentatively assigned the α configuration, also appeared ca. 0.4 ppm downfield when compared to the positions of the corresponding protons of the isomer **9b**. Since coupling between a fluorine substituent in the 2′-arabino configuration and the H-8 proton of the purine base of β purine nucleosides, but not of the α purine nucleosides, has been previously reported,[19,20] the observed splitting of the H-6 resonance in the ^{1}H NMR spectrum of **9b** and not in the spectrum of **9a** provided additional support for the anomeric assignment. The assignment was then chemically confirmed, as described previously for the 6-bromo derivatives by converting the two isomers **9a,b** into their 5′-tosylate derivatives followed by heating the solution of each individual tosylate in *p*-dioxane. As expected, the tosylate derived from nucleoside **9a** was stable for 6 h at reflux. However, the tosylate derived from **9b** reacted rapidly, under the same conditions, to provide a bathochromic shift of 9 nm in the absorption maximum of the UV spectrum of the *p*-dioxane solution.

This established that we had prepared the desired 2′-deoxy-2′-fluoro-ara-toyocamycin (**9b**). We then converted[25] the cyano moiety of **9b** into the carboxamide moiety with hydrogen peroxide in a solution of methanol and ammonium hydroxide. This provided the desired 2′-deoxy-2′-fluoro-ara-sangivamycin (**10**). We then converted the cyano moiety of **9b** into a thioamide group with dihydrogen sulfide to obtain 2′-deoxy-2′-fluoro-ara-thiosangivamycin (**11**).

Table 1. Antiproliferative Activity of α- and β-2′-Deoxy-2′-fluoroarabinosides of 5-Substituted and 5,6-Disubstituted 4-Aminopyrrolo[2,3-*d*]pyrimidines in L1210 Murine Leukemia *in Vitro*



compd	R_1	R_2	configuration[a]	screen GR[b] (% of control)	IC_{50}[c] (μM)
4b	CN	Br	β	21	66
4a	CN	Br	α	45	90
9b	CN	H	β	11	39
9a	CN	H	α	98	
10	$CONH_2$	H	β	9.0	3.2
11	$CSNH_2$	H	β	5.0	5.0

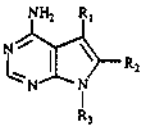
[a] Configuration of the glycosidic bond. [b] Screen GR, growth rate in the presence of the test compound at 100 μM. See the Experimental Section for definition. [c] IC_{50}, concentration required to decrease growth rate to 50% of control.

Antiproliferative Studies. The antitumor potential of this group of compounds was assessed by evaluating their capacity to slow proliferation of L1210 cells *in vitro*. As shown in Table 1, the target compounds **10** and **11** had significant antiproliferative activity. Their IC_{50}'s are comparable to the value of 5 μM obtained previously for arabinosylthiosangivamycin,[26] showing that replacement of a 2′-OH with a 2′-F (**11**) did not affect the potency of this compound. The stereochemistry of the glycosidic bond had the expected influence on antiproliferative potency. The α anomers (**4a** and **9a**) of the 5-CN derivatives were less potent than their respective β anomers (**4b** and **9b**), with **9a** completely lacking antiproliferative activity at 100 μM.

The 5 substituent played an important role in determining the antiproliferative potency of this series of compounds. The 5-CN derivative **9b** had an IC_{50} on the order of 10-fold higher than that of the 5-$CONH_2$ derivative **10** and the 5-$CSNH_2$ derivative **11**. This finding confirmed the correlation noted previously that a 5-$CSNH_2$ substituent conferred greater antiproliferative potency than a 5-CN among 2′-modified pyrrolo-[2,3-*d*]pyrimidine adenosine analogs, specifically, the 2′-NH_2-2′-deoxyarabinosyl and 2′-deoxy derivatives.[26]

Antiviral Studies. The antiviral potential of the compounds was investigated by examining their activity against selected herpes viruses and human immunodeficiency virus (HIV). Data in Table 2 establish that certain compounds have potent antiviral activity. All compounds, both α and β anomers, showed some activity against both human cytomegalovirus (HCMV) and HIV. The activity of the α anomers was surprising and was confirmed in replicate experiments. The activity against HCMV was investigated more extensively using yield reduction assays. In these assays the α anomers were only slightly active, indicating that activity in plaque assays might have involved inhibition of cell to cell spread of the virus and not reflecting inhibition of infectious virus production. The more potent activity of the β anomers against HCMV, particularly in the

Table 2. Antiviral Activity and Cytotoxicity of 2'-Fluoroarabinosylpyrrolo[2,3-*d*]pyrimidine Analogs



				50% or 90% inhibitory concentration (μM)					
				antiviral activity[a]					
				HCMV		HIV-1[c]	HSV-1[b]	cytotoxicity[d]	
compd	R_1	R_2	R_3	plaque	yield	RT	ELISA	visual	growth
4a	CN	Br	α-D-2-F-arabinosyl	3.2	>100	32	>100[e]	55	>150
4b	CN	Br	β-D-2-F-arabinosyl	12	7	12	>100	21	144
9a	CN	H	α-D-2-F-arabinosyl	16	100	>100	46	100	>100
9b	CN	H	β-D-2-F-arabinosyl	1.0	1.1	12	6.5	6.8	8.8
10	$CONH_2$	H	β-D-2-F-arabinosyl	0.025	0.075	0.02	0.47	5.5	1.9
11	$CSNH_2$	H	β-D-2-F-arabinosyl	0.22	1.5	0.07	8.7	3.1	3
zidovudine[f]						0.011 ± 0.0008		>100	
ganciclovir[g]				7.4 ± 6.5	1.6 ± 1.2		3.5 ± 2.1	>100	>100

[a] Assays were performed in duplicate as described in the text. Results from plaque, syncytium, and ELISA are reported as IC_{50}'s, those for yield reduction experiments as IC_{90}'s. [b] All compounds were assayed by ELISA in quadruplicate wells. [c] Assay for HIV-1 by measurement of RT activity in culture supernatant. [d] Visual cytotoxicity was scored on HFF cells at time of HCMV plaque enumeration for numbered compounds. For zidovudine scoring was on CEM cells at time of RT assay. (Results of visual cytotoxicity scoring in CEM cells for numbered compounds gave IC_{50}'s of >100, 33, >100, 33, 0.4, and 2 μM, respectively.) Cell growth inhibition was determined in KB cells in quadruplicate assays as described in the text. All results are presented as IC_{50}'s. [e] > indicates IC_{50} not reached at the noted (highest) concentration tested. [f] Mean ± standard deviation from four experiments. [g] Mean ± standard deviation from 108, 33, and 3 experiments, respectively, in which ganciclovir was used as a positive control.

Table 3. Antiviral Activity of 2'-Deoxy-2'-fluoroarabinosylsangivamycin (**10**)

	50% inhibitory concentration[a] (μM)	
virus and cell line	antiviral	cytotoxicity
Antiviral Activity		
HSV-1	0.03	
HSV-2	0.03	
VZV	0.003	
HIV-1[b]	0.0037 ± 0.0017	
Cytotoxicity		
human foreskin fibroblasts		
stationary cells, neutral red uptake		6.7
stationary cells, MTT assay		0.13
proliferating cells, MTT assay		0.16
CEM-SS cells		
visual inspection		0.4
[^{3}H]dThd uptake[c]		0.193 ± 0.074

[a] All assays performed in duplicate as described in the text. [b] Mean ± standard deviation from quadruplicate assays in the syncytial plaque assay using CEM-SS cells. [c] Mean ± standard deviation from triplicate assays.

yield reduction assay (Table 2), established their capacity to inhibit infectious virus production at micromolar and lower concentrations. Compounds **9b**, **10**, and **11** were the most potent with activity extending to HIV and possibly herpes simplex virus type 1 (HSV-1) (Table 2). The activity of **9b**, however, was not well separated from cytotoxicity (Table 2). Consequently, activity against HIV and HSV-1 may be only a manifestation of cytotoxicity for this compound. Additional experiments showed that it also inhibited [^{3}H]dThd incorporation into DNA (IC_{50} = 3.3 μM).

In contrast, the difference between cytotoxicity and activity of compounds **10** and **11** (particularly **10**) against both HCMV and HIV was sufficient to indicate specific antiviral activity in the low nanomolar range. Activity against HSV-1 was less apparent in an ELISA assay (Table 2) and could reflect cytotoxicity. More extensive examination of compound **10** revealed that it also was highly active against HSV-2 and varicella zoster virus (VZV) in a plaque assay (Table 3). Interestingly, it was also more active against HSV-1 in the plaque assay (Table 3) compared to weaker activity in the ELISA assay (Table 2). Because plaque assays measure more parameters needed for viral replication than an ELISA assay, the plaque results may reflect more closely the effect of the compound on replicating virus. Likewise, compound **10** also was more active against HIV in a syncytial plaque assay (Table 3) than in an assay which used reverse transcriptase (RT) activity to detect the virus (Table 2). In an additional series of experiments, compound **10** was much less active in cells persistently infected with HIV. In such cells, 100 nM **10** inhibited virus production by 23–50% in U1 cells and 0–32% in $H9III_B$ cells based on inhibition of p24 antigen production, RT activity, and infectious virus production measured in the syncytial plaque assay. We have reported previously that zidovudine (AZT) gave a similar pattern of potent inhibition in acutely infected cells and weaker activity in persistently infected cells (20–40% inhibition of the three parameters at 1 μM).[27] These data show that like AZT compound **10** does not inhibit processes which are needed only in persistently infected cells which already contain the integrated viral genome. Because RT activ-

ity is required for genome integration and not for virus production in persistently infected cells, the data suggest that compound **10** or a metabolite inhibits RT.

There may be another factor affecting the antiviral activity and cytotoxicity of compound **11**. In other studies we have shown that certain 7-substituted 4-aminopyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamides, such as **11**, are converted to their corresponding 5-carbonitrile derivatives *in vitro*.[28] Although we did not examine compound **11** for this phenomenon, we expect it would undergo such a conversion in cell culture medium. Hence, the biological data for compound **11** may be the result of a mixture of 5-thioamide (**11**) and 5-carbonitrile (**9b**) derivatives. Because the nitrile analog **9b** is less active, the activity of **11** must be greater than we observed if conversion from the thioamide to the nitrile occurred.

Compounds **10** and **11** both are moderately potent cytotoxic agents in L1210 murine leukemic cells (Table 1), CEM human leukemic cells, HFF human foreskin fibroblasts, and KB human nasopharyngeal carcinoma cells (Tables 2 and 3). In L1210 cells, compound **10** is 1000-fold less potent than the parent ribosyl nucleoside antibiotic sangivamycin,[3] and compound **11** is 100-fold less potent than its ribosyl counterpart, thiosangivamycin.[26] These compounds appear to be more cytotoxic in CEM and KB cells suggesting that they might be of interest for cancer chemotherapy if they retained sufficient efficacy *in vivo* and also caused less host toxicity than the parent compounds.

The antiviral activity of most compounds was separated from cytotoxicity by ca. 10–100-fold. Compounds which had the most potent antiviral activity also were the most cytotoxic, but some of the less active and cytotoxic compounds such as **4b** and **9b** had the poorest differential between the two parameters (Table 2). In contrast, the most active and cytotoxic compound (**10**) had the best difference between activity against HCMV and HIV and cytotoxicity measured in our initial tests (Table 2). To better understand the cytotoxicity of compound **10**, other cytotoxicity tests were performed. Data in Table 3 show that the effect on human foreskin fibroblasts and KB cells measured by dye uptake was similar to visual effects on HFF cells (Table 2) and similar to effects on proliferating L1210 cells as well (Table 1). In contrast, measurement of more biochemical parameters such as mitochondrial dehydrogenase (MTT assay) and cell DNA synthesis ([^{3}H]dThd uptake) revealed more potent effects (IC_{50}'s = 0.13–0.19 μM, Table 3). To better understand the cytotoxicity of this compound, its effect on growing KB cells was determined in a traditional cell growth experiment. Data in Figure 1 show that little to no growth inhibition occurred up to 0.1 μM but that 1.0 μM blocked cell growth completely. Higher concentrations were clearly cytotoxic (Figure 1). Thus by all measures, cytotoxicity was not observed at concentrations which gave activity against HCMV, VZV, and HIV leading us to conclude that compound **10** could act against a specific viral function. Further structure–activity relationship studies among analogs of compound **10** are warranted.

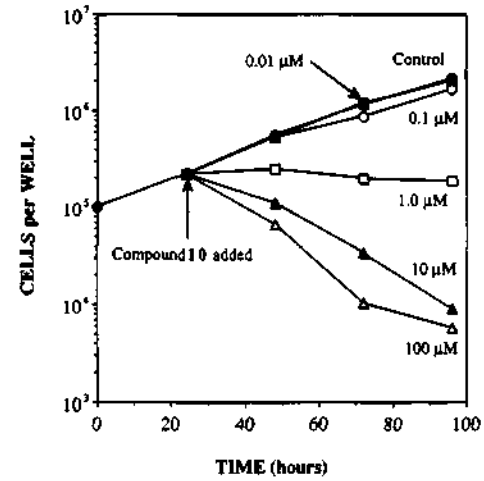


Figure 1. Effect of compound **10** on the growth of KB cells. Cells were planted as described in the text at 1 × 10^5 cells/well in 6-well cluster plates. Following one population doubling, medium was removed and replaced with medium containing compound **10** in the noted concentrations in duplicate. Cells were harvested at the times indicated and enumerated by a Coulter counter.

Experimental Section

General Methods. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. The silica gel used for chromatography was silica gel 60 230–400 mesh (E. Merck, Darmstadt, West Germany). Thin layer chromatography (TLC) was performed on prescored SilicAR 7GF plates (Analtech, Newark, DE). Compounds were visualized by illuminating under UV light (254 nm) and spraying with 20% methanolic sulfuric acid followed by charring via a Bunsen burner. The following solvent designations are used throughout the manuscript: solvent system A, ethyl acetate/cyclohexane (1:4, v:v); solvent system B, ethyl acetate/cyclohexane (1:3, v:v); solvent system C, chloroform/methanol (9:1, v:v); solvent system D, chloform/methanol (4:1, v:v); solvent system E, chloroform/methanol (16:1, v:v); solvent system F, ethyl acetate/cyclohexane (1:2, v:v); solvent system G, ethyl acetate/methanol (9:1, v:v). All evaporations were carried out using a rotary evaporatory connected to a water aspirator pump. The water bath temperature was maintained between 40 and 50 °C. Determination of UV spectra was performed on a Hewlett-Packard 8450-A UV/vis spectrophotometer. IR spectra were obtained on a Nicolet 5 DXB Fourier transform spectrophotometer. ^{1}H NMR spectra were obtained using an IBM WP 270-SY spectrometer operating in the FT mode at 270 MHz. Where necessary, deuterium exchange and homonuclear decoupling experiments were used to obtain proton shift assignments of the ribose moiety.

4-Amino-6-bromo-5-cyano-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (4b) and 4-Amino-6-bromo-5-cyano-1-(2-deoxy-2-fluoro-α-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (4a). To a solution of 2-bromo-3,4-dicyano-5-[(ethoxymethylene)amino]pyrrole[23] (**1**; 0.267 g, 1 mmol) in acetonitrile (25 mL) was added sodium hydride (0.040 g, 1 mmol, 60% oil dispersion). After the evolution of hydrogen ceased (ca. 15 min), a solution of 1-bromo-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranose[22] (**2**; 0.464 g, 1 mmol) in acetonitrile (10 mL) was added, and the temperature was maintained at 80 °C for 4 h. After this time, the mixture was partitioned between ethyl acetate (50 mL) and water (50 mL), and the organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed on a silica gel column (25 mm × 300 mm) using solvent system A to afford two fractions as determined by TLC (solvent B). The fraction containing the faster moving product (R_f = 0.35, solvent system B) was evaporated to yield 0.23 g of a white foam: ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1H, CH=N), 8.10–7.30 (m, 10H, benzoyl), 6.44 (dd, 1H, H-1, $J_{1',2'}$ = 5.4 Hz, $J_{1,F}$ = 17.0 Hz), 6.10 (ddd, 1H, H-2', $J_{2',F}$ = 53.9 Hz), 5.86 (ddd, 1H, H-3', $J_{3',F}$ = 21.2 Hz) 4.80–4.60 (m, 3H, H-4', H-5'a,b), 4.20 (m, 2H, CH_2), 1.20 (t, 3H, CH_3).

This material was dissolved in methanolic ammonia (saturated at 0 °C), and the solution was kept in a sealed bottle at

room temperature for 16 h. The solution was then evaporated, the residue was triturated with acetonitrile, and the solid which had formed was collected by filtration to yield 0.073 g of the α anomer **4a**: mp 206–207 °C; ^{1}H NMR (DMSO-d_6) δ 8.25 (s, 1H, H-2), 7.07 (bs, 2H, NH_2), 6.26 (dd, 1H, H-1', $J_{1',2'}$ = 5.4 Hz, $J_{1',F}$ = 16.7 Hz), 6.08 (bs, 1H, 3'-OH), 6.05 (ddd, 1H, H-2', $J_{2',F}$ = 55.9 Hz), 4.93 (bs, 1H, 5'-OH), 4.47 (m, 1H, H-3'), 4.39 (m, 1H, H-4'), 3.56 (m, 2H, H-5'a,b); UV λ_{max} (nm) methanol, 285; IR (KBr) 2224 cm^{-1} (cyano); TLC R_f = 0.31, solvent system C. Anal. ($C_{12}H_{11}N_5O_3FBr$) C,H,N.

The fraction containing the slower running product (R_f = 0.20, solvent system B) was also evaporated to yield a crisp foam (0.1 g): ^{1}H NMR ($CDCl_3$) δ 8.36 (s, 1H, CH=N), 8.10–7.30 (m, 10H, benzoyl), 6.47 (dd, 1H, H-1', $J_{1',2'}$ = 4.9 Hz, $J_{1',F}$ = 17.5 Hz), 5.88 (ddd, 1H, H-3', $J_{3',F}$ = 19.7 Hz), 5.38 (ddd, 1H, H-2', $J_{2',F}$ = 52.1 Hz), 4.76 (ddd, 2H, H-5'ab), 4.41 (m, 3H, H-4', CH_2), 1.41 (t, 3H, CH_3).

This material was dissolved in methanolic ammonia (saturated at 0 °C), and the solution was kept in a sealed bottle at room temperature for 16 h. The solution was then evaporated, the residue was triturated with acetonitrile, and the solid which had formed was collected by filtration to yield 0.032 g of the β anomer **4b**: mp 220–223 °C; ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1H, H-2), 7.02 (bs, 2H, NH_2), 6.69 (dd, 1H, H-1', $J_{1',2'}$ = 6.2 Hz, $J_{1',F}$ = 11.2 Hz), 5.93 (d, 1H, 3'-OH), 5.29 (ddd, 1H, H-2', $J_{2',F}$ = 55.4 Hz), 5.00 (t, 1H, 5'-OH), 4.66 (ddd, 1H, H-3', $J_{3',F}$ = 23.3 Hz), 3.75 (m, 3H, H-4', H-5'a,b); UV λ_{max} (nm) methanol, 285; IR (KBr) 2228 cm^{-1} (cyano); TLC R_f = 0.31, solvent system C. Anal. ($C_{12}H_{11}N_5O_3FBr$) C,H,N.

Anomeric Confirmation of 4a,b. A solution of nucleoside **4a** (12 mg) in pyridine (0.4 mL) was treated with *p*-toluenesulfonyl chloride (20 mg). After ca. 5 h, water (3 mL) was added, and the precipitate was collected by filtration to afford a white solid (14 mg; R_f = 0.42, solvent system C; UV λ_{max} (nm) methanol, 285). A solution of this material (5 mg) in *p*-dioxane (2 mL) was heated at reflux for 18 h. TLC analysis of the solution (solvent system C), after this time, showed the presence of only starting material, and the UV spectrum of the solution was unchanged.

Likewise, a solution of nucleoside **4b** (12 mg) in pyridine (0.4 mL) was treated with *p*-toluenesulfonyl chloride (20 mg). After 5 h, water was added (5 mL), and the cloudy emulsion was extracted with ethyl acetate (3 × 5 mL). The combined extracts were washed with water (10 mL) and brine (10 mL), dried over sodium sulfate, and evaporated to yield a foam (11 mg; R_f = 0.47, solvent system C; UV λ_{max} (nm) methanol, 285). A solution of this foam (5 mg) was heated at reflux in *p*-dioxane for 1 h. TLC analysis (solvent system C) of the solution after this time revealed an absence of starting material and the presence of a new product at R_f = 0.0. The UV absorbtion maximum of the solution was observed at 294 nm.

3,4-Dicyano-2-[(ethoxymethylene)amino]pyrrole (7). A suspension of 2-amino-5-bromo-3,4-dicyanopyrrole[23] (**5**; 20.3 g, 96.2 mmol) and zinc dust (50 g, 765 mmol) in acetic acid (250 mL) was heated at reflux under argon for 1 h. The suspension was filtered, and the zinc bed was washed with acetic acid (2 × 50 mL). The combined filtrates were evaporated *in vacuo* to afford a thick syrup, and this syrup was coevaporated three times with a mixture of ethanol and toluene (1:1, 50 mL) to afford a dark foam. This foam was dissolved in hot ethyl acetate (50 mL), and the resulting warm slurry was filtered. The filtrate was evaporated, and the residue was dissolved in acetonitrile (250 mL). The solution was treated with triethyl orthoformate (20 mL, 120 mmol), and the mixture was heated at reflux for 0.5 h. After this time, the solution was evaporated, and the residue was coevaporated with toluene (2 × 50 mL). The residue was then triturated with warm ethyl acetate (250 mL), the suspension was filtered, and to the filtrtate was added a saturated aqueous solution of sodium bicarbonate (100 mL). The resulting emulsion was vacuum-filtered through a fritted glass funnel, and brine was added to the biphasic mixture. The organic layer was then separated and washed with brine (200 mL), dried over sodium sulfate, filtered, and evaporated. The residue was treated with solvent system C (100 mL), and the mixture was momentarily heated at reflux on a steam bath. The warm suspension was applied to a silica gel column (21 cm × 10 cm) which had been slurry packed in chloroform. The column was eluted with solvent system E, and the fraction containing the product (R_f = 0.45, solvent system E) was collected and evaporated. The residue was triturated with cyclohexane (50 mL), and the product was collected to afford 4.02 g (22%) of a light yellow solid: mp 120–121 °C; ^{1}H NMR (DMSO-d_6) δ 12.58 (bs, 1H, NH), 8.39 (s, 1H, CH=), 7.66 (s, 1H, H-5), 4.28 (q, 2H, CH_2), 1.29 (t, 3H, CH_3); UV λ_{max} (nm) (log ε) methanol, 274 (4.05), 213 (4.26); pH 1, 271 (4.05), 214 (4.22); pH 11, 281 (4.08), 247 (4.01), 230 (4.05); IR (KBr) 2213, 2226 cm^{-1} (cyano); TLC R_f = 0.45, solvent system E. Anal. ($C_9H_8N_4O$) C,H,N.

4-Amino-5-cyano-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9b) and 4-Amino-5-cyano-1-(2-deoxy-2-fluoro-α-D-arabinofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (9a). Sodium hydride (1.05 g, 50% oil dispersion, 22 mmol) was added to a solution of 3,4-dicyano-2-[(ethoxymethylene)amino]pyrrole (**7**; 3.76 g, 20 mmol) in acetonitrile (250 mL). The mixture was stirred for 0.5 h with the exclusion of moisture, and after this time, a solution of 1-bromo-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranose (8.4 g, 20 mmol) in acetonitrile (125 mL) was added. The mixture was stirred at room temperature for 16 h, and the resulting suspension was evaporated to yield a thick paste. This paste was partitioned between ethyl acetate (500 mL) and water (250 mL). The organic layer was washed with brine (250 mL), dried over sodium sulfate, filtered, and evaporated to yield a foam. This foam was dissolved in solvent system F (100 mL), and the solution was applied to a silica gel column (10 cm × 35 cm) which had been slurry packed in solvent system B. The column was eluted first with solvent system B (1 L) and then with solvent system F. The fraction containing the faster running product (R_f = 0.32, solvent system B) was evaporated to afford 2.34 g (22%) of **8a** as a foam: ^{1}H NMR ($CDCl_3$) δ 8.47 (s, 1H, CH=N), 8.2–7.4 (3m, 10H, benzoyl), 7.29 (s, 1H, H-5), 6.27 (d, 1H, H-1', $J_{1',2'}$ < 1 Hz, $J_{1',F}$ = 14.0 Hz), 5.67 (dd, 1H, H-3', $J_{3',F}$ = 15.7 Hz), 5.35 (dd, 1H, H-2', $J_{2',F}$ = 48.1 Hz), 4.95 (m, 1H, H-4'), 4.67 (m, 2H, H-5'ab), 4.34 (q, 2H, CH_2), 1.38 (t, 3H, CH_3); UV λ_{max} (nm) (log ε) methanol, 282 (3.93), 274 (3.91), 226 (4.55); pH 1, 240 (4.45), 206 (4.44); pH 11, 284 (4.11), 234 (4.53); IR (KBr) 2228 cm^{-1} (cyano); TLC R_f = 0.32 solvent system B. Anal. ($C_{28}H_{23}N_4O_6F\cdot H_2O$) C,H,N.

This material (1.4 g, 2.6 mmol) was dissolved in methanolic ammonia (50 mL, saturated at 0 °C), and the solution was kept at room temperature in a pressure bottle for 16 h. The solution was then evaporated, and the residue was dissolved in water (50 mL). The aqueous solution was treated with triethylamine (5 mL), and the mixture was allowed to stand at room temperature for 4 h. The solid which had precipitated from the solution was collected and washed with ether (2 × 20 mL) to yield 0.56 g of the α anomer **9a** as a solid (74%): mp 228.5–229 °C; ^{1}H NMR (DMSO-d_6) δ 8.36, 8.24 (2s, 2H, H-2, H-6), 6.95 (bs, 2H, NH_2), 6.36 (dd, 1H, H-1', $J_{1',2}$ = 3.1 Hz, $J_{1',F}$ = 15.4 Hz), 5.98 (d, 1H, 3'-OH), 5.54 (ddd, 1H, H-2', $J_{2',F}$ = 52.1 Hz), 5.02 (t, 1H, 5'-OH), 4.34 (m, 1H, H-3'), 4.29 (m, 1H, H-4'), 3.53 (m, 2H, H-5'ab); UV λ_{max} (nm) (log ε) methanol, 279 (4.15), 229 (4.01), 210 (4.21); pH 1, 273 (4.07), 234 (4.21), 205 (4.09); pH 11, 278 (4.14), 231 (3.98); IR (KBr) 2228 cm^{-1} (cyano); TLC R_f = 0.64 solvent system C. Anal. ($C_{12}H_{12}N_5O_3F$) C,H,N.

Further elution of the column afforded a fraction containing the slower running product (R_f = 0.16, solvent system B) which upon evaporation yielded 7.34 g (69%) of **8b** as a solid: ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1H, CH=N), 8.1–7.4 (3m, 10H, benzoyl), 7.34 (d, 1H, H-5, $J_{5',F}$ = 2.1 Hz), 6.23 (dd, 1H, H-1', $J_{1',2}$ = 2.9 Hz, $J_{1',F}$ = 17.4 Hz), 5.63 (dd, 1H, H-3', $J_{3',F}$ = 16.0 Hz), 5.17 (dd, 1H, H-2', $J_{2',F}$ = 50.1 Hz), 4.73 (m, 2H, H-5'ab), 4.53 (m, 1H, H-4'), 4.33 (q, 2H, CH_2), 1.37 (t, 3H, CH_3); UV λ_{max} (nm) (log ε) methanol, 282 (3.93), 276 (3.93), 226 (4.57); pH 1, 241 (4.47), 208 (4.45); pH 11, 284 (4.13), 235 (4.54); IR (KBr) 2229 cm^{-1} (cyano); TLC R_f = 0.16, solvent system B. Anal. ($C_{28}H_{23}N_4O_6F$) C,H,N.

This material (7.2 g, 13.6 mmol) was suspended in methanol (50 mL), and methanolic ammonia (50 mL, saturated at 0 °C) was added. The solution was kept at room temperature in a pressure bottle for 16 h and then evaporated. The residue was dissolved in water (100 mL), the aqueous solution was treated

with triethylamine (10 mL), and the mixture was allowed to stand at room temperature for 1 h. After this time, the cloudy mixture was evaporated, the resulting syrup was triturated with acetonitrile (25 mL), and the solid which had precipitated was collected. The process of evaporation and trituration was repeated twice more, and the combined product was recrystallized from water/ethanol (4:1, v:v, 50 mL) to yield 1.72 g (43%) of the β anomer **9b**, mp 188–190 °C. An analytical sample was obtained by recrystallizing the material (1.05 g) from methanol (15 mL) to yield 0.91 g of material: mp 225.5–227.5 °C; ^{1}H NMR (DMSO-d_6) δ 8.33 (d, 1H, H-6, $J_{6,F}$ = 1.5 Hz), 8.22 (s, 1H, H-2), 6.95 (bs, 2H, NH_2), 6.56 (dd, 1H, H-1′, $J_{1',2'}$ = 4.5 Hz, $J_{1',F}$ = 13.3 Hz), 5.94 (d, 1H, 3′-OH), 5.17 (ddd, 1H, H-2′, $J_{2',F}$ = 52.7 Hz), 5.11 (t, 1H, 5′-OH), 4.36 (ddd, 1H, H-3′, $J_{3',F}$ = 18.7 Hz), 3.84 (m, 1H, H-4′), 3.66 (m, 2H, H-5′ab); UV λ_{max} (nm) (log ϵ) methanol, 278 (4.23), 229 (4.08), 208 (4.32); pH 1, 272 (4.12), 232 (4.24), 205 (4.11); pH 11, 277 (4.23), 231 (4.05); IR (KBr) 2223 cm^{-1} (cyano); TLC R_f = 0.57, solvent system C. Anal. ($C_{12}H_{12}N_5O_3F$) C,H,N.

Anomeric Configuration of 9a,b. To a solution of nucleoside **9a** (50 mg) in pyridine (1 mL) was added *p*-tolunesulfonyl chloride (90 mg). The solution was kept at room temperature for 4 h, and the mixture was then partitioned between water (20 mL) and ethyl acetate (20 mL). The organic layer was washed with water (3 × 20 mL), saturated aqueous sodium bicarbonate solution (20 mL), and brine (20 mL) and then dried over sodium sulfate, filtered, and evaporated to yield 59 mg of a foam (R_f = 0.72, solvent system G; UV λ_{max} (nm) methanol 278). A solution of this foam (20 mg) in *p*-dioxane (2.5 mL) was heated at reflux for 6 h. TLC (solvent system G) analysis of the solution after this time revealed only starting material, and the UV spectrum was unchanged.

To a solution of nucleoside **9b** (50 mg) in pyridine (1 mL) was added *p*-toluenesulfonyl chloride (90 mg). The solution was kept at room temperature for 4 h, and the mixture was then partitioned between water (20 mL) and ethyl acetate (20 mL). The organic layer was washed with water (3 × 20 mL), saturated aqueous sodium bicarbonate solution (20 mL), and brine (20 mL) and then dried over sodium sulfate, filtered, and evaporated to yield 78 mg of a foam (R_f = 0.61, solvent system G; UV λ_{max} (nm) methanol, 278). A solution of this foam (20 mg) in *p*-dioxane (2.5 mL) was heated at reflux for 6 h. TLC (solvent system G) analysis of the solution after this time revealed no starting material and one product with R_f = 0.0. The UV spectrum (methanol) showed a new absorbtion maximum at 287 nm.

4-Amino-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (10). To a stirred suspension of 4-amino-5-cyano-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**9b**; 0.292 g, 1 mmol) in water/methanol (2:1, v:v, 15 mL) was added concentrated ammonium hydroxide (0.15 mL) followed by three portions of hydrogen peroxide (30%, 0.2 mL) at 0.5 h intervals. The solution was stirred for an additional 1 h, and the solution was then evaporated using an ambient temperature water bath. The resulting syrup was coevaporated with ethanol (2 × 10 mL), and the residue was triturated with ethanol (10 mL). The resulting solid was crystallized from water (10 mL) with the aid of decolorizing carbon to afford 0.13 g (42%) of product **10**: mp 271 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.90 (bs, 2H, $CONH_2$), 8.10 (s, 1H, H-2), 8.08 (d, 1H, H-6, $J_{6,F}$ = 1.9 Hz), 7.38 (bs, 2H, NH_2), 6.58 (d, 1H, H-1′, $J_{1',2'}$ = 4.1 Hz, $J_{1',F}$ = 17.2 Hz), 5.95 (d, 1H, 3′-OH), 4.68 (ddd, 1H, H-2′, $J_{2',F}$ = 56.3 Hz), 4.98 (t, 1H, 5′-OH), 4.34 (ddd, 1H, H-3′, $J_{3',F}$ = 17.9 Hz), 3.84 (m, 1H, H-4′), 3.67 (m, 2H, H-5′ab); UV λ_{max} (nm) (log ϵ) methanol, 280 (4.17), 230 (4.01), 206 (4.32); pH 1, 275 (4.12), 226 (4.19), 204 (4.18); pH 11, 279 (4.17), 232 (4.00); IR (KBr) no cyano absorbtion observed; TLC R_f = 0.30, solvent system G. Anal. ($C_{12}H_{14}N_5O_4F$) C,H,N.

4-Amino-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-(thiocarboxamide) (11). Hydrogen sulfide gas was bubbled through a solution of 4-amino-5-cyano-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**9b**; 0.292 g, 1 mmol) in pyridine (15 mL) containing triethylamine (0.1 mL) over a period of 2 h. The solution was evaporated, and the residue was triturated with ethanol (10 mL). The solid which formed was collected and crystallized from water/ethanol (3:1, v:v, 40 mL) to yield 0.16 g (49%) of the product **11** as a light yellow solid: mp 227.5–228.5 °C; ^{1}H NMR (DMSO-d_6) δ 9.65, 9.50 (2bs, 2H, $CSNH_2$), 8.13 (s, 1H, H-2), 7.97 (bs, 2H, NH_2), 7.85 (d, 1H, H-6, $J_{6',F}$ = 1.4 Hz), 6.59 (dd, 1H, H-1′, $J_{1',2'}$ = 4.3 Hz, $J_{1',F}$ = 15.4 Hz), 6.49 (d, 1H, H-3′, $J_{2',3'}$ = 5.8 Hz), 5.93 (d, 1H, 5′-OH), 5.14 (ddd, 1H, H-2′, $J_{2',F'}$ = 55.1 Hz), 5.03 (t, 1H, 5′-OH), 4.38 (ddd, 1H, H-3′, $J_{3',F'}$ = 18.2 Hz), 3.84 (m, 1H, H-4′), 3.65 (m, 2H, H-5′ab); UV λ_{max} (nm) (log ϵ) MeOH, 285 (4.11), 246 (4.06), 207 (4.44); pH 1, 291 (4.14), 241 (4.26), 205 (4.41); pH 11, 281 (4.18), 244 (4.11); IR (KBr) no cyano absorbtion; TLC R_f = 0.37, solvent C. Anal. ($C_{12}H_{12}N_5O_2S$) C,H,N.

***In Vitro* Antiproliferative Studies.** The *in vitro* cytotoxicity against L1210 was evaluated as described previously.[30] L1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice with 10% heat-inactivated (56 °C, 30 min) horse serum. The growth rate is defined as the number of cell doublings in the treated cultures as a percent of the number in the control culture and was calculated from determinations of cell number at 0, 48, and 96 h in the presence of various concentrations of the test compound. Control population-doubling time was 12 h. When the growth rate in the treated cultures decreased during the experiment, the rate used was that between 48 and 96 h. The IC_{50} is defined as the concentration required to decrease the growth rate to 50% of the control.

***In Vitro* Antiviral Studies. Cell Culture Procedures.** The routine growth and passage of KB and HFF cells was performed in monolayer cultures using minimal essential medium (MEM) with either Hanks salts [MEM(H)] or Earle salts [MEM(E)] supplemented with 10% calf serum or 10% fetal bovine serum (HFF cells). The sodium bicarbonate concentration was varied to meet the buffering capacity required. Cells were passaged at 1:2–1:10 dilutions according to conventional procedures by using 0.05% trypsin plus 0.02% EDTA in a HEPES-buffered salt solution. Similar suspension culture conditions were employed for CEM cells.

Virological Procedures. The Towne strain, plaque-purified isolate P_o of HCMV was kindly provided by Dr. Mark Stinski, University of Iowa, IA. The KOS strain of HSV-1 was used in most experiments and was provided by Dr. Sandra K. Weller, University of Connecticut, CT. Stock HCMV was prepared by infecting HFF cells at a multiplicity of infection (MOI) of <0.01 plaque-forming units (PFU)/cell as detailed previously.[1] High-titer HSV-1 stocks were prepared by infecting KB cells at an MOI of <0.1 also as detailed previously.[1] Virus titers were determined using monolayer cultures of HFF cells for HCMV and monolayer cultures of BSC-1 cells for HSV-1 as described earlier.[31] U1 and H9III$_B$ cells persistently infected with HIV-1 and strain III$_B$ of HIV-1 were obtained and propagated as described previously.[27]

Antiviral Assays: HCMV. Both plaque and yield reduction assays were used. HFF cells in 24-well cluster dishes were infected with ca. 100 PFU of HCMV/well of cell sheet using the procedures detailed above. Following virus adsorption, compounds dissolved in growth medium were added to duplicate wells in four to eight selected concentrations. After incubation at 37 °C for 7–10 days, cell sheets were fixed and stained with crystal violet and microscopic plaques enumerated as described above. Drug effects were calculated as a percentage of reduction in number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug. For yield reduction assay, the procedure devised by us[31] was used. HFF cells were planted as described above in 96-well cluster dishes and incubated overnight, the medium was removed, and the cultures were inoculated with HCMV at a MOI of 0.5–1 PFU/cell. After virus adsorption, inoculum was replaced with 0.2 mL of fresh medium containing test compounds. The first row of 12 wells was left undisturbed and served as virus controls. Each well in the second row received an additional 0.1 mL of medium with test compound at 3 times the desired final concentration. The contents of the 12 wells were mixed by repeated pipetting and then serially diluted 1:3 along the remaining wells. In this manner, six compounds could be tested in duplicate on a

single plate with concentrations from 100 to 0.14 μM. Plates were incubated at 37 °C for 7 days and subjected to one cycle of freezing and thawing; aliquots from each of the eight wells of a given column were transferred to the first column of a fresh 96-well monolayer culture of HFF cells. Contents were mixed and serially diluted 1:3 across the remaining 11 columns of the secondary plate. Each column of the original primary plate was diluted across a separate plate in this manner. Cultures were incubated, plaques were enumerated, and titers were calculated as described.[31]

HSV-1. An ELISA assay developed by us[32] was employed to detect HSV-1. Ninety-six-well cluster dishes were planted with 10 000 BSC-1 cells/well in 200 μL/well MEM(E) plus 10% calf serum. After overnight incubation at 37 °C, selected drug concentrations in quadruplicate and HSV-1 at a concentration of 100 PFU/well were added. Following a 3 day incubation at 37 °C, medium was removed, plates were blocked and rinsed, and horse radish peroxidase-conjugated rabbit anti-HSV-1 antibody was added. Following removal of the antibody-containing solution, plates were rinsed and then developed by adding 150 μL/well of a solution of tetramethylbenzidine as substrate. The reaction was stopped with H_2SO_4, and absorbance was read at 450 and 570 nm. Drug effects were calculated as a percentage of the reduction in absorbance in the presence of each drug concentration compared to absorbance obtained with virus in the absence of drug.

Other Herpes Viruses. The activity of compound **10** against other herpes viruses was determined by plaque assay. The sensitivity of HSV-1 (E-377 strain), HSV-2 (MS strain), and VZV (Ellen strain) to compounds was determined in HFF cells by a plaque reduction assay. Confluent cell monolayers in 6-well plates were inoculated with 20–50 PFU of the appropriate virus and incubated at 37 °C and 90% humidity for 1 h. Serial 5-fold dilutions of each drug were prepared in 2× concentrated MEM(E) and mixed with an equal volume of 1% agarose, and 2 mL of the mixture was added to the monolayer cultures. An additional overlay mixture without drug (1 mL) was added on days 3 and 6 for VZV. At the appropriate time (HSV, 3 days; VZV, 10 days) monolayers were stained with neutral red and plaques enumerated either visually or with the aid of a stereodissecting microscope. Drug-treated cultures were compared to untreated control cultures, and IC_{50} values were calculated using a dose–effect analysis software program (Elsevier-Biosoft, Cambridge, U.K.).

HIV. Two separate assays were employed to evaluate the activities of compounds in cells acutely infected with HIV. The syncytial plaque assay performed as previously described by us[27] was used to measure the effect of compounds on HIV-1. Briefly, CEM-SS cells rendered adherent to substrate by means of poly(L-lysine) were infected with HIV-1 strain III_B, in the presence and absence of selected concentrations of compounds. Syncytial plaques were enumerated at 30-fold magnification 7 days postinfection. The second assay measured the presence of HIV in supernatants of CEM cells infected with strain III_B by the amount of RT activity. Cells were grown, infected, and incubated in the presence of seven concentrations (one-half $\log_{10}$ dilutions) beginning at a 1 or 100 μM concentration of the compounds to be assayed. Procedures and the RT assay were performed as detailed previously by White et al.[33] Techniques for determination of activity of test compounds in cells persistently infected with HIV, including assays for p24 antigen, have been detailed previously.[27]

Cytotoxicity Assays. Several different assays were used to explore cytotoxicity of selected compounds using methods we have detailed previously. (i) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[1] (ii) The effect of compounds during two population doublings of KB cells was determined by crystal violet staining and spectrophotometric quantitation of dye eluted from stained cells as described earlier.[34] Briefly, 96-well cluster dishes were planted with KB cells at 3000–5000 cells/well. After overnight incubation at 37 °C, test compound was added in quadruplicate at six to eight concentrations. Plates were incubated at 37 °C for 48 h in a CO_2 incubator, rinsed, fixed with 95% ethanol, and stained with 0.1% crystal violet. Acidified ethanol was added, and plates were read at 570 nm in a spectrophotometer designed to read 96-well ELISA assay plates. (iii) The MTT dye assay also was used to determine the effect of compound **10** on selected cells lines. Briefly, cells were grown as described above, and compound **10** was added in one-half $\log_{10}$ dilutions. Plates were incubated for one to three population doublings in a humidified atmosphere of 5% CO_2 in air. Viable cells were measured with the tetrazolium salt MTT. Plates were read at a wavelength of 570 nm on a plate reader, and the percent inhibition per drug concentration was measured as test over control and expressed in percent. (iv) Inhibition of DNA synthesis was measured by determining the effect of compounds on the uptake of [^{3}H]dThd into total acid-precipitable DNA as detailed earlier.[27]

Data analysis. Dose–response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. Fifty percent inhibitory (IC_{50}) concentrations were calculated from the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

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JM950293K

BEFORE THE OFFICE OF THE CONTROLLER OF PATENTS, DELHI

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, 2024

AND

IN THE MATTER OF:

Indian Patent No. 564721 (Formerly Indian Application No. 201617031899) filed by MERCK SHARP & DOHME LLC.

AND

IN THE MATTER OF:

MERCK SHARP & DOHME LLC. ..... **APPLICANT**

vs

LOW COST STANDARD THERAPEUTICS .....**OPPONENT**

AFFIDAVIT

[A] **INTRODUCTION:**

I, Debkumar Nandi, age 45 years, S/o Anil Nandi, residing at R/0 Village Kaliyan, Po-Santrapur, Ps-Keshiary, Dist.-West Midnapore, West Bengal, India, Pin Code-721133 and working for gain at TCG Lifesciences Pvt. Ltd. Chembiotek, and presently at New Delhi do hereby state as under:

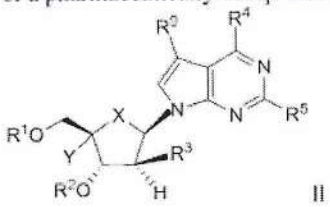
1. I am serving as senior research scientist at TCG Lifesciences Pvt. Ltd. Chembiotek. I hold PhD in the field of Organic Chemistry with extensive experience of in synthesis and purification, and characterization of a broad range of heterocyclic small molecules. I have over 16 years of research and industrial experience.

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2. I have been approached by Low Cost Standard Therapeutics (hereinafter referred as the "Opponent") regarding an Indian patent no. 564721 (formerly Indian patent application No. 201617031899) in the name of MERCK SHARP & DOHME LLC. (hereinafter referred as "Patentee/Applicant"). The relevant documents like specification, claims and certain prior art was provided by Opponent. The impugned patent claims compounds purportedly active against HIV, the claims of which is reproduced below-

1. A compound

or a pharmaceutically acceptable salt thereof, which has structural formula II:



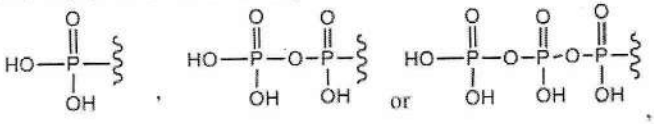
II

;

X is O;

Y is –C≡C-R^8;

R^1 is -H, -C(O)R^6, -C(O)OR^6, -C(O)N(R^6)$_2$,



or a pro-drug modification of the mono-, di- or triphosphate;

R^2 is -H, -C(O)R^{6a}, -C(O)OR^{6a} or -C(O)N(R^{6a})$_2$;

R^3 is –H;

R^4 is -N(R^X)$_2$, -NHC(O)OR^{6b},

-N(C(O)OR^{6b})$_2$, -NHC(O)N(R^{6b})$_2$, or -NHC(O)R^{6b};

R^5 is -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -C_3-C_7 cycloalkyl, halo,

-OR^X, -CN, -N(R^X)$_2$-NHC(O)OR^{6b}, -N(C(O)OR^{6b})$_2$, -NHC(O)N(R^{6b})$_2$, or -NHC(O)R^{6b};

R^6, **R^{6a}** and **R^{6b}** are each independently selected at each occurrence from -H, -C_1-C_6 alkyl, or -C_1-C_6 haloalkyl;

R^8 is -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -C_3-C_7 cycloalkyl;

R^9 is -H, halo, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -CN, -OR^Y or -N(R^Y)$_2$;

R^X is independently selected at each occurrence from -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, aryl, or 5- or 6- membered monocyclic heteroaryl;

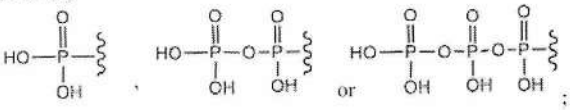
or when either or both of **R^4** or **R^5** is -N(R^X)$_2$, each R^X may optionally be joined together with the nitrogen to which they are both attached to form a 5- or 6-membered monocyclic heteroaryl or 9- or 10-membered bicyclic heteroaryl; and

R^Y is -H, -C_1-C_6 alkyl or -C_1-C_6 haloalkyl.



2. The compound as claimed in claim 1, or or a pharmaceutically acceptable salt thereof, wherein:

R^1 is –H,



; and

R^4 is $-N(R^X)_2$.

3. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein R^2 is -H.

4. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof wherein R^4 is $-N(R^X)_2$.

5. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein R^5 is –H, halo, $-C_1$-C_6 alkyl, $-OR^X$, or $-N(R^X)_2$.

6. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein R^8 is–H.

7. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein R^9 is –H, halo, $-C_1$-C_3 alkyl, $-C_1$-C_3 haloalkyl, -CN, $-OR^Y$ or $-N(R^Y)_2$.

8. The compound as claimed in claim 1 to 7 or a pharmaceutically acceptable salt thereof, wherein

R^X is -H, $-C_1$-C_6 alkyl or $-C_1$-C_6 haloalkyl.

9. The compound as claimed in claim 1 or 2 or a pharmaceutically acceptable salt thereof, wherein:

R^5 is –H, halo, $-C_1$-C_6 alkyl, $-C_1$-C_6 haloalkyl, -CN, $-OR^X$, $-N(R^X)_2$, $-NHC(O)OR^{6b}$, $-N(C(O)OR^{6b})_2$, $-NHC(O)N(R^{6b})_2$, or $-NHC(O)R^{6b}$; and

R^9 is –H, halo, $-C_1$-C_3 alkyl, $-C_1$-C_3 haloalkyl, -CN, $-OR^Y$ or $-N(R^Y)_2$.

10. The compound as claimed in claim 9 or a pharmaceutically acceptable salt thereof wherein R^4 is $-N(R^X)_2$.

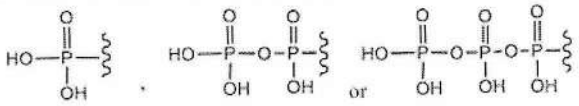
11. The compound as claimed in claim 9 or a pharmaceutically acceptable salt thereof wherein R^1 is –H and R^4 is $-N(R^X)_2$.



12. The compound as claimed in claim 1 or a pharmaceutically acceptable salt thereof, wherein:

Y is –C≡CH;

R^1 is -H, $-C(O)R^6$, $-C(O)OR^6$, $-C(O)N(R^6)_2$,



;

R^2 is H, $-C(O)R^{6a}$, $-C(O)OR^{6a}$ or $-C(O)N(R^{6a})_2$;

R^3 is –H;

R^4 is $-N(R^X)_2$, $-NHC(O)OR^{6b}$ or $-NHC(O)N(R^{6b})_2$;

R^5 is –H, halo, $-C_1$-C_6 alkyl, $-C_1$-C_6 haloalkyl, -CN, $-OR^X$ or $-N(R^X)_2$;

R^6, R^{6a}, and R^{6b} are each independently selected at each occurrence from -H, $-C_1$-C_6 alkyl, or $-C_1$-C_6 haloalkyl;

R^9 is –H, halo, $-C_1$-C_3 alkyl, $-C_1$-C_3 haloalkyl, -CN, $-OR^Y$, or $-N(R^Y)_2$;

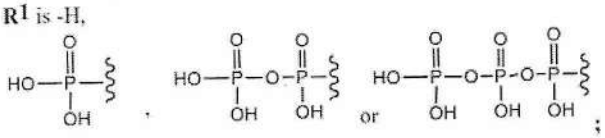
R^X is independently selected at each occurrence from -H, $-C_1$-C_6 alkyl, or $-C_1$-C_6 haloalkyl; and

R^Y is independently selected at each occurrence from -H, $-C_1$-C_6 alkyl or $-C_1$-C_6 haloalkyl.

13. The compound as claimed in claim 1, or a pharmaceutically acceptable salt thereof, wherein:

Y is -C≡CH;

R^1 is -H,



;

R^2 is H;

R^3 is -H;

R^4 is NH_2;

R^5 is –H, halo or $-NH_2$; and

R^9 is –H, halo or $-CH_3$.

14. The compound as claimed in claims 1 or a pharmaceutically acceptable salt thereof, wherein one or more of R1, R2, R4 or R5 is selected as follows:

R^1 is $-C(O)R^6$, $-C(O)OR^6$, $-C(O)N(R^6)_2$; and/or

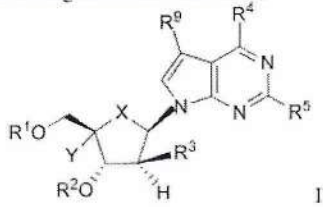
R^2 is $-C(O)R^{6a}$, $-C(O)OR^{6a}$ or $-C(O)N(R^{6a})_2$; and/or

R^4 is $-NHC(O)OR^{6b}$, $-N(C(O)OR^{6b})_2$, $-NHC(O)N(R^{6b})_2$, or $-NHC(O)R^{6b}$; and/or

R^5 is $-NHC(O)OR^{6b}$, $-N(C(O)OR^{6b})_2$, $-NHC(O)N(R^{6b})_2$, or $-NHC(O)R^{6b}$.

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15. The compound as claimed in claim 1 or a pharmaceutically acceptable salt thereof, wherein the compound having structural Formula II,



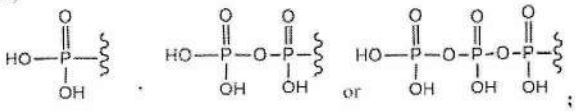
II,

or a pharmaceutically acceptable salt thereof, wherein:

X is O;

Y is –C≡CH;

R1 is –H,



;

R2 is –H;

R3 is –H;

R4 is –NH_2;

R5 is –H, halo or –NH_2; and

R9 is –H, halo or –CH_3.

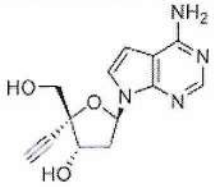
16. The compound as claimed in claim 1 that is:

1)	(2*R*,3*S*,5*R*)-5-(4-amino-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
2)	(2*R*,3*S*,5*R*)-5-(4-amino-5-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
3)	(2*R*,3*S*,5*R*)-5-(4-amino-5-methyl-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
4)	(2*R*,3*S*,5*R*)-5-(4-amino-2-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
5)	(2*R*,3*S*,5*R*)-5-(4-amino-5-bromo-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
6)	(2*R*,3*S*,5*R*)-5-(4-amino-5-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
7)	(2*R*,3*S*,5*R*)-5-(4-amino-5-iodo-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
8)	(2*R*,3*S*,5*R*)-5-(2,4-diamino-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
9)	(2*R*,3*S*,5*R*)-5-(4-amino-2-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol ;
10)	((2*R*,3*S*,5*R*)-5-(4-amino-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
11)	((2*R*,3*S*,5*R*)-5-(4-amino-5-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
12)	((2*R*,3*S*,5*R*)-5-(4-amino-5-methyl-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
13)	((2*R*,3*S*,5*R*)-5-(4-amino-2-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;
14)	((2*R*,3*S*,5*R*)-5-(4-amino-5-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate;

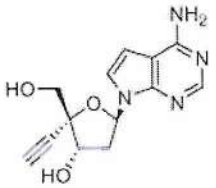
or a pharmaceutically acceptable salt thereof

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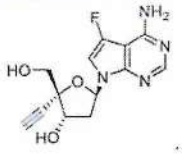
17. The compound as claimed in claim 1 that is:



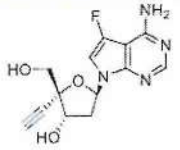
18. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



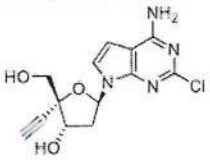
19. The compound as claimed in claim 1 that is:



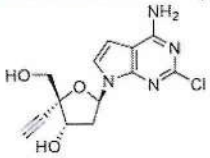
20. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



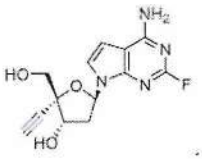
21. The compound as claimed in claim 1 that is:



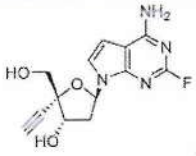
22. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



23. The compound as claimed in claim 1 that is:



24. The compound as claimed in claim 1 that is a pharmaceutically acceptable salt of:



25. A pharmaceutical composition comprising a compound as claimed in any one of claims 1 to 16, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier and optionally comprising at least one anti-HIV agent selected from an HIV antiviral agent, an immunomodulator, and an anti-infective agent such as herein described.

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3. I have been apprised that a case of lack of novelty and inventive step is made out, where the subject matter of Indian patent no. 564721 (formerly Indian patent application No. 201617031899) is devoid of any novelty and inventive step.

4. I also want to make it clear that my opinion is purely based on my education, knowledge, training and experiences in the relevant scientific field.

5. I also want to declare that for consulting the matter described herein on this case, I am being compensated but neither is my compensation related to the outcome of these proceedings, nor do I have a personal interest with bad faith.

6. I am no stranger to the requirements of an invention as per Indian Patents Act, 1970, (herein after referred as "the Patents Act") that to be patentable, an invention must be novel, inventive/non-obvious and have industrial applicability. More importantly, the invention shall also cross the barrier of non-patentable subject matters in accordance with those that are described in 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 skilled in the art (PHOSITA).

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9. Thus, from above information, it may be clear to the Learned Controller that I am well mindful of Indian patents law also.

10. I am advised that this scrutiny is also to be done by a person skilled in the art who may be one with Ph.D. degree in pharmacy or chemistry, or a Masters degree (in pharmacy or chemistry), and few years (2-5) of experience of working in laboratories. I have also been exposed to patents and I am familiar with reading and understanding pharmaceutical patents as I have gone through the same in my career. Any 'skilled person' as described above is one who would be able to understand patents and their working. I believe I would meet these criteria.

[B] EDUCATIONAL AND PROFESSIONAL BACKGROUND:

11. My educational qualifications, work experience, associations are more particularly stated in Exhibit-1, hereto. In view thereof, I am the ordinarily skilled person in the art who is the addressee of the invention disclosed and claimed in the opposed patent application and I am competent to make this Affidavit, *inter alia* expressing an opinion, as an expert in the field of pharmaceutical Sciences on the issue stated herein below.

12. My previous work experiences are mentioned in detail in the form of curriculum vitae enclosed herewith as Exhibit-1. A representative list of my career achievements is also attached herewith as Exhibit-1.

[C] TECHNICAL:

13. In view of my position, qualifications, experience and knowledge in the field of Chemistry, I am competent to depose this Affidavit by expressing my opinions on the discussed matter.

14. I have carefully reviewed the documents provided to me and based on their content and my expertise in the field, I understand that the impugned patent concerns the field of compounds meant to be effective against HIV. These molecules belong to the broader class of nucleoside

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antivirals, many of which have been employed over the years to address treatment needs in diseases caused by viruses other than HIV also.

BACKGROUND ON COMPOUNDS FOR HIV TREATMENT

15. HIV is a pathogenic virus which belongs to the Retroviridae family. Infection can be acquired through infected needles, through unprotected sexual contact and through maternal to fetal route of the mother is infected. Over a long period of time, it causes a syndrome of immunodeficiency, which leads to other opportunistic infections resulting in death. Treatment for HIV, referred to as antiretroviral therapy (ART) has evolved through the years and today, an infected person's viral load can be reduced enough that they are able to lead a relatively normal life. However, a high pill burden and numerous adverse effects associated with the current drugs are a significant roadblock in achieving a good quality of life for those afflicted with the disease.

16. I note that the compounds claimed in IN'721 are nucleoside analogues used as reverse transcriptase inhibitors. This class of compounds has been extensively investigated as antivirals including for addressing HIV infections. Incorporation into viral DNA/RNA as a result of their similarity with natural nucleosides is the chief mechanism by which these compound exert their therapeutic effect.

17. The common structural feature of these compounds is the sugar, the base, and sometimes, the phosphate group. Substitutions on all these moieties, and modification thereof has been thoroughly investigated for obtaining compounds with better potency and other optimized properties. Modifications of the base (both purines and pyrimidines) and sugar going back more than 50 years has led to significant progress where substitution on the2′ and 4′ positions of sugar by Ohrui et al. and Kodama et al. led to compounds with considerably improved activity, including for targeting HIV.

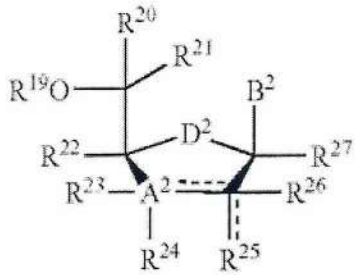
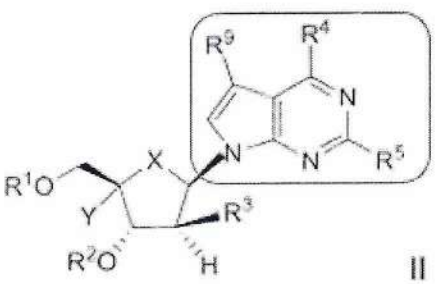
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18. Further improvements led to modulated enzyme binding, cellular uptake, and metabolic stability of these class of compounds. Thus, a person skilled in the art had a plethora of options for influencing the design of nucleoside analogues at the priority date of the impugned patent.

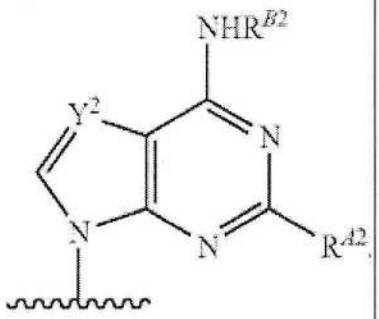
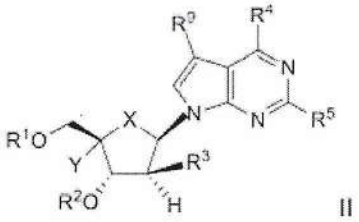
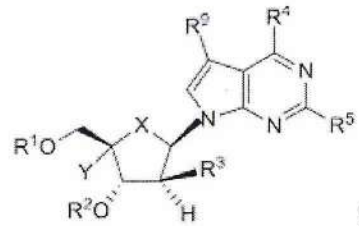
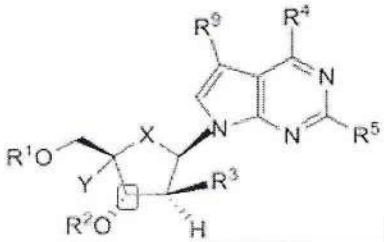
Novelty

19. I find that claim 1 of the impugned patent covers a large range of compounds that are represented by the Markush formula therein. However, despite this, I find that the Patentee has made reference to specific compounds in the written submission subsequent to the section 14 hearing. I understand that claim 1 stands on its own and thus ought to be evaluated without necessarily referring to only a few compounds. In my view, the claim lacks novelty in light of the contents of the following prior art

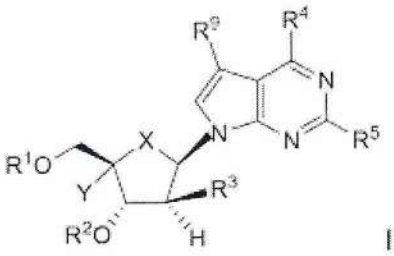
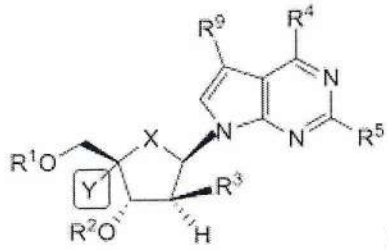
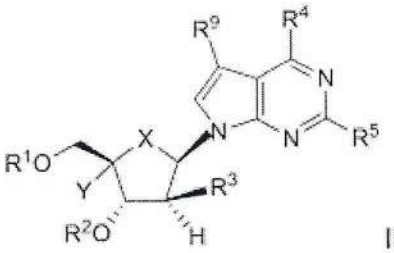
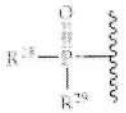
US20100249068

US20100249068	Claim 1
	
B^2 is an optionally substituted **heterocyclic base** or a derivative thereof (para [0099] internal page 11)	 II
Various optionally substituted heterocyclic bases and optionally substituted heterocyclic base derivatives can be present in a compound	R^4 is $-N(R^x)2$, $-NHC(O)OR^{6b}$, $-N(C(O)OR^{6b})2$, $-NHC(O)N(R^{6b})2$, or $-NHC(O)R^{6b}$

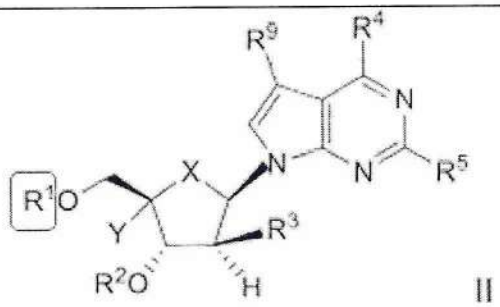
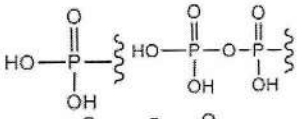
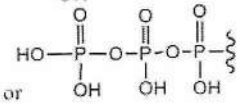
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of Formula (II). Examples of suitable optionally substituted heterocyclic bases (para[0108], internal page 15)  R^{A2} can be **hydrogen** or **halogen** R^{B2} can be **hydrogen** Y^2 can be CR^{F2}, wherein R^{F2} can be **hydrogen** (para [0108], internal page 16)	R^5 is -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -C_3-C_7 cycloalkyl, halo, -OR^X, -CN, -$N(R^X)2$-$NHC(O)OR^{6b}$, -$N(C(O)OR^{6b})2$, -$NHC(O)N(R^{6b})2$, or -$NHC(O)R^{6b}$; R^9 is -H, halo, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -CN, -OR^Y or -$N(R^Y)2$; R^X is independently selected at each occurrence from -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, aryl, or 5- or 6- membered monocyclic heteroaryl
D^2 is selected from the group consisting of C=CH_2, CH_2, **O (oxygen)**, S (sulfur), CHF, and CF_2; (para [0099] internal page 11)	 II ; **X** is O;
R^{27} can be **hydrogen** (para [0099] internal page 11) R^{26} and R^{25} can be **hydrogen** (para [0099] internal page 12)	 II **R3** is –H;
A^2 is selected from the group consisting of **C (carbon)**, O (oxygen) and S (sulfur) (para [0099] internal page 11)	 II

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R^{23} and R^{24} can **independently** be **hydrogen** **—OR ^{a2},** **—O—(C=O)R ^{a2},** **—O—C(=O)OR ^{a2},** **—O—C(=O)NR ^{a2}R ^{b2}** R ^{a2} and R ^{b2} can be **hydrogen** and can also be an optionally substituted **alkyl** (para [0099] internal page 12)	 II **R2** is -H, -C(O)R^{6a}, -C(O)OR^{6a} or -C(O)N$(R^{6a})_2$; R^6, R^{6a} and R^{6b} are each independently selected at each occurrence from -H, -C_1-C_6 alkyl, or -C_1-C_6 haloalkyl
R^{22} can be an **optionally substituted C $_{2-6}$ alkynyl** (para [0099], internal page 11)	 II **Y** is –C≡C-R^8; R^8 is -H, -C_1-C_6 alkyl, -C_1-C_6 haloalkyl, -C_3-C_7 cycloalkyl
R^{20} and R^{21} are independently selected from the group consisting of **hydrogen** (para [0099], internal page 11)	 II
R ^{19} is selected from the group consisting of **hydrogen** (para [0099], internal page 11) 	

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diphosphate, triphosphate or **derivatives** thereof; R^{28} and R^{29} can be **OH** (para [0100], internal page 12)	 II R^1 is -H, -C(O)R^6, -C(O)OR^6, -C(O)N(R^6)2,  or  or a pro-drug modification of the mono-, di- or triphosphate;

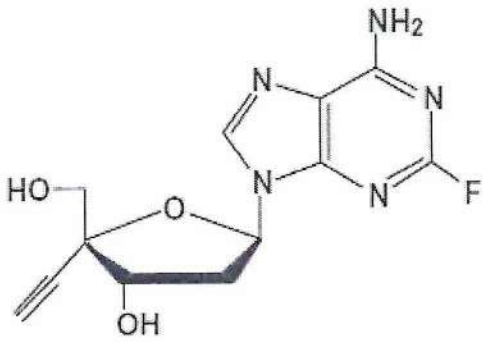
Inventive step

20. I say that the claimed subject matter is obvious and devoid of any technical advancement in light of prior arts that are discussed below, and due to following reasons:

Hiroshi Ohrui, "2′-Deoxy-4′-C-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, is highly potent against all human immunodeficiency viruses type 1 and has low toxicity," The Chemical Record, Vol. 6, No. 3, 2006, pp. 133–143

21. Ohrui discloses development of 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, which is highly potent to all human immunodeficiency viruses type 1 (HIV-1s) including multidrug-resistant HIV-1 and has a low toxicity, which is shown below





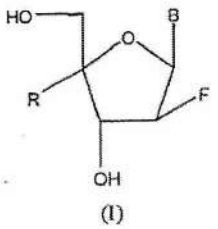
4'Ed2FA

22. It is further disclosed that the author designed this compound based on a proposed working hypothesis based on the fundamentals of both organic chemistry and biochemistry. Further, scientific evidence proved the validity of the working hypothesis and resulted in the development of 4'Ed2FA, which is highly potent against all HIV-1s, is stable to intracellular enzymatic catabolism and acidic degradation, has a very long intracellular *T*1/2, does not greatly inhibit DNA polymerase γ, and does not have acute mouse toxicity.

23. It can be seen that this compound, which has extremely favourable properties would be a good starting point for further modifications and development of compounds against HIV with improved potency and better pharmacokinetic properties.

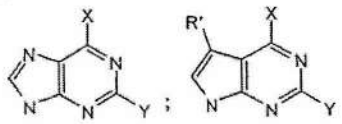
EP2177527 (EP'527) published 18 Dec 2013

24. EP'527 discloses 2'-fluoro-4'-substituted nucleoside derivatives for the treatment of viral infections including HIV. The structure of these compounds is depicted below



(I)

wherein:

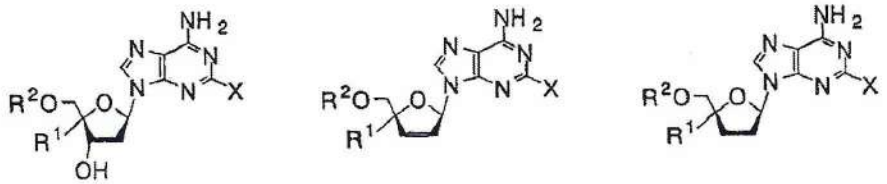
B= 

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25. EP'527 teaches substitution at the 4′-position of the sugar with ethynyl (–C≡CH). In conjunction with the teachings of Ohrui above, it can be seen that 4′-ethynyl substitution is a well explored substituent in the class of compounds intended for HIV. Further, the 7-deazaadenosine nucleobase is part of the Markush depicted above, and thus represents one of only two options above.

CA2502109 (CA'109) published 23 Feb 2010

26. CA'109 discloses 4'-C-substituted-2-haloadenosine derivatives which as per the inventors were developed in order to attain further enhancement of selectivity index; i.e., (concentration at which cytotoxicity is obtained)/(concentration at which anti-HIV activity is obtained) and to provide resistance to inactivation by adenosine deaminase, through chemical modification of 4'-C-substituted-2'-deoxyadenosine (a lead compound), which, among various 4'-C-substituted purine nucleosides, exhibits potent anti-HIV activity and less toxicity.



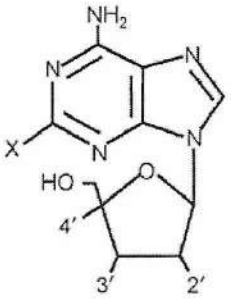
[I] [II] [III]

Kawamoto et al. *"2'-Deoxy-4'-C-ethynyl-2-halo-adenosines active against drug-resistant human immunodeficiency virus type 1 variants"*; The International Journal of Biochemistry & Cell Biology 40 (2008) 2410–2420

27. Kawamoto et al. is a publication reporting on work done in continuation to previous syntheses of 2'-deoxy-4'-C-ethynylnucleosides against NRTI-resistant HIV strains. They also report that other work in this direction has resulted in compounds such as 2',3'-didehydro-3'-deoxy-4'-*C*-ethynyl-thymidine (Ed4T) active against wild-type and drug-resistant strains and less toxic than the drug stavudine) *in vitro*.

28. Based on the above work, the authors synthesized 4'-substituted adenosine analogs shown below

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Base X	Sugar 2′	Sugar 3′	4′	Compound abbreviation
-H	-H	-OH	-C ≡ CH	*E*dA
-F	-H	-OH	-C ≡ CH	2F-*E*dA
-F	-H	-H	-C ≡ CH	2F-*E*ddA
-F	C	C	-C ≡ CH	2F-*E*d4A
-F	-H	-OH	-C ≡ N	2F-CNdA
-Cl	-H	-OH	-C ≡ CH	2Cl-*E*dA

Fig. 1. Structures of 4′-substituted adenosine analogs. All nucleoside analogs discussed here have substitutions at the 4′-position of the sugar ring.

29. The activity of these compounds against HIV-1 and HIV-2 strains in MT-4 cells is shown below

Table 1
Antiviral activity against HIV-1 and HIV-2 strains in MT-4 cells

Compound (abbreviation)	EC_{50} (μM)[a]		Selectivity[b]	
	HIV-1_{IIIB}	HIV-2_{ROD}	CC_{50} (μM)[c]	index
2′-Deoxy-4′-C-ethynyl-adenosine (EdA)	0.0095 ± 0.0027[d]	0.006 ± 0.0015	104 ± 6.2	11,000
2′-Deoxy-4′-C-ethynyl-2-fluoroadenosine (EFdA)	0.000073 ± 0.000017	0.000098 ± 0.000022	9.8 ± 3.4	134,000
2′,3′-Dideoxy-4′-C-ethynyl-2-fluoroadenosine (EFddA)	1.17 ± 0.29	1.07 ± 0.23	230 ± 33	196
2′,3′-Didehydro-3′-deoxy-4′-C-ethynyl-2-fluoroadenosine (EFd4A)	0.11 ± 0.033	0.089 ± 0.0007	98 ± 26	890
2′-Deoxy-4′-C-cyano-2-fluoroadenosine (CNFdA)	0.1 ± 0.034	0.09 ± 0.0087	>340	>3,300
2′-Deoxy-4′-C-ethynyl-2-chloroadenosine (ECldA)	0.00089 ± 0.00018	0.0006 ± 0.0000028	230 ± 16	330,000
2′,3′-Dideoxyinosine (ddI)	27 ± 12	24 ± 4.4	>100	>4
3′-Azido-3′-deoxythymidine (AZT)	0.0028 ± 0.00062	0.0022 ± 0.00073	30 ± 7.2	10,800

Anti-HIV activity was determined by the MTT method.
[a] EC_{50} represents the concentration that blocks HIV-1 replication by 50%.
[b] Selectivity index is calculated by the CC_{50}/EC_{50} for HIV-1_{IIIB}.
[c] CC_{50} represents the concentration that suppress the viability of HIV-1-unexposed cells by 50%.
[d] Data shown are mean values with standard deviations for at least three independent experiments.

30. From the above data, it can be seen that, although EFdA demonstrated the best EC_{50} against both HIV-1 and HIV-2, the best selectivity index was demonstrated by ECldA, which is indicative of low toxicity. ECldA also demonstrated good activity against both strains and thus showed a good combination of properties. This is echoed by the authors who observed (*Results, 3.1*) that the 2-chloro (Cl) substitution also provided enhanced activity but with a decreased toxicity.

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31. The authors note that modifications of the sugar ring from2'-deoxyribose to 2',3'-dideoxy- or 2', 3'-didehydro-2',3'-dideoxy-ribose

(EFddA or EFd4A) resulted in a drastic decrease of inhibitory potential. Substitution of the 4'-E group with a structurally similar 4'-cyano group also resulted in markedly decreased inhibitory activity. These results indicate that the 3'-OH and 4'-E moieties in the sugar ring are indispensable for high efficacy, and that antiviral activities are augmented by the modification with F- or Cl-moiety at the adenine 2-position.

32. Further, they observed that the two highly active 4'-E analogs, (EFdA or ECldA) were even more effective against the dideoxy-type NRTI resistant variants K65R, L74V, and Q151M complex than they were against WT RT.

Table 2
Anti-HIV-1 activity against drug-resistant infectious clones

	EC_{50} (μM)[a]								
	EdA	EFdA	EFddA	EFd4A	CNFdA	ECldA	ddI	AZT	3TC
WT	0.021	0.0011	1.2	0.35	0.21	0.0004	4.1	0.015	0.71
K65R	0.0092	0.00022 (×0.2)	1.56	0.2	ND[b]	0.0017 (×0.3)	ND	0.0030 (×0.2)	ND
L74V	0.01	0.00048 (×0.4)	2.52	0.54	ND	0.0015 (×0.2)	14.6	0.019 (×1.3)	ND
V75T	0.0075	0.00067 (×0.6)	0.13	0.05	ND	0.005 (×0.8)	ND	0.047 (×3.1)	ND
M41L/T215Y	0.062	0.0015 (×1.5)	6.7	1.67	ND	0.0065 (×0.1)	ND	0.12 (×8)	ND
M41L/T69SSC/T215Y[c]	0.15	0.0065 (×6)	ND	ND	ND	0.025 (×4)	21	0.20 (×13)	0.9
MDR[d]	0.011	0.00074 (×0.7)	16	0.46	0.09	0.0057 (×0.9)	40	18 (×1200)	1.1
P119S	0.018	0.00067 (×0.6)	ND	ND	ND	0.0062 (×1)	ND	0.0033 (×0.2)	0.6
T165A	0.045	0.001 (×0.9)	ND	ND	ND	0.0082 (×1.3)	ND	ND	0.66
I142V	0.077	0.001 (×0.9)	ND	ND	ND	0.0062 (×1)	ND	0.016 (×1)	0.36
T165R	0.088	0.0016 (×1.5)	ND	ND	ND	0.012 (×1.9)	ND	0.011 (×0.7)	0.26
M184V	0.088	0.0083 (×7.5)	101	6.41	1.76	0.064 (×13)	ND	0.0021 (×0.1)	>100
T165R/M184V	0.6	0.014 (×13)	ND	ND	ND	0.17 (×27)	ND	0.0053 (×0.4)	>100
I142V/T165R/M184V	0.31	0.023 (×22)	ND	ND	ND	0.41 (×65)	ND	0.0076 (×0.5)	>100
T165A/M184V	0.43	0.015 (×14)	ND	ND	ND	0.16 (×25)	ND	0.0049 (×0.3)	>100
P119S/T165A/M184V[e]	0.5	0.015 (×14)	ND	ND	ND	0.20 (×32)	ND	0.0047 (×0.3)	>100

33. Thus, based on the above findings, a person skilled in the art would understand that the best balance of activity, reduced toxicity and resistance to mutations is demonstrated by ECldA and that 3'-OH and 4'-E moieties in the sugar ring being important for high efficacy lead to retaining these moieties.

Vittori et al. "*Antiviral Properties of Deazaadenine Nucleoside Derivatives*"; Current Medicinal Chemistry, 2006, 13, 3529-3552

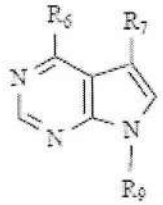
34. Vittori et al. is a review of studies conducted to investigate the antiviral activity of deazapurine nucleoside derivatives. In relation to HIV, They report that (at the time of publication) although more than 25 drugs, many of which are structurally related to nucleosides are used in the treatment of HIV infections, new drugs are nonetheless needed because

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existing ones have severe side effects and because of the emergence of viral resistance. They also add that other unmet needs drive this search for new antivirals such as finding antivirals with a broad spectrum of action.

35. With respect to the different approaches for discovering new antiviral purine nucleosides, it is disclosed that most modifications have been to the heterocycle and to the presence of hydroxyl groups in the sugar moiety. Further, they report that, in particular, deaza analogues of natural purine nucleosides have been thoroughly investigated from both the synthetic and the biological point of view.

36. Specifically, in relation to 7-deazapurine derivatives, they report work done on analogues and derivatives of tubercidin, toyocamycin, and sangivamycin (Table 9, Table 10 and Table 11 internal page 3536-3541).



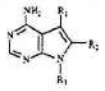
37. The skilled person would understand that exploring 7-deazaadenosine analogues is a viable path to obtaining new antiviral compounds, and would consider its substitution as a base in the compounds of for e.g. Kawamoto et al.

Krawczyk et al "*Synthesis and Antiproliferative and Antiviral Activity of 2'-Deoxy-2'-fluoroarabinofuranosyl Analogs of the Nucleoside Antibiotics Toyocamycin and Sangivamycin*" *J. Med. Chem. 1996,38,* 4106-4114

38. Krawczyk et al. is an article detailing the research work 2'-deoxy-2'-substituted arabinofuranosyl analogs of toyocamycin and sangivamycin for evaluation of their antiviral activities.

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Table 2. Antiviral Activity and Cytotoxicity of 2'-Fluoroarabinosylpyrrolo[2,3-*d*]pyrimidine Analogs



				50% or 90% inhibitory concentration (μM)					
				antiviral activity[a]					
				HCMV		HIV-1[c]	HSV-1[b]	cytotoxicity[d]	
compd	R_1	R_2	R_3	plaque	yield	RT	ELISA	visual	growth
4a	CN	Br	α-D-2-F-arabinosyl	3.2	>100	32	>100[e]	55	>150
4b	CN	Br	β-D-2-F-arabinosyl	12	7	12	>100	21	144
9a	CN	H	α-D-2-F-arabinosyl	16	100	>100	46	100	>100
9b	CN	H	β-D-2-F-arabinosyl	1.0	1.1	12	6.5	6.8	8.8
10	$CONH_2$	H	β-D-2-F-arabinosyl	0.025	0.075	0.02	0.47	5.5	1.9
11	$CSNH_2$	H	β-D-2-F-arabinosyl	0.22	1.5	0.07	8.7	3.1	3
zidovudine[f]						0.011 ± 0.0008		>100	
ganciclovir[g]				7.4 ± 6.5	1.6 ± 1.2		3.5 ± 2.1	>100	>100

39. It is reported that the β anomers were most active among all analogs, and also active against HIV.

Table 3. Antiviral Activity of 2'-Deoxy-2'-fluoroarabinosylsangivamycin (10)

	50% inhibitory concentration[a] (μM)	
virus and cell line	antiviral	cytotoxicity
Antiviral Activity		
HSV-1	0.03	
HSV-2	0.03	
VZV	0.003	
HIV-1[b]	0.0037 ± 0.0017	
Cytotoxicity		
human foreskin fibroblasts		
stationary cells, neutral red uptake		6.7
stationary cells, MTT assay		0.13
proliferating cells, MTT assay		0.16
CEM-SS cells		
visual inspection		0.4
[^{3}H]dThd uptake[c]		0.193 ± 0.074

[a] All assays performed in duplicate as described in the text. [b] Mean ± standard deviation from quadruplicate assays in the syncytial plaque assay using CEM-SS cells. [c] Mean ± standard deviation from triplicate assays.

40. It can be seen that the analogues of toyocamycin and sangivamycin demonstrate antiviral activity including against HIV. In conjunction with the extensive data as detailed in Vittori et al. indicates that this nucleobase can be substituted with other nucleobases to obtain better antiviral activity.Ohrui, EP'527, CA'109 and Kawamoto et al. already demonstrates the excellent activity of ECldA, and the necessity of retaining 3'-OH and 4'-E moieties in the sugar ring, as well as chloro on the adenosine to get good activity and reduced toxicity. Thus the substitutable moiety therein would be the base. In view of the teachings of Vittori et al. and Krawczyk et al, 7-deazadenosine would be a viable substitution for adenosine in Kawamoto et al. Thus, the compounds claimed in the impugned patent are obvious in light of the teachings of Kawamoto, Vittori and Krawczyk.

41. In view of the above submissions, impugned patent lacks novelty and inventive step and therefore, should be revoked on this ground alone.

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Debkumar Nandi

DEPONENT

VERIFICATION

I, Dr. Debkumar Nandi, the Deponent do hereby verify that the contents of my affidavit at para 01 to 41 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 there from.

02 APR 2026

Verified at _____ on this _____ day of _____, 2026

Debkumar Nandi

DEPONENT

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ATTESTED

NOTARY PUBLIC
GOVT. OF INDIA

02 APR 2026

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HIGHLIGHTS

- PhD in Organic chemistry with 7 years research and 6 year industrial experience
- Sound knowledge and experience in natural products isolation
- Extensive experience in synthesis, purification, and characterization of a broad range of heterocyclic small molecules
- Synthesis of oligo-saccharides and glycosides
- Experience of operating and maintaining lab equipment: NMR, HPLC, FTIR, HPTLC.
- Software expertise: Bielstein cross fire, SciFinder, Reaxys, Chemdraw, MS Office
- Highly self-motivated and unyielding commitment to innovation
- Demonstrated ability to perform independent research and supervisory skills
- Highly productive team worker with solid written/oral communication skills
- Conversant with literature and practice of organic synthesis procedures, solving synthetic problems as they apply to the multi-gram through pilot plant scale preparation of active pharmaceutical agents, synthetic route design and implementation, reaction trouble-shooting and optimization through reported and self-developed procedures with suitable modification as and when necessary.

RESEARCH EXPERIENCE

1. **Senior research Scientis (Tcg Lifesciences Chembiotek, Salt Lake Electronics Complex, Plot No-7, Street Number 18, BN Block, Sector V, Bidhannagar, Kolkata, West Bengal 700091) 2020-till date: Engaged in different CRO projects.**
2. **Scientist-C (Natural product Chemistry), Natreon Inc., branch office, India**

Prof. S. Ghosal research centre, **Rishi tech park, premises no- 02/360, action area 1D, New town, Kolkata -700 160: September 2017-2019.**

Identification and quantification of biomarkers of Herbal and Herbo-mineral Ayurvedic medicines and semi syntheses of neuroprotective molecules.

3. **Global Excellence Stature (GES) Postdoctoral Research Fellow, University Of Johannesburg, AKP Campus; Advisor: Prof. KaushikMallick; February 2015-August 2017.**

   Application of polymer/support based transition metal nano-materials in organic methodologies

4. **Research Associate, Indian Institute of Chemical Biology: September, 2013-January, 2015**

   "PDE-IV as target for perkinson's disease: Synthesis of congeners of irsogladine, and their evaluation in cellular and animal models of the disease"

5. **Postdoctoral Research Fellow, National Changhua University of Education (NSC Fellowship, Taiwan); Advisor: Prof. Hon Man Lee; October, 2010-July 2013**

   Transition metal (namely Pd-NHC complexes or Pd(II) salts) as a catalyst for C-H, C-C bond activation and synthesis of heterocyclic compounds.

6. **Senior Research Scientist, Fresenius-Kabi Oncology (API industry), Ghaziabad (near Delhi), Uttar Pradesh., India, January, 2010 to September, 2010.**

   Developed and optimized new methodologies in connection with the ongoing discovery program. Developed a new synthetic route of API on Cladribine, an FDA approved drug used to treat hairy cell leukemia.

Research Fellow (Ph.D.), Indian Institute of Chemical Biology (under Jadavpur University), Kolkata, India; Advisor: Dr. P. Jaisankar and Dr. J. R. Vedasiromoni; January, 2005- December 2009.

- Developed novel synthetic methodologies.
- Isolation and characterization of natural products from Indian plants
- Synthesis of oligo-saccharides, and glycosides

EDUCATION:

PhD (Organic Chemistry and Biotechnology), Jadavpur University, Kolkata, India (Research performed at Indian Institute of Chemical Biology, Kolkata, India) 2005-2009.
M. Sc. (Chemistry, Organic chemistry Specialization) Bengal Engineering and Science University, India, 2003-2004
B. Sc. (major: Chemistry Honors, minor: Physics and Mathematics), BehalaParnasree College, Calcutta University, India 2000-2002

Awards/ Honors

- Junior Research Fellowship -Awarded by Council of Scientific & Industrial Research (CSIR), INDIA, 2005-2006
- Senior Research Fellowship -Awarded by Council of Scientific & Industrial Research (CSIR), INDIA, 2007-2009.

- NSC(National Science Council) Postdoctoral fellowship, Taiwan, 2010-2013
- DBT research associate, Indian Institute of Chemical biology, India 2013-2014
- GES (Global Excellence Stature) Postdoctoral fellowship, University of Johannesburg, South Africa, 2015-2017.

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