

September 28, 2022

THE CONTROLLER OF PATENT
THE PATENT OFFICE
BOUDHIK SAMPADA BHAWAN, PLOT NO. 32
SECTOR 14, DWARKA, NEW DELHI-110078

Re: REPRESENTATION u/s 25(1) of the Patent act – By SANKALP
REHABILITATION TRUST against Indian Patent Application No.
201917029812 filed on 23/07/2019
Title: NUCLEOTIDE HEMI-SULFATE SALT FOR THE TREATMENT OF
HEPATITIS C VIRUS
Applicant: ATEA PHARMACEUTICALS, INC.

Respected Madam,

We are filing this representation by way of Pre-Grant Opposition along with annexures u/s 25 (1) of the Patents Act, 1970 and Rule 55 of the Patent Rules, 2003 in Form 7A.


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,



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

Encl: As stated

C.C: LAKSHMI KUMARAN & SRIDHARAN
Email: iprdel@lakshmisri.com;

Also at: A - 202, First Floor, Shivalik Enclave, Malviya Nagar, New Delhi-110017

BEFORE THE CONTROLLER OF PATENTS, THE PATENT OFFICE,
NEW 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 Pre-grant opposition under Section 25(1)

AND

IN THE MATTER of Indian Patent Application No. 201917029812

IN THE MATTER OF:

SANKALP REHABILITATION TRUST

.....OPPONENT

VS.

ATEA PHARMACEUTICALS, INC.

.....APPLICANT

PRE-GRANT OPPOSITION BY SANKALP REHABILITATION TRUST

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Dated this day 28th of September, 2022



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

TO,
THE CONTROLLER OF PATENTS
THE PATENT OFFICE, NEW DELHI

FORM 7A
THE PATENTS ACT,
1970 (39 OF 1970)
AND
THE PATENTS RULES, 2003
REPRESENTATION FOR OPPOSITION TO GRANT OF PATENT
[See Rule 55]

We, **SANKALP REHABILITATION TRUST**, having its registered office at SS Bengali Municipal School, First Floor, Thakurdwar Road, Charni Road East, Mumbai – 400002, hereby give Notice of opposition to the grant of patent in respect of Indian Patent Application No. 201917029812 filed on 23/07/2019 made by ATEA PHARMACEUTICALS, INC. on the grounds.

- (a) Section 25(1)(b): Lack of novelty
- (b) Section 25(1)(e): Lack of inventive step
- (c) Section 25(1)(f): Invention is not patentable under section 3(d) and 3(e)
- (d) Section 25(1)(g): The complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed.
- (e) Section 25(1)(h): Failed to disclose to the Controller the information required by section 8.

(Detailed grounds are set out in the Opposition)

Our address for service in India is:

RAJESHWARI H.
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Dated this 28th day of September, 2022



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

TO
THE CONTROLLER OF PATENTS
PATENT OFFICE, NEW DELHI

BEFORE THE CONTROLLER OF PATENTS, THE PATENT OFFICE,
NEW DELHI

In the matter of Section 25(1) 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, 2006

And

IN THE MATTER of Indian Patent Application 201917029812 dated 23/07/2019 in the name of **ATEA PHARMACEUTICALS, INC.**

REPRESENTATION BY:

SANKALP REHABILITATION TRUST

.....OPPONENT

VS.

ATEA PHARMACEUTICALS, INC.

.....APPLICANT

REPRESENTATION BY WAY OF PRE-GRANT OPPOSITION UNDER
SECTION 25(1) OF THE PATENTS ACT, 1970

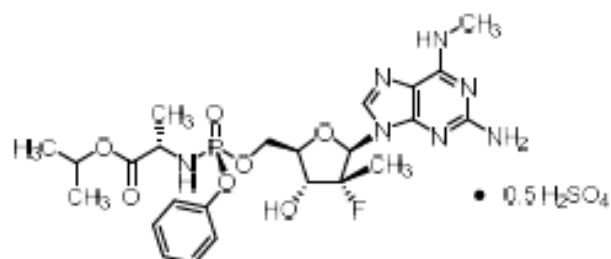
We, Sankalp Rehabilitation Trust an Indian Non-Government Organization, hereby submit our representation by way of opposition to the grant of patent in respect of Indian Patent Application 201917029812 filed on 23/07/2019 in the name of ATEA PHARMACEUTICALS, INC. entitled "Nucleotide Hemi-Sulfate Salt for the Treatment of hepatitis C Virus".

STATEMENT OF CASE OF OPPONENT

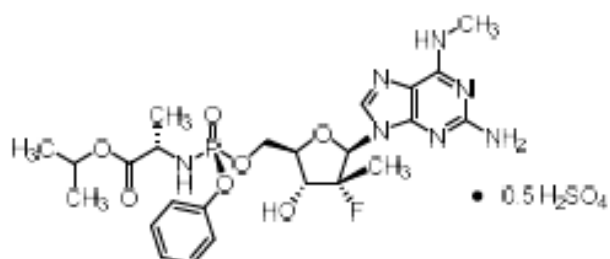
1. The Opponent has learnt that the Applicant has filed an Indian Patent Application No. 201917029812 (hereinafter “the Impugned Patent Application”) on 23/07/2019. The impugned patent application was published in the official journal of the patent office on 18/10/2019, which is currently pending before the Patent Office. The Impugned Patent Application has a priority date of 01/02/2017.
2. The Impugned Patent Application is entitled “NUCLEOTIDE HEMI-SULFATE SALT FOR THE TREATMENT OF HEPATITIS C VIRUS”.
3. The Opponent by way of this present pre-grant opposition submits that the claims currently pending on record are not patentable under the provisions provided in this Act. The claims as filed and currently on record are annexed herewith as **Annexure-1** and reproduced herein below for ready reference:

I/We Claim:

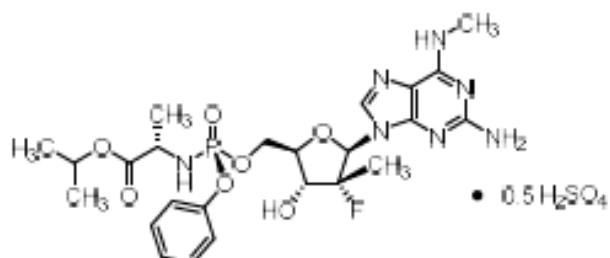
1. A compound of the formula:



2. A solid dosage form comprising a compound of the formula



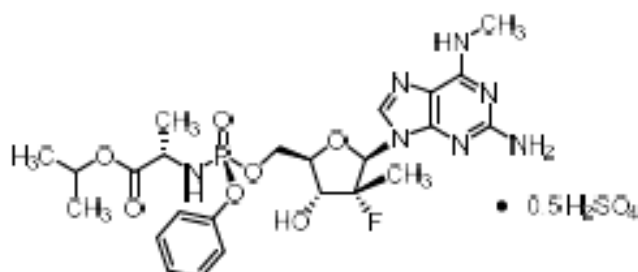
3. A pharmaceutical composition comprising from 10 to 1000 mg of a compound of the formula



in a pharmaceutically acceptable carrier.

4. The pharmaceutical composition of claim 3, in a solid dosage form that delivers from 100 mg to 800 mg of the compound.

5. The pharmaceutical composition of claim 3, in a solid dosage form that delivers at least 600 mg of the compound.
6. The pharmaceutical composition of claim 3, in a solid dosage form that delivers at least 700 mg of the compound.
7. The pharmaceutical composition of claim 3, in a solid dosage form that delivers at least 800 mg of the compound.
8. The pharmaceutical composition of any one of claim 3-7, wherein the pharmaceutically acceptable carrier is suitable for oral delivery.
9. The pharmaceutical composition of claim 8, wherein the pharmaceutically acceptable carrier is in the form of a tablet.
10. The compound of formula



optionally in a pharmaceutically acceptable carrier, for use in the treatment of a hepatitis C infection or a condition resulting from a hepatitis C infection, in a host in need thereof.

11. The compound of claim 10, wherein the compound is administered orally.
12. The compound of claim 10, wherein the compound is administered via controlled release.
13. The compound of claim 10, wherein the condition resulting from a hepatitis C infection has resulted in antibody positive and antigen positive conditions, a viral-based chronic liver inflammation, a liver cancer resulting from advanced hepatitis C, cirrhosis, or fatigue.
14. The compound of any one of claims 10-13, wherein at least 300 mg of the compound is administered.

15. The compound of any one of claims 10-13, wherein at least 400 mg of the compound is administered.
 16. The compound of any one of claims 10-13, wherein at least 500 mg of the compound is administered.
 17. The compound of any one of claims 10-13, wherein at least 600 mg of the compound is administered.
 18. The compound of any one of claims 10-17, further comprising administering the compound in combination with another anti-HCV agent.
 19. The compound of claim 18, wherein the additional anti-HCV agent is selected from the group consisting of a protease inhibitor, an NS5A inhibitor, another NS5B polymerase inhibitor, a non-substrate inhibitor, interferon alfa-2a, ribavirin, a helicase inhibitor, an antisense oligodeoxynucleotide, an aptamer, a nuclease-resistant ribozyme, iRNA, an antibody to HCV, a partial antibody to HCV, and a domain antibody to HCV.
4. **Impugned Patent Application:** The present pre-grant opposition is against Indian Patent Application 201917029812 dated 23/07/2019 in the name of ATEA PHARMACEUTICALS, INC. entitled “Nucleotide Hemi-Sulfate Salt for The Treatment of hepatitis C Virus” and is drawn towards the hemi-sulfate salt of a selected nucleotide compound that has therapeutic properties to treat a host infected with hepatitis C, as well as pharmaceutical compositions and dosage forms thereof.
 5. The impugned patent application further discloses that the hemisulfate salt of Compound 1, which is provided below as Compound 2, exhibits unexpected advantageous therapeutic properties, including enhanced bioavailability and target organ selectivity, over its free base (Compound 1). Compound 2 is referred to as the hemi -sulfate salt of isopropyl((S)-(((2R,3R,4R,5R)-5-(2-amino-6-(methylamino)-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-Z-alaninate.

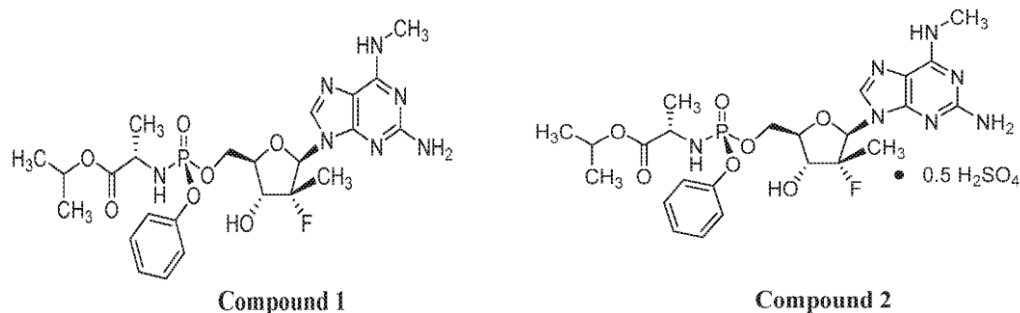


Figure 1

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

- i. **D1-** WO2016144918A1 (WO'918) published on 15 September 2016 (annexed herewith as **Annexure -2**)
- ii. **D2-** G. Steffen Paulekuhn et al; Trends in Active Pharmaceutical Ingredient Salt Selection based on Analysis of the Orange Book Database, *J. Med. Chem.* **2007**, *50*, 6665–6672 (annexed herewith as **Annexure -3**).
- iii. **D3-** Herve Rebiere et al; Determination of 19 antiretroviral agents in pharmaceuticals or suspected products with two methods using high-performance liquid chromatography; *Journal of Chromatography B*, **850** (2007) 376–383 (annexed herewith as **Annexure -4**).
- iv. **D4-** US 2014/0187773 (US'773) published on 03 July 2014 (annexed herewith as **Annexure – 5**).

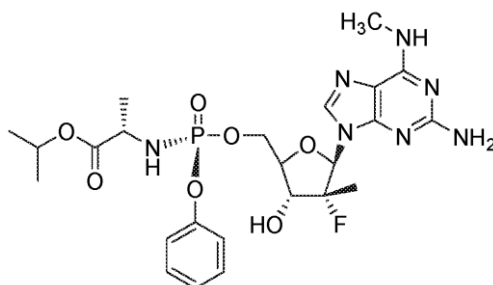
7. It is submitted that the claims of impugned patent application are liable to be refused on following grounds as below, which are without prejudice to each other:

- (a) Section 25(1)(b): Lack of novelty
- (b) Section 25(1)(e): Lack of inventive step
- (c) Section 25(1)(f): Invention is not patentable under section 3(d) and 3(e)

- (d) Section 25(1)(g): The complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed.
- (e) Section 25(1)(h): Failed to disclose to the Controller the information required by section 8.

GROUND 1: SECTION 25(1)(B) LACK OF NOVELTY

8. It is submitted that claim 1 to 19 are not novel, and therefore have to be rejected under Section 25(1)(b) of the Act.
9. It is submitted that claim 1 to 19 are not novel in view of WO 2016144918 (WO'918) published on 15 September 2016 which is before the priority date of impugned patent application i.e. 01/02/2017.
10. It is further submitted that WO'918 discloses the impugned molecule and its pharmaceutically acceptable salts like sulphate salt. The said document not only encompassed the mono salt but also encompassed the hemi-salt of compound. The structure of impugned molecule is given below.



Compound 5-2 (Table 7)

Figure 1

11. Moreover, WO'918 further discloses the pharmaceutical compositions that comprise an anti-HCV virus effective amount of β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate compound, optionally in combination with a pharmaceutically acceptable carrier, additive, or excipients. In addition to this, it further discloses that the said composition optionally in combination with at least one other antiviral, such as an anti-HCV agent.

12. Furthermore, WO'918 also states that the amount of compound included within therapeutically active formulations is an effective amount for treating the Hepatitis C virus (HCV) infection, reducing the likelihood of a HCV infection or the inhibition, reduction, and/or abolition of HCV or its secondary effects, including disease states, conditions, and/or complications which occur secondary to HCV. In general, a therapeutically effective amount of the present compound in pharmaceutical dosage form usually ranges from about 0.001 mg/kg to about 100 mg/kg per day or **more**. The average body weight of a human being is considered as 70 kg approximately. Therefore, therapeutically effective amount of the present compound for a human being could be 700mg or more.
13. The above paragraph discloses that the amount of compound which is delivered through solid dosage forms as claimed in impugned patent application is overlapping with the disclosure of WO'918.
14. WO'918 further discloses that it may be useful to use β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate compound in combination with other therapeutically active agents like protease inhibitor, NS5A inhibitor, Another NS5B polymerase inhibitor, NS5B non-substrate inhibitor, ribavirin Helicase inhibitor, Antisense oligodeoxynucleotide, Aptamer, Nuclease-resistant ribozyme, iRNA, Antibody, and partial antibody or domain antibody to the virus.
15. WO'918 further discloses that it may be useful to use β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate compound in combination with a protease inhibitor, such as an NS3/4A protease inhibitor (for example, telaprevir (Incivek®) boceprevir (Victrelis™) simeprevir (Olysio™), or paritaprevir.
16. WO'918 further discloses that compounds of the present invention are often administered orally, for example in tablet form (Page 20).
17. In addition to above, WO'918 further discloses that for solid oral preparations such as tablet suitable carriers and additives including starches, sugar carriers, such as dextrose,

manifold, lactose, and related carriers, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like may be used (Page 108).

18. Opponent submits that each and every feature of the claimed subject matter is disclosed and encompassed in cited prior art. Therefore, the subject matter claimed in impugned patent application is disclosed by WO'918. Thus, claimed subject matter is anticipated and lacks novelty in view of WO'918.
19. Thus, a case of lack of novelty is made out and the impugned patent application ought to be refused on this ground alone.

GROUND 3: SECTION 25(1)(E) LACK OF INVENTIVE STEP

20. It is submitted that the invention as claimed in claim 1-19 of impugned patent application is obvious and does not involve any inventive step in view of whatever was known and published in India or elsewhere prior to the priority date of impugned patent application i.e. prior to 01/02/2017 the earliest claimed priority.
21. Without prejudice, the impugned patent application lacks inventive step in view of disclosure of WO'918.
22. WO'918 discloses that compound is highly active against the HCV virus when administered in an effective amount to a host in need thereof. The host can be a human or any animal that carries the viral infection.
23. WO'918 further states that the activity of the parent nucleoside β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine in a replicon assay (EC_{50} = 15.7 micromolar) indicates that it is not suitable for use as a human drug due to insufficient activity. However, the stabilized racemic phosphate prodrug (phosphoramidate) exhibits an EC_{50} = 26 nanomolar (nM), in a replicon assay, which is at least 600 fold increase in activity. The corresponding (S)-phosphoramidate exhibits an EC_{50} = 4 nM, which is at least a 3,900 fold increase in activity.

24. It is further submitted that the compound and its pharmaceutically active salt i.e sulphate salt is disclosed in WO'918. Moreover, the significance of this compound is disclosed in the document and it would be sufficient to motivate a person skilled in the art to use the impugned compound and make its preferential pharmaceutically acceptable salt to get better property. It is common in practice to make a salt of known compound to enhance the solubility of that compound.
25. Furthermore, WO'918 also discloses that compounds of the present invention are typically administered through oral route in the tablet form in the range of 700mg or more to a human patient.
26. G. Steffen Paulekuhn et al discloses the concepts and importance of salt selection of active pharmaceutical ingredient. Salt formation is a well-known technique to modify and optimize the physical chemical properties of an ionisable research or development compound. Properties such as solubility, dissolution rate, hygroscopicity, stability, impurity profiles, and crystal habit can be influenced by using a variety of pharmaceutically acceptable counter ions. The crystal structure of a salt is usually completely different from the crystal structure of the conjugate base or acid and also differs from one salt to another. The modification of physical chemical properties, mainly solubility and dissolution rate, may also lead to changes in biological effects such as pharmacodynamics and pharmacokinetics, including bioavailability and toxicity profile.
27. G. Steffen Paulekuhn et al further discloses that there are only two anions with an average incidence of more than 5% over the whole period. These are the chlorides and sulfates. The anion encountered with highest frequency after chloride is sulfate. The table 2 in the said document further discloses the distribution of anions used in active pharmaceutical ingredients of category I, where chloride possesses 53.4% and sulphate 7.5%. For oral delivery two most common anions are chloride and sulphate. This document motivate to a person skilled in the art to select chloride or sulphate as a preferential anion to make salt.

28. Furthermore, the selection of salt for active pharmaceutical ingredient is routine practice for a person skilled in the art which depends on the property any nature of active pharmaceutical ingredient.
29. Moreover, Herve Rebiere et al. discloses various antiviral drugs -
- ❖ **Aatazanavir sulfate,**
 - ❖ Didanosine,
 - ❖ Efavirenz
 - ❖ Stavudine
 - ❖ Zidovudine,
 - ❖ Lamivudine,
 - ❖ **Abacavir sulfate,**
 - ❖ Amprenavir and
 - ❖ Fosamprenavir;
 - ❖ Amdoxovir,
 - ❖ Emtricitabine
 - ❖ Tenofovir disoproxil fumarate;
 - ❖ Enfuvirtide,
 - ❖ Nelfinavir mesylate,
 - ❖ Saquinavir mesylate
 - ❖ Zalcitabine from
 - ❖ **Indinavir sulfate,**
 - ❖ Lopinavir
 - ❖ Ritonavir;
 - ❖ nevirapine anhydre,
 - ❖ nevirapine
 - ❖ Tipranavir
30. Opponent submits that among the list of various antiviral drugs, the sulfate salt is one of the common salt for antiviral drugs. Therefore, it would motivate to a person skilled in the art to opt sulphate salt for antiviral drugs.

31. US'773 disclose the hemi-salt of tenofovir which is an antiviral drug. This document teaches the importance of hemi-salt over mono-salt of tenofovir alafenamide. The hemifumarate form of tenofovir alafenamide can be more readily and easily separated from impurities than the monofumarate form. Other major advantages of tenofovir alafenamide hemifumarate over the monofumarate form include improved thermodynamic and chemical stability (including long-term storage stability), Superior process reproducibility, Superior drug product content uniformity, and a higher melting point. The hemifumarate form of tenofovir alafenamide was compared with the monofumarate form. Under identical conditions, the hemifumarate form of tenofovir alafenamide was chemically more stable and exhibited better long-term storage stability, with significantly less degradation (%Total Deg. Products) than the monofumarate form.
32. The hemifumarate form of tenofovir alafenamide has a melting point that is about 10°C higher than that of the monofumarate form, indicating that the hemifumarate form has improved thermal stability as compared with the monofumarate form.
33. The preceding paragraphs are evident that the stability of the hemi salt form is more than the mono salt form.
34. US'773 clearly motivates to person skilled in the art to make hemi-salt of a well known molecule i.e. the compound as disclosed in WO'918.
35. It is submitted that the dosage form of the compound 2 is same as disclosed in WO'918. Moreover, the effective blood level concentration of active compound is also same. The relevant paragraphs are reproduced here for reference.

"In general, a therapeutically effective amount of the present compound in a pharmaceutical dosage form may range from about 0.001 mg/kg to about 100 mg/kg per day or more, more often, slightly less than about 0.1 mg/kg to more than about 25 mg/kg per day of the patient or considerably more, depending upon the compound used, the condition or infection treated and the route of administration. Compound 2 is often administered in amounts ranging from about 0.1 mg/kg to about 15 mg/kg per day of

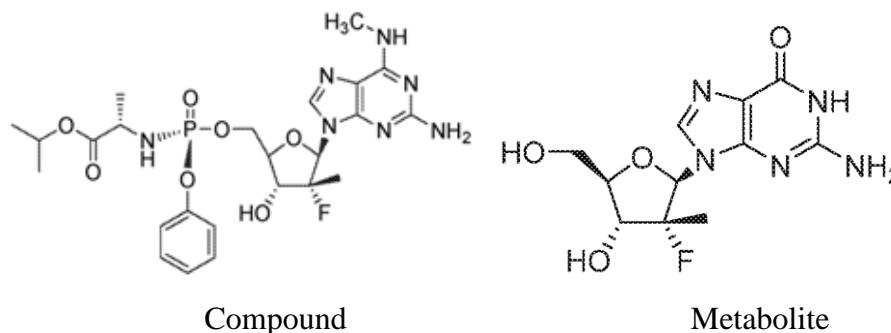
the patient, depending upon the pharmacokinetic of the agent in the patient. This dosage range generally produces effective blood level concentrations of active compound which may range from about 0.001 to about 100, about 0.05 to about 100 micrograms/cc of blood in the patient.” **From Impugned patent application**

“In general, a therapeutically effective amount of the present compound in pharmaceutical dosage form usually ranges from about 0.001 mg/kg to about 100 mg/kg per day or more, more often, slightly less than about 0.1 mg/kg to more than about 25 mg/kg per day of the patient or considerably more, depending upon the compound used, the condition or infection treated and the route of administration. The active nucleoside compound according to the present invention is often administered in amounts ranging from about 0.1 mg/kg to about 15 mg/kg per day of the patient, depending upon the pharmacokinetics of the agent in the patient. This dosage range generally produces effective blood level concentrations of active compound which may range from about 0.001 to about 100, about 0.05 to about 100 micrograms/cc of blood in the patient.” **From Prior art WO’918**

The above mentioned paragraphs disclose that dosage form ranges are same in both conditions.

36. The above mentioned paragraph discloses that there is no technical advancement in impugned patent application.
37. The combined teaching of the cited prior arts are given below-

❖ WO’918 discloses the compound and its active metabolite.



- ❖ G. Steffen Paulekuhn et al discloses two preferred anions for salt preparation i.e. chloride and sulphates. Selection of salt is common in practice for a person skilled in the art.
 - ❖ Herve Rebiere et al discloses various antiviral drugs among them sulphate is the preferred salt for antiviral drugs.
 - ❖ US'773 disclose the importance and significance of hemi-salt over mono salt.
38. It is clear from the above paragraphs that each and every feature of the claimed subject matter was already known and taught by cited prior arts. Therefore, the claimed subject matter is obvious and devoid of any inventive merit.
39. Thus, the subject matter claimed in impugned patent application is obvious and lacks inventive step.
40. In view of the above submissions, impugned application lacks inventive step and therefore, should be rejected on this ground alone.

GROUND 3: CLAIMS NOT PATENTABLE UNDER SECTION 25(1)(F)

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

41. It is submitted that the impugned patent application should not be allowed under Section 3(d) 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.”

42. As submitted under the grounds of lack of novelty by prior publication and lack of inventive step the subject matter claimed in impugned application is neither novel nor inventive and hence, squarely falls under the ambit of Section 3(d) of the Act.
43. It is further submitted that Applicant has provided AUC data for hemisulfate salt of the compound with respect to free base but failed to provide any data in the specification to demonstrate enhanced therapeutic efficacy.
44. Therefore, the claims of impugned patent application squarely fall under the ambit of section 3(d). The impugned patent application should be rejected on this ground alone.

Claims of impugned application is not patentable as per Section 3(e) of the Act

45. Section 3(e) which clearly states that a substance obtained by a mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance is not patentable.
46. The Opponent state that the subject matter of impugned application is drawn to composition which is an admixture of various components. It is further submitted that the Applicant has failed to demonstrate any unexpected effect of the claimed composition in the impugned specification as compared to other formulations.
47. In absence of any unexpected effect of the claimed composition established in the impugned specification, the claimed subject matter of the impugned application is merely an admixture which falls under the prohibition of Section 3(e) read with Section 25(1)(f) of the Act. Thus, impugned application is liable to be rejected on this ground alone.

GROUND 5: INSUFFICIENCY OF DISCLOSURE

48. The complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed.
49. The Opponent states that it is a well settled rule that the specification should clearly and fairly describe the invention and disclose the best mode of working the invention so that the person skilled in the art could perform the invention without any undue efforts and it is hereby stated that the Applicant has failed to do so.
50. It is submitted that claims are not fairly based on the specification and complete specification does not describe the invention and the method of performing the invention.
51. It is submitted that Applicant has not provided C¹³ NMR, and IR data for the synthesized compounds.
52. It is submitted that Applicant has merely mentioned the effectiveness of the claimed subject matter in combination therapies, but failed to provide any relevant or supporting data.
53. Therefore, the impugned specification does not enable a person of average skill in the art to make or use the invention as claimed without undue experimentation. Consequently, the specification as filed does not provide enabling disclosure of the claims at hand.
54. The impugned patent application does not provide adequate teaching to a person of skill in the art to practice the invention. Considering above, impugned application does not sufficiently and clearly describe the invention. Therefore, the impugned application should be refused on this ground alone.

GROUND 5 - SECTION 25(1)(H)

55. 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.
56. 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.
57. 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.
58. It is submitted that the Applicant has failed to disclose the details of corresponding foreign applications and impugned patent application to be refused.
59. The opponents crave leave to file further submissions and evidence with respect to this ground.

CONCLUSION

60. In view of the above, the claims are not novel, inventive and not patentable and insufficient. The pre-grant opposition as filed may be allowed and the subject patent application may be refused.

HEARING REQUESTED

61. The Opponent hereby requests a hearing under section 25(1) 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 application 201917029812, be rejected under Section 25(1) 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 28th day of September, 2022



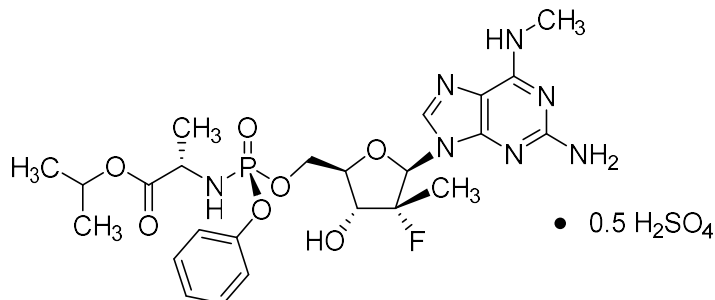
RAJESHWARI H. IN/PA – 0358
OF RAJESHWARI AND ASSOCIATES
AGENT FOR THE OPPONENT

TO
THE CONTROLLER OF PATENTS
PATENT OFFICE, NEW DELHI

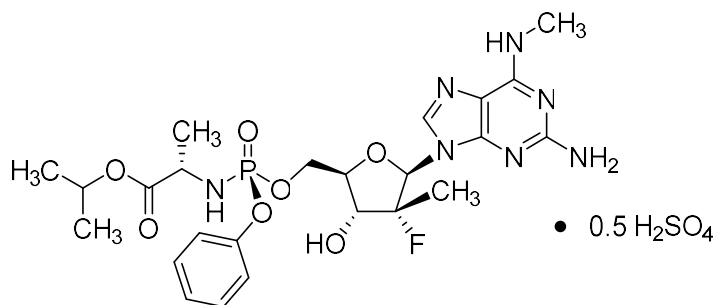
Annexure - 1

I/We Claim:

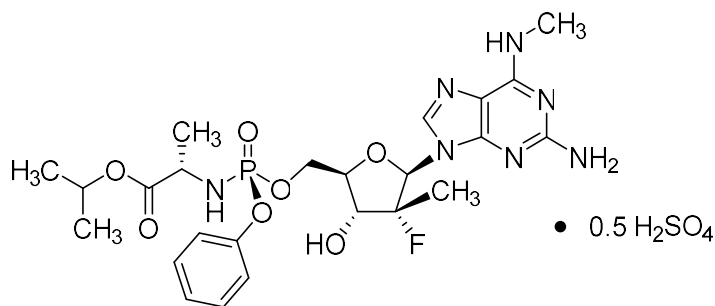
1. A compound of the formula:



2. A solid dosage form comprising a compound of the formula



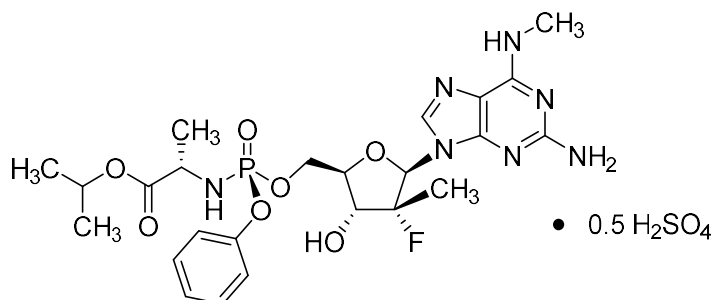
3. A pharmaceutical composition comprising from 10 to 1000 mg of a compound of the formula



in a pharmaceutically acceptable carrier.

4. The pharmaceutical composition of claim 3, in a solid dosage form that delivers from 100 mg to 800 mg of the compound.

5. The pharmaceutical composition of claim 3, in a solid dosage form that delivers at least 600 mg of the compound.
6. The pharmaceutical composition of claim 3, in a solid dosage form that delivers at least 700 mg of the compound.
7. The pharmaceutical composition of claim 3, in a solid dosage form that delivers at least 800 mg of the compound.
8. The pharmaceutical composition of any one of claim 3-7, wherein the pharmaceutically acceptable carrier is suitable for oral delivery.
9. The pharmaceutical composition of claim 8, wherein the pharmaceutically acceptable carrier is in the form of a tablet.
10. The compound of formula



optionally in a pharmaceutically acceptable carrier, for use in the treatment of a hepatitis C infection or a condition resulting from a hepatitis C infection, in a host in need thereof.

11. The compound of claim 10, wherein the compound is administered orally.
12. The compound of claim 10, wherein the compound is administered via controlled release.
13. The compound of claim 10, wherein the condition resulting from a hepatitis C infection has resulted in antibody positive and antigen positive conditions, a viral-based chronic liver inflammation, a liver cancer resulting from advanced hepatitis C, cirrhosis, or fatigue.
14. The compound of any one of claims 10-13, wherein at least 300 mg of the compound is administered.

15. The compound of any one of claims 10-13, wherein at least 400 mg of the compound is administered.
16. The compound of any one of claims 10-13, wherein at least 500 mg of the compound is administered.
17. The compound of any one of claims 10-13, wherein at least 600 mg of the compound is administered.
18. The compound of any one of claims 10-17, further comprising administering the compound in combination with another anti-HCV agent.
19. The compound of claim 18, wherein the additional anti-HCV agent is selected from the group consisting of a protease inhibitor, an NS5A inhibitor, another NS5B polymerase inhibitor, a non-substrate inhibitor, interferon alfa-2a, ribavirin, a helicase inhibitor, an antisense oligodeoxynucleotide, an aptamer, a nuclease-resistant ribozyme, iRNA, an antibody to HCV, a partial antibody to HCV, and a domain antibody to HCV.

Dated **23 July 2019**

MALATHI LAKSHMIKUMARAN
IN/PA-1433
AGENT FOR THE APPLICANT

To,
The Controller of Patents
 The Patent Office at **New Delhi**

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

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(54) **Title:** β -D-2'-DEOXY-2' α -FLUORO-2'- β -C-SUBSTITUTED-2-MODIFIED-N⁶-SUBSTITUTED PURINE NUCLEOTIDES FOR HCV TREATMENT

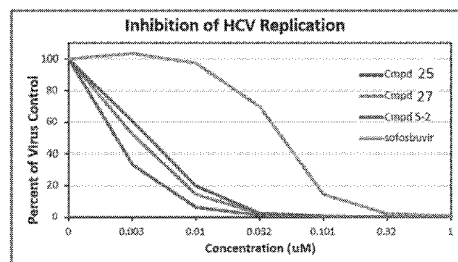
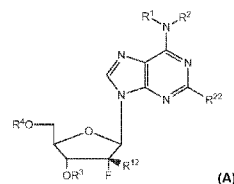


FIG. 4



(57) **Abstract:** A compound of the structure (A), or a pharmaceutically acceptable salt or composition thereof for the treatment of a host infected with or exposed to an HCV virus or other disorders more fully described herein.

**β -D-2'-DEOXY-2'- α -FLUORO-2'- β -C-SUBSTITUTED-2-MODIFIED-N⁶-SUBSTITUTED
PURINE NUCLEOTIDES FOR HCV TREATMENT**

5 **PRIORITY**

This application claims priority to U.S.S.N. 62/129,319 filed on March 6, 2015, U.S.S.N. 62/253,958 filed on November 11, 2015, and U.S.S.N 62/276,597 filed on January 8, 2016, each of which is incorporated herewith in their entirety.

10 **FIELD OF THE INVENTION**

The present invention is directed to nucleotide compounds and compositions and uses thereof to treat the Hepatitis C virus ("HCV").

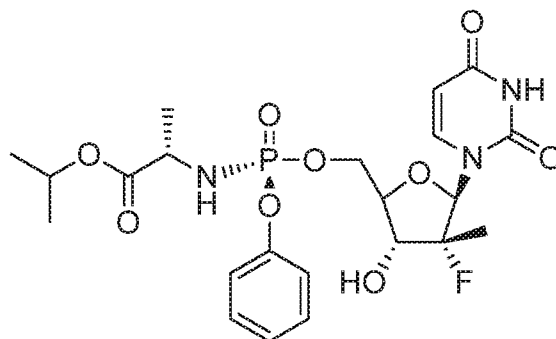
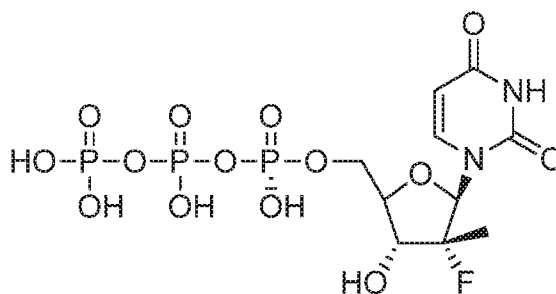
BACKGROUND OF THE INVENTION

15 Hepatitis C (HCV) is an RNA single stranded virus and member of the Hepacivirus genus. It is estimated that 75% of all cases of liver disease are caused by HCV. HCV infection can lead to cirrhosis and liver cancer, and if left to progress, liver failure which may require a liver transplant. Approximately 170-200 million people worldwide are infected, with an estimated 3-4 million infections in the United States.

20 RNA polymerase is a key component in the targeting of RNA single stranded viruses. The HCV non-structural protein NS5B RNA-dependent RNA polymerase is a key enzyme responsible for initiating and catalyzing viral RNA synthesis. As a result, HCV NS5B is an attractive target for the current drug discovery and development of anti-HCV agents. There are two major subclasses of NS5B inhibitors: nucleoside analogs, which are anabolized to their
25 active triphosphates – which act as alternative substrates for the polymerase – and non-nucleoside inhibitors (NNIs), which bind to allosteric regions on the protein. Nucleoside or nucleotide inhibitors mimic natural polymerase substrate and act as chain terminators. They inhibit the initiation of RNA transcription and elongation of a nascent RNA chain.

In addition to targeting RNA polymerase, other RNA viral proteins may also be targeted
30 in combination therapies. For example, HCV proteins that are additional targets for therapeutic approaches are NS3/4A (a serine protease) and NS5A (a non-structural protein that is an essential component of HCV replicase and exerts a range of effects on cellular pathways).

In December 2013, the first nucleoside NS5B polymerase inhibitor sofosbuvir (Sovaldi[®], Gilead Sciences) was approved. Sovaldi[®] is a uridine phosphoramidate prodrug that is taken up by hepatocytes and undergoes intracellular activation to afford the active metabolite; 2'-deoxy-2'- α -fluoro- β -C-methyluridine-5'-triphosphate; see structures below:

Sovaldi[®]2'-Deoxy-2'- α -fluoro- β -C-methyluridine-5'-triphosphate

Sovaldi[®] is the first drug that has demonstrated safety and efficacy to treat certain types of HCV infection without the need for co-administration of interferon. Sovaldi[®] is the third drug with breakthrough therapy designation to receive FDA approval.

In 2014, the U.S. FDA approved Harvoni[®] (ledipasvir, a NS5A inhibitor, and sofosbuvir) to treat chronic hepatitis C virus genotype 1 infection. Harvoni[®] is the first combination pill approved to treat chronic HCV genotype 1 infection. It is also the first approved regimen that does not require administration with interferon or ribavirin. In addition, the FDA approved simeprevir (Olysio[™]) in combination with sofosbuvir (Sovaldi[®]) as a once-daily, all oral, interferon and ribavirin-free treatment for adults with genotype 1 HCV infection.

The U.S. FDA also approved AbbVie's VIEKIRA Pak[™] in 2014, a multipill pack containing dasabuvir (a non-nucleoside NS5B polymerase inhibitor), ombitasvir (a NS5A

inhibitor), paritaprevir (a NS3/4A inhibitor), and ritonavir. The VIEKIRA PakTM can be used with or without the ribavirin to treat genotype 1 HCV infected patients including patients with compensated cirrhosis. VIEKIRA PakTM does not require interferon co-therapy.

In July 2015, the U.S. FDA approved TechnivieTM and DaklinzaTM for the treatment of
 5 HCV genotype 4 and HCV genotype 3 respectively. TechnivieTM (Ombitasvir/paritaprevir/ritonavir) was approved for use in combination with ribavirin for the treatment of HCV genotype 4 in patients without scarring and cirrhosis and is the first option for HCV-4 infected patients who do not require co-administration with interferon. DaklinzaTM was approved for use with Sovaldi[®] to treat HCV genotype 3 infections. DaklinzaTM is the first drug
 10 that has demonstrated safety and efficacy in treating HCV genotype 3 without the need for co-administration of interferon or ribavirin.

In October 2015, the U.S. FDA warned that HCV treatments Viekira Pak and Technivie can cause serious liver injury primarily in patients with underlying advanced liver disease, and required that additional information about safety be added to the label.

15 Other current approved therapies for HCV include interferon alpha-2b or pegylated interferon alpha-2b (Pegintron[®]), which can be administered with ribavirin (Rebetol[®]), NS3/4A telaprevir (Incivek[®], Vertex and Johnson & Johnson), boceprevir (VictrelisTM, Merck), simeprevir (OlysioTM, Johnson & Johnson), paritaprevir (AbbVie), Ombitasvir (AbbVie), (NNI) Dasabuvir (ABT-333) and Merck's ZepatierTM (a single-tablet combination of the two drugs
 20 grazoprevir and elbasvir).

Additional NS5B polymerase inhibitors are currently under development. Merck is developing the uridine nucleotide prodrug MK-3682 (formerly Idenix IDX21437). The drug is currently in Phase II combination trials.

United States patents and WO applications which describe nucleoside polymerase
 25 inhibitors for the treatment of Flaviviridae, including HCV, include those filed by Idenix Pharmaceuticals (6,812,219; 6,914,054; 7,105,493; 7,138,376; 7,148,206; 7,157,441; 7,163,929; 7,169,766; 7,192,936; 7,365,057; 7,384,924; 7,456,155; 7,547,704; 7,582,618; 7,608,597; 7,608,600; 7,625,875; 7,635,689; 7,662,798; 7,824,851; 7,902,202; 7,932,240; 7,951,789; 8,193,372; 8,299,038; 8,343,937; 8,362,068; 8,507,460; 8,637,475; 8,674,085; 8,680,071;
 30 8,691,788; 8,742,101; 8,951,985; 9,109,001; 9,243,025; US2016/0002281; US2013/0064794; WO/2015/095305; WO/2015/081133; WO/2015/061683; WO/2013/177219; WO/2013/039920;

WO/2014/137930; WO/2014/052638; WO/2012/154321); Merck (6,777,395; 7,105,499; 7,125,855; 7,202,224; 7,323,449; 7,339,054; 7,534,767; 7,632,821; 7,879,815; 8,071,568; 8,148,349; 8,470,834; 8,481,712; 8,541,434; 8,697,694; 8,715,638, 9,061,041; 9,156,872 and WO/2013/009737); Emory University (6,348,587; 6,911,424; 7,307,065; 7,495,006; 7,662,938; 5 7,772,208; 8,114,994; 8,168,583; 8,609,627; US 2014/0212382; and WO2014/1244430); Gilead Sciences/ Pharmasset Inc. (7,842,672; 7,973,013; 8,008,264; 8,012,941; 8,012,942; 8,318,682; 8,324,179; 8,415,308; 8,455,451; 8,563,530; 8,841,275; 8,853,171; 8,871,785; 8,877,733; 8,889,159; 8,906,880; 8,912,321; 8,957,045; 8,957,046; 9,045,520; 9,085,573; 9,090,642; and 9,139,604) and (6,908,924; 6,949,522; 7,094,770; 7,211,570; 7,429,572; 7,601,820; 7,638,502; 10 7,718,790; 7,772,208; RE42,015; 7,919,247; 7,964,580; 8,093,380; 8,114,997; 8,173,621; 8,334,270; 8,415,322; 8,481,713; 8,492,539; 8,551,973; 8,580,765; 8,618,076; 8,629,263; 8,633,309; 8,642,756; 8,716,262; 8,716,263; 8,735,345; 8,735,372; 8,735,569; 8,759,510 and 8,765,710); Hoffman La-Roche (6,660,721), Roche (6,784,166; 7,608,599, 7,608,601 and 8,071,567); Alios BioPharma Inc. (8,895,723; 8,877,731; 8,871,737, 8,846,896, 8,772,474; 15 8,980,865; 9,012,427; US 2015/0105341; US 2015/0011497; US 2010/0249068; US2012/0070411; WO 2015/054465; WO 2014/209979; WO 2014/100505; WO 2014/100498; WO 2013/142159; WO 2013/142157; WO 2013/096680; WO 2013/088155; WO 2010/108135), Enanta Pharmaceuticals (US 8,575,119; 8,846,638; 9,085,599; WO 2013/044030; WO 2012/125900), Biota (7,268,119; 7,285,658; 7,713,941; 8,119,607; 8,415,309; 8,501,699 and 20 8,802,840), Biocryst Pharmaceuticals (7,388,002; 7,429,571; 7,514,410; 7,560,434; 7,994,139; 8,133,870; 8,163,703; 8,242,085 and 8,440,813), Alla Chem, LLC (8,889,701 and WO 2015/053662), Inhibitex (8,759,318 and WO/2012/092484), Janssen Products (8,399,429; 8,431,588, 8,481,510, 8,552,021, 8,933,052; 9,006,29 and 9,012,428) the University of Georgia Foundation (6,348,587; 7,307,065; 7,662,938; 8,168,583; 8,673,926, 8,816,074; 8,921,384 and 25 8,946,244), RFS Pharma, LLC (8,895,531; 8,859,595; 8,815,829; 8,609,627; 7,560,550; US 2014/0066395; US 2014/0235566; US 2010/0279969; WO/2010/091386 and WO 2012/158811) University College Cardiff Consultants Limited (WO/2014/076490, WO 2010/081082; WO/2008/062206), Achillion Pharmaceuticals, Inc. (WO/2014/169278 and WO 2014/169280), Cocrystal Pharma, Inc. (US 9,173,893), Katholieke Universiteit Leuven (WO 2015/158913), 30 Catabasis (WO 2013/090420) and the Regents of the University of Minnesota (WO 2006/004637).

Nonetheless, there remains a strong medical need to develop anti-HCV therapies that are safe, effective and well-tolerated. The need is accentuated by the expectation that drug resistance. More potent direct-acting antivirals could significantly shorten treatment duration and improve compliance and SVR rates for patients infected with all HCV genotypes.

5 It is therefore an object of the present invention to provide compounds, pharmaceutical compositions, and methods and uses to treat and/or prevent infections of HCV.

SUMMARY OF THE INVENTION

10 It has been discovered that the compounds of Formula I, Formula II, Formula III, Formula IV, Formula V, Formula VI, Formula VII and including β -D-2'-deoxy-2'- α -fluoro-2'- β -C-substituted-N⁶-(mono- or di-methyl) purine nucleotides, are highly active against the HCV virus when administered in an effective amount to a host in need thereof. The host can be a human or any animal that carries the viral infection.

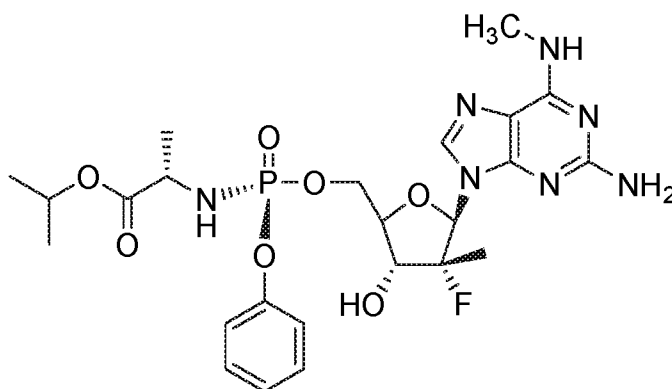
15 Disclosed nucleotides include those with nanomolar activity against HCV in vitro and therapeutic indices that range to 25,000 or more.

20 Surprisingly, the parent N⁶-(methyl) purine nucleosides of disclosed compounds had not been developed or specifically disclosed as drug candidates prior to this invention. For example, it was reported in 2010 that 3'-azido-N⁶-dimethyl-2,6-diaminopurine is not substantially deaminated by adenosine deaminase over a long period (120 minutes), and for that reason it had been considered an inappropriate compound to derivatize as a drug (see for example, WO 2010/091386, page 86 and corresponding US Patent 8,609,627).

25 However, it has now been discovered that compounds of the present invention are anabolized to a 5-monophosphate of the N⁶-substituted-purine without substantial N⁶-deamination and then subsequently anabolized at the 6-position to generate active guanine triphosphate compounds, in a manner that provides exceptional activity and therapeutic index.

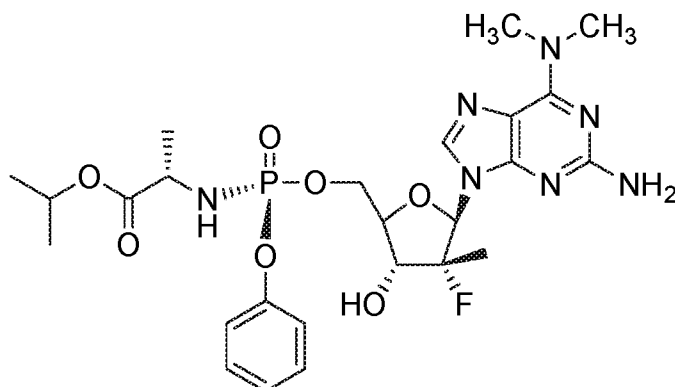
30 In particular, it has been discovered that a 5'-stabilized phosphate prodrug or derivative of β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine nucleotide, as well as β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-dimethyl-2,6-diaminopurine nucleotide, and other β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleotides as described below, are highly active against HCV. This is surprising because the activity of the parent nucleoside β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine in a replicon

assay (EC_{50} = 15.7 micromolar) indicates that it is not suitable for use as a human drug due to insufficient activity (in combination with the reference WO 2010/091386, page 86 and corresponding US Patent 8,609,627 that suggests that N^6 -methyl-2,6-diaminopurines are not deaminated in vivo) however, the stabilized racemic phosphate prodrug (phosphoramidate) exhibits an EC_{50} = 26 nanomolar (nM), in a replicon assay, which is at least an 600 fold increase in activity. The corresponding (S)-phosphoramidate exhibits an EC_{50} = 4 nM, which is at least a 3,900 fold increase in activity; see the structure below and compound 5-2 in Table 7. With a TC_{50} greater than one hundred micromolar, the compound thus has a therapeutic index of greater than 25,000. For comparison, Sofosbuvir has an EC_{50} = 53 nM, a TC_{50} greater than one hundred micromolar and a therapeutic index greater than 1,920.

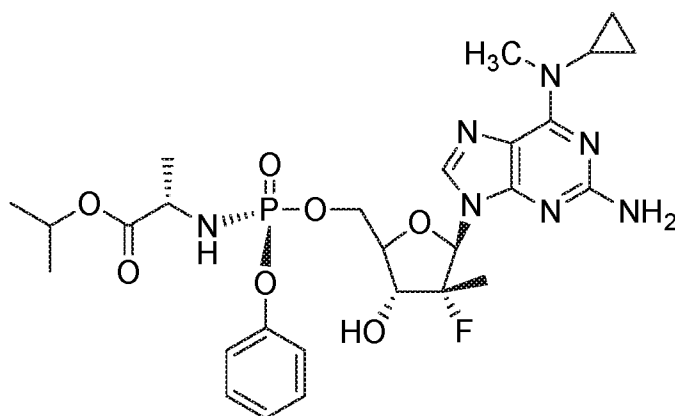


Compound 5-2 (Table 7)

Likewise, the activity of the parent nucleoside β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl- N^6 -dimethyl-2,6-diaminopurine in a replicon assay (EC_{50} = 10.7 micromolar, " μ M") indicates that it is also not suitable for use as a human drug due to insufficient activity, however, the stabilized racemic phosphate prodrug (phosphoramidate) exhibits an EC_{50} = 12 nM, in a replicon assay, which is more than a 890 fold increase in activity. The corresponding (S)-phosphoramidate (compound 25, Table 7) also exhibits an EC_{50} = 4 nM, which is at least a 2,600 fold increase in activity; see the structure below. In addition, compound 25 also has a therapeutic index of greater than 25,000.

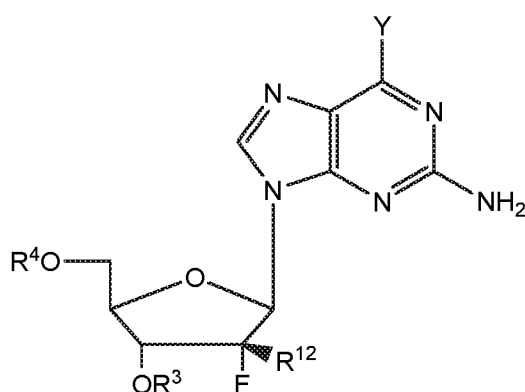


In another example, the compound isopropyl (((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methyl-*N*-cyclopropyl-amino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate exhibited an $EC_{50} = 7$ nM and the corresponding
 5 (S)-phosphoramidate exhibited an $EC_{50} = 5$ nM in a replicon assay; see compound 27 in Table 7 and the structure below.



As stated above, the metabolism of the β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl- N^6 -methyl-2,6-diaminopurine nucleoside as a phosphoramidate involves the production of a 5'-
 10 monophosphate and the subsequent anabolism of the N^6 -methyl-2,6-diaminopurine base to generate the β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-guanine nucleoside as the 5'-monophosphate. The monophosphate is then further anabolized to the active species; the 5'-triphosphate. The β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-guanine triphosphate has an $IC_{50} = 0.15$ μ M against the HCV genotype 1b NS5B polymerase.

Thus, in one embodiment, the invention is:



Formula I

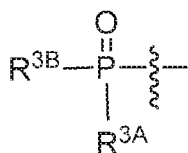
wherein:

Y is NR^1R^2 ;

5 R^1 is $\text{C}_1\text{-C}_5$ alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), $\text{C}_1\text{-C}_5$ haloalkyl (including CH_2F , CHF_2 , CF_3 , CH_2CF_3 , CF_2CH_3 and CF_2CF_3), $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{C}_3\text{-C}_6\text{cycloalkyl})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{heterocycle})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{aryl})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{heteroaryl})$, $-\text{OR}^{25}$, $-\text{C}(\text{O})\text{R}^{3\text{C}}$ (including $-\text{C}(\text{O})\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{OCH}_3$, $-\text{C}(\text{O})\text{OC}_2\text{H}_5$, $-\text{C}(\text{O})\text{OC}_3\text{H}_7$, $-\text{C}(\text{O})\text{OC}_4\text{H}_9$, and $-\text{C}(\text{O})\text{OC}_5\text{H}_{11}$), $-\text{C}(\text{S})\text{R}^{3\text{D}}$, or $-\text{SO}_2\text{R}^{28}$ each of which can be optionally substituted;

R^2 is hydrogen, $\text{C}_1\text{-C}_5$ alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), $\text{C}_1\text{-C}_5$ haloalkyl (including CHF_2 , CHF_2 , CF_3 , CH_2CF_3 and CF_2CF_3), $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{C}_3\text{-C}_6\text{cycloalkyl})$, $-\text{C}(\text{O})\text{R}^{3\text{C}}$ (including $-\text{C}(\text{O})\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{OCH}_3$, $-\text{C}(\text{O})\text{OC}_2\text{H}_5$, $-\text{C}(\text{O})\text{OC}_3\text{H}_7$, $-\text{C}(\text{O})\text{OC}_4\text{H}_9$, and $-\text{C}(\text{O})\text{OC}_5\text{H}_{11}$), $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{aryl})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{heterocycle})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{heteroaryl})$; and

wherein at least one of R^1 and R^2 is methyl, CH_2F , CHF_2 or CF_3 ;



R^3 is hydrogen, $\text{R}^{3\text{A}}$, diphosphate, triphosphate, an optionally substituted carbonyl linked amino acid, or $-\text{C}(\text{O})\text{R}^{3\text{C}}$;

20 $\text{R}^{3\text{A}}$ can be selected from O^- , OH , an $-\text{O}$ -optionally substituted aryl, an $-\text{O}$ -optionally substituted heteroaryl, or an optionally substituted heterocyclyl;

R^{3B} can be selected from O^- , OH , an optionally substituted N-linked amino acid or an optionally substituted N-linked amino acid ester;

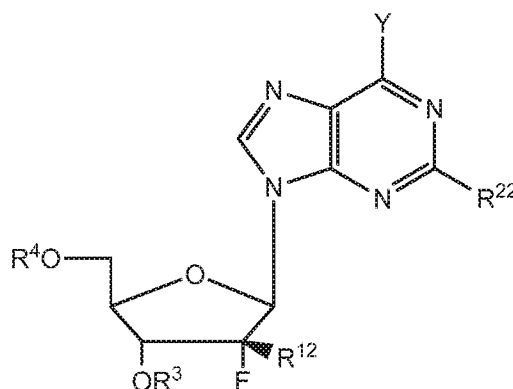
R^{3C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(cycloalkyl)$, $-(C_0-C_2)(heterocyclo)$, $-(C_0-C_2)(aryl)$, $-(C_0-C_2)(heteroaryl)$, $-O-alkyl$, $-O-alkenyl$, $-O-alkynyl$, $-O-(C_0-C_2)(cycloalkyl)$, $-O-(C_0-C_2)(heterocyclo)$, $-O-(C_0-C_2)(aryl)$, or $-O-(C_0-C_2)(heteroaryl)$, each of which can be optionally substituted;

R^4 is a monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, including but not limited to a phosphoramidate, a thiophosphoramidate, or any other moiety that is metabolized to a monophosphate, diphosphate or triphosphate *in vivo* in the host human or animal; or

R^3 and R^4 together with the oxygens that they are bonded to can form a 3',5'-cyclic prodrug, including but not limited to, a 3',5'-cyclic phosphate prodrug;

R^{12} is CH_3 , CH_2F , CHF_2 , CF_3 , or ethynyl.

In one embodiment, the invention is:



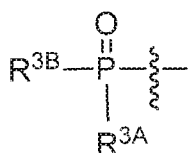
Formula II

wherein:

Y is NR^1R^2 ;

R^1 is C_1-C_5 alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C_1-C_5 haloalkyl (including CH_2F , CHF_2 , CF_3 , CH_2CF_3 , CF_2CH_3 and CF_2CF_3), C_2-C_6 alkenyl, C_2-C_6 alkynyl, $-(C_0-C_2alkyl)(C_3-C_6cycloalkyl)$, $-(C_0-C_2alkyl)(heterocycle)$, $-(C_0-C_2alkyl)(aryl)$, $-(C_0-C_2alkyl)(heteroaryl)$, $-OR^{25}$, $-C(O)R^{3C}$ (including $-C(O)CH_3$, $-C(O)CH_2CH_3$, $-C(O)CH(CH_3)_2$, $-C(O)OCH_3$, $-C(O)OC_2H_5$, $-C(O)OC_3H_7$, $-C(O)OC_4H_9$, and $-C(O)OC_5H_{11}$), $-C(S)R^{3D}$, or $-SO_2R^{28}$ each of which can be optionally substituted;

R^2 is hydrogen, optionally substituted C_1 - C_5 alkyl (including methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C_1 - C_5 haloalkyl (including CHF_2 , CHF_2 , CF_3 , CH_2CF_3 and CF_2CF_3), optionally substituted $-(C_0-C_2alkyl)(C_3-C_6cycloalkyl)$, optionally substituted $-(C_0-C_2alkyl)(heterocycle)$, optionally substituted $-(C_0-C_2alkyl)(aryl)$,
 5 optionally substituted $-(C_0-C_2alkyl)(heteroaryl)$, $-C(O)R^{3C}$ (including $-C(O)CH_3$, $-C(O)CH_2CH_3$, $-C(O)CH(CH_3)_2$, $-C(O)OCH_3$, $-C(O)OC_2H_5$, $-C(O)OC_3H_7$, $-C(O)OC_4H_9$, and $-C(O)OC_5H_{11}$), $-C(S)R^{3D}$, or $-SO_2R^{28}$; and
 wherein at least one of R^1 and R^2 is methyl, CH_2F , CHF_2 or CF_3 ;



R^3 is hydrogen, R^{3A} , diphosphate, triphosphate, an optionally substituted
 10 carbonyl linked amino acid, or $-C(O)R^{3C}$;

R^{3A} can be selected from O^- , OH, an $-O$ -optionally substituted aryl, an $-O$ -optionally substituted heteroaryl, or an optionally substituted heterocyclyl;

R^{3B} can be selected from O^- , OH, an optionally substituted N-linked amino acid or an
 15 optionally substituted N-linked amino acid ester;

R^{3C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(cycloalkyl)$, $-(C_0-C_2)(heterocyclo)$, $-(C_0-C_2)(aryl)$, $-(C_0-C_2)(heteroaryl)$, $-O$ -alkyl, $-O$ -alkenyl, $-O$ -alkynyl, $-O$ -(C_0-C_2)(cycloalkyl), $-O$ -(C_0-C_2)(heterocyclo), $-O$ -(C_0-C_2)(aryl), $-O$ -(C_0-C_2)(heteroaryl), $-S$ -alkyl, $-S$ -alkenyl, $-S$ -alkynyl, $-S$ -(C_0-C_2)(cycloalkyl), $-S$ -(C_0-C_2)(heterocyclo), $-S$ -(C_0-C_2)(aryl), or $-S$ -(C_0-C_2)(heteroaryl) each of
 20 which can be optionally substituted;

R^{3D} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(cycloalkyl)$, $-(C_0-C_2)(heterocyclo)$, $-(C_0-C_2)(aryl)$, $-(C_0-C_2)(heteroaryl)$, $-O$ -alkyl, $-O$ -alkenyl, $-O$ -alkynyl, $-O$ -(C_0-C_2)(cycloalkyl), $-O$ -(C_0-C_2)(heterocyclo), $-O$ -(C_0-C_2)(aryl), or $-O$ -(C_0-C_2)(heteroaryl), each of which can be optionally substituted;

25 R^4 is a monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, including but not limited to a phosphoramidate, a thiophosphoramidate, or any other moiety that is metabolized to a monophosphate, diphosphate or triphosphate *in vivo* in the host human or animal; or

R^3 and R^4 together with the oxygens that they are bonded to can form a 3',5'-cyclic prodrug, including but not limited to, a 3',5'-cyclic phosphate prodrug;

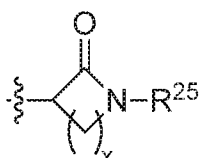
R^5 is C_1 - C_5 alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C_1 - C_5 haloalkyl (including CHF_2 , CHF_2 , CF_3 , CH_2CF_3 and CF_2CF_3),
 5 C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, $-(C_0-C_2alkyl)(C_3-C_6cycloalkyl)$, $-(C_0-C_2alkyl)(heterocycle)$, $-(C_0-C_2alkyl)(aryl)$, $-(C_0-C_2alkyl)(heteroaryl)$, $-OR^{25}$, $-C(O)R^{3C}$ (including $-C(O)CH_3$, $-C(O)CH_2CH_3$, $-C(O)CH(CH_3)_2$, $-C(O)OCH_3$, $-C(O)OC_2H_5$, $-C(O)OC_3H_7$, $-C(O)OC_4H_9$, and $-C(O)OC_5H_{11}$), $-C(S)R^{3D}$, or $-SO_2R^{28}$ each of which can be optionally substituted;

R^6 is hydrogen, optionally substituted C_1 - C_5 alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C_1 - C_5 haloalkyl (including CHF_2 ,
 10 CHF_2 , CF_3 , CH_2CF_3 and CF_2CF_3), optionally substituted $-(C_0-C_2alkyl)(C_3-C_6cycloalkyl)$, optionally substituted $-(C_0-C_2alkyl)(heterocycle)$, optionally substituted $-(C_0-C_2alkyl)(aryl)$, optionally substituted $-(C_0-C_2alkyl)(heteroaryl)$, $-C(O)R^{3C}$ (including $-C(O)CH_3$, $-C(O)CH_2CH_3$, $-C(O)CH(CH_3)_2$, $-C(O)OCH_3$, $-C(O)OC_2H_5$, $-C(O)OC_3H_7$, $-C(O)OC_4H_9$, and $-C(O)OC_5H_{11}$), $-C(S)R^{3D}$, or $-SO_2R^{28}$; or

R^5 and R^6 together with the nitrogen that they are bonded to can form a heterocyclic ring;

R^{12} is CH_3 , CH_2F , CHF_2 , CF_3 , or ethynyl;

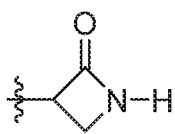
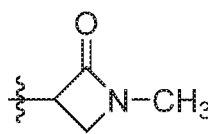
R^{22} is Cl, Br, F, CN, N_3 , C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, $-(C_1-C_2alkyl)(C_3-C_6cycloalkyl)$, $-(C_0-C_2alkyl)(C_3-C_6heterocycle)$, $-(C_0-C_2alkyl)(aryl)$, $-(C_0-C_2alkyl)(heteroaryl)$;
 20 $-ONHC(=O)OR^{23}$, $-NHOR^{24}$, $-OR^{25}$, $-SR^{25}$, $-NH(CH_2)_{1-4}N(R^{26})_2$, $-NHNHR^{26}$, $-N=NR^{27}$, $-NHC(O)NHNHR^{27}$, $-NHC(S)NHNHR^{27}$, $-C(O)NHNHR^{27}$, $-NR^{27}SO_2R^{28}$, $-SO_2NR^{27}R^{29}$,



$-C(O)NR^{27}R^{29}$, $-CO_2R^{29}$, $-SO_2R^{29}$, $-P(O)H(OR^{29})$, $-P(O)(OR^{29})(OR^{30})$,
 $-P(O)(OR^{29})(NR^{29}R^{30})$ or $-NR^{5}R^6$;

for example including but not limited to the following embodiments, chloro, bromo,
 25 fluoro, cyano, azido, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and n-pentyl, 1,1-dimethylpropyl, 2,2-dimethylpropyl, 3-methylbutyl, 1-methylbutyl, 1-ethylpropyl, vinyl, allyl, 1-butyryl, 2-butyryl, acetylenyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, $-(CH_2)$ -cyclopropyl, $-(CH_2)$ -cyclobutyl, $-(CH_2)$ -cyclopentyl, $-(CH_2)$ -cyclohexyl, aziridine, oxirane, thiirane, azetidine, oxetane, thietane, pyrrolidine, tetrahydrofuran, thiolane,

pyrazolidine, piperidine, oxane, thiane, $-(CH_2)$ -aziridine, $-(CH_2)$ -oxirane, $-(CH_2)$ -thiirane, $-(CH_2)$ -azetidide, $-(CH_2)$ -oxetane, $-(CH_2)$ -thietane, $-(CH_2)$ -pyrrolidine, $-(CH_2)$ -tetrahydrofuran, $-(CH_2)$ -thiolane, $-(CH_2)$ -pyrazolidine, $-(CH_2)$ -piperidine, $-(CH_2)$ -oxane, $-(CH_2)$ -thiane, phenyl, pyridyl, $-ONHC(=O)OCH_3$, $-ONHC(=O)OCH_2CH_3$, $-NHOH$, $NHOCH_3$, $-OCH_3$, OC_2H_5 , $-OPh$,
 5 OCH_2Ph , $-SCH_3$, $-SC_2H_5$, $-SPh$, SCH_2Ph , $-NH(CH_2)_2NH_2$, $-NH(CH_2)_2N(CH_3)_2$, $-NHNH_2$, $-NHNHCH_3$, $-N=NH$, $-N=NCH_3$, $-N=NCH_2CH_3$, $-NHC(O)NHNH_2$, $-NHC(S)NHNH_2$, $-C(O)NHNH_2$, $-NHSO_2CH_3$, $-NHSO_2CH_2CH_3$, $-SO_2NHCH_3$, $-SO_2N(CH_3)_2$, $-C(O)NH_2$, $-C(O)NHCH_3$, $-C(O)N(CH_3)_2$, $-CO_2CH_3$, $-CO_2CH_2CH_3$, $-CO_2Ph$, $-CO_2CH_2Ph$, $-SO_2CH_3$,

$-SO_2CH_2CH_3$, $-SO_2Ph$, $-SO_2CH_2Ph$, , , $-P(O)H(OH)$, $-P(O)H(OCH_3)$, $-P(O)(OH)(OH)$, $-P(O)(OH)(OCH_3)$, $-P(O)(OCH_3)(OCH_3)$, $-P(O)(OH)(NH_2)$, $-P(O)(OH)(NHCH_3)$, $-P(O)(OH)N(CH_3)_2$, $-NHC(O)CH_3$, $-NHC(O)CH_2CH_3$, $-NHC(O)CH(CH_3)_2$, $-NHC(O)OCH_3$, $-NHC(O)OCH_2CH_3$, $-NHC(O)OCH(CH_3)_2$, $-NHC(O)OCH_2CH_2CH_3$, $-NHC(O)OCH_2CH_2CH_2CH_3$ and $-NHC(O)OCH_2CH_2CH_2CH_2CH_3$;

R^{23} is C_1 - C_5 alkyl, $-(C_0$ - C_2 alkyl)(C_3 - C_6 cycloalkyl), $-(C_0$ - C_2 alkyl)(heterocycle)-(C_0 -
 15 2 alkyl)(aryl) or $-(C_0$ - C_2 alkyl)(heteroaryl) each of which can be optionally substituted;

R^{24} is hydrogen, C_1 - C_6 alkyl, $-(C_1$ - C_2 alkyl)(C_3 - C_6 cycloalkyl), $-(C_1$ - C_2 alkyl)(C_3 - C_6 heterocycle) $-(C_0$ - C_2 alkyl)(aryl) or $-(C_0$ - C_2 alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted;

R^{25} is hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, $-(C_0$ - C_2 alkyl)(C_3 -
 20 C_6 cycloalkyl), $-(C_0$ - C_2 alkyl)(C_3 - C_6 heterocycle), $-(C_0$ - C_2 alkyl)(aryl) or $-(C_0$ - C_2 alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted;

R^{26} is independently selected from hydrogen, C_1 - C_6 alkyl, $-(C_0$ - C_2 alkyl)(C_3 -
 C_6 cycloalkyl), $-(C_0$ - C_2 alkyl)(heterocycle), $-(C_0$ - C_2 alkyl)(aryl), or $-(C_0$ - C_2 alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted;

R^{27} hydrogen or optionally substituted C_1 - C_6 alkyl;

R^{28} is C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, $-(C_0$ - C_2 alkyl)(C_3 - C_6 cycloalkyl), $-(C_0$ - C_2 alkyl)(C_3 - C_6 heterocycle), $-(C_0$ - C_2 alkyl)(aryl) or $-(C_0$ - C_2 alkyl)(heteroaryl) each of which can be optionally substituted;

R²⁹ is hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted; or

R²⁷ and R²⁹ together with the nitrogen that they are bonded to can form a heterocyclic ring;

R³⁰ is hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted; or

R²⁹ and R³⁰ can be bonded together to form a heterocyclic ring;
x is 1, 2 or 3.

The metabolism of the β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-dimethyl-2,6-diaminopurine nucleotide involves both the formation of the β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-dimethyl-2,6-diaminopurine nucleoside triphosphate as well as the generation of the corresponding guanine nucleoside triphosphate. See Scheme 2 and 3.

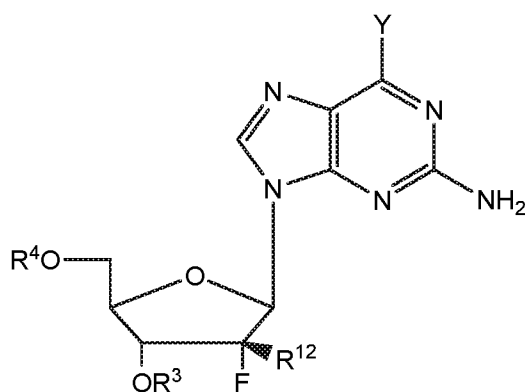
2'-Deoxy-2'- α -fluoro-2'- β -C-substituted-N⁶-substituted-2,6-diaminopurine nucleotides can be further substituted at the N²-position by alkylation or acylated which can modify the lipophilicity, pharmacokinetics and/or targeting of the nucleotide to the liver. It has been discovered that 2'-deoxy-2'- α -fluoro-2'- β -C-substituted-N⁶-substituted-2,6-diaminopurine nucleotides modified at the 2-position of the diaminopurine can be dealkylated or deacylated by hepatic enzymes to further increase the specificity of the nucleotide derivatives both in vitro and in vivo, unless the N²-amino group is completely replaced by a different moiety, as described herein, such as fluoro. For example, the nucleoside phosphoramidate 2'-deoxy-2'- α -fluoro-2'- β -methyl-N²-methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate is dealkylated to 2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate when incubated with a human liver S9 fraction in vitro, up to 60 minutes, these conditions mimics in vivo conditions. In one embodiment, N² modifications will increase cell permeability and hepatic targeting.

Despite the volume of antiviral nucleoside literature and patent filings, the 5'-stabilized phosphate derivative of 2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine

nucleoside, 2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-dimethyl-2,6-diaminopurine nucleoside, and other β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleoside derivatives as described herein have not been specifically disclosed, nor have their advantageous activities been described.

5 Unless otherwise specified, the compounds described herein are provided in the β -D-configuration. Likewise, when in phosphoramidate or thiophosphoramidate form, the amino acid portion can be in the L- or D-configuration. In an alternative embodiment, the compounds can be provided in a β -L-configuration. Likewise, any substituent group that exhibits chirality can be provided in racemic, enantiomeric, diastereomeric form or any mixture thereof. Where a
10 phosphoramidate, thiophosphoramidate or other stabilized phosphorus prodrug in which the phosphorus exhibits chirality is used as the R⁴ stabilized phosphate prodrug, it can be provided as an R or S chiral phosphorus derivative or a mixture thereof, including a racemic mixture. All of the combinations of these stereoconfigurations are included in the invention described herein.

15 Accordingly, the present invention includes a compound of Formula I-VII, or a pharmaceutically acceptable composition, salt, or prodrug thereof, as described herein:



Formula I

20 In one specific embodiment, the parent nucleoside, i.e., the nucleoside wherein R⁴ is hydrogen and the 5'-position thus has a hydroxyl group, is not substantially deaminated by adenosine deaminase under conditions that mimic the *in vivo* environment (e.g., ambient temperature and aqueous physiological pH), for a period of 7 minutes, 10 minutes, 30 minutes, 60 minutes or 120 minutes. Unless otherwise stated, the time period is 30 minutes. In this embodiment, the term "not substantially deaminated" means that the parent compound is not

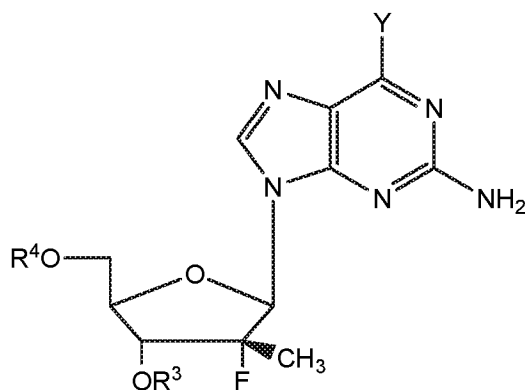
converted to the corresponding guanine derivative, or 6-oxo derivative, in an amount sufficient to provide a therapeutic effect *in vivo*.

Compounds, methods, and compositions are provided for the treatment of a host infected with a HCV virus via administration of an effective amount of the compound or its pharmaceutically acceptable salt.

The compounds and compositions can also be used to treat related conditions such as anti-HCV antibody positive and antigen positive conditions, viral-based chronic liver inflammation, liver cancer resulting from advanced hepatitis C, cirrhosis, chronic or acute hepatitis C, fulminant hepatitis C, chronic persistent hepatitis C and anti-HCV-based fatigue.

The compound or formulations that include the compounds can also be used prophylactically to prevent or restrict the progression of clinical illness in individuals who are anti-HCV antibody or antigen positive or who have been exposed to hepatitis C.

In another embodiment, compounds of Formula Ia are disclosed:



Formula Ia

wherein:

Y, R³ and R⁴ are as defined above.

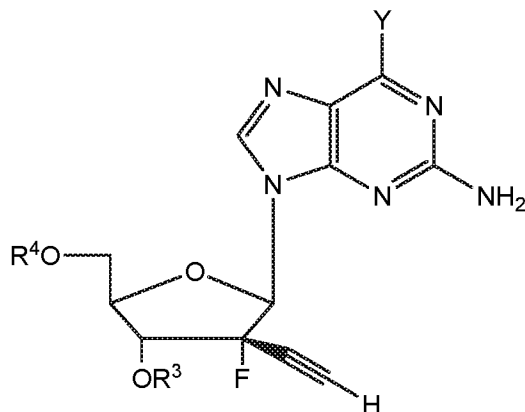
In one embodiment of Formula Ia, R³ is hydrogen.

In one embodiment of Formula Ia, when Y is NR¹R², R¹ is methyl and R² is hydrogen.

In one embodiment of Formula Ia, when Y is NR¹R², both R¹ and R² are methyl.

In one embodiment of Formula Ia, when Y is NR¹R², R¹ is methyl and R² is cyclopropyl.

In another embodiment, compounds of Formula Ib are disclosed:



Formula Ib

wherein:

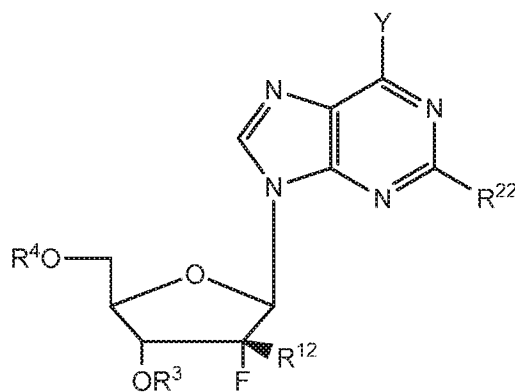
5 Y, R³ and R⁴ are as defined above.

In one embodiment of Formula Ib, R³ is hydrogen.

In one embodiment of Formula Ib, when Y is NR¹R², R¹ is methyl and R² is hydrogen.

In one embodiment of Formula Ib, when Y is NR¹R², both R¹ and R² are methyl.

In one embodiment, compounds of Formula II are disclosed:

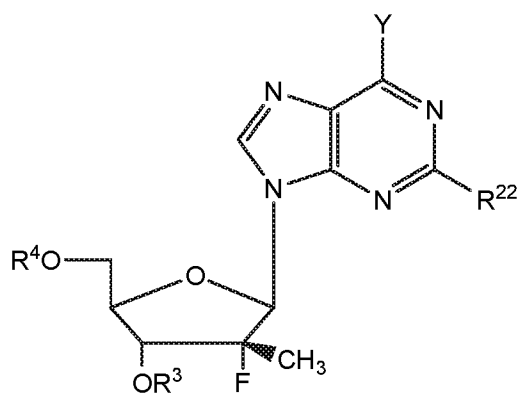


Formula II

wherein:

10 Y, R³, R⁴, R¹² and R²² are as defined above.

In another embodiment, compounds of Formula IIa are disclosed:

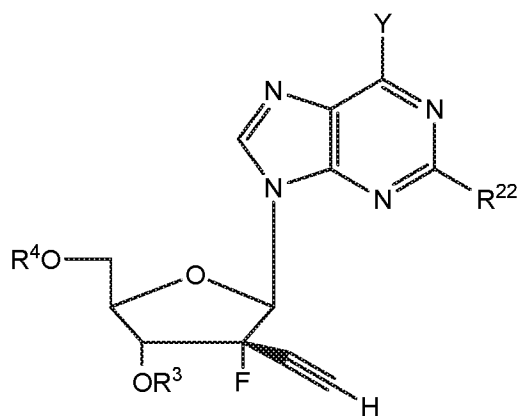


Formula IIa

5 wherein:

Y, R³, R⁴ and R²² are as defined above.

In another embodiment, compounds of Formula IIb are disclosed:

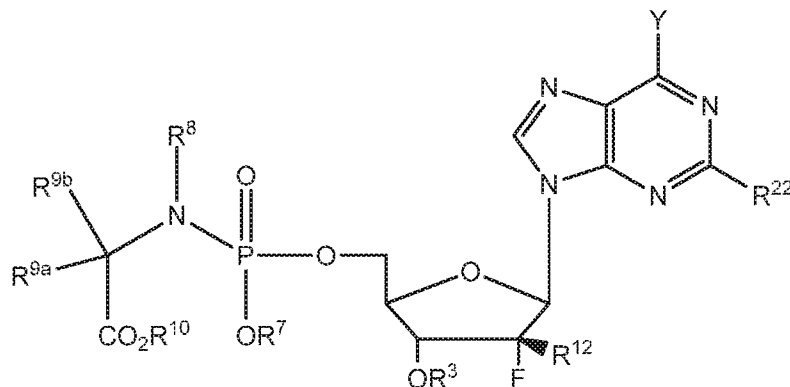


Formula IIb

10 wherein:

Y, R³, R⁴, and R²² are as defined above.

In one embodiment, compounds of Formula III are disclosed:

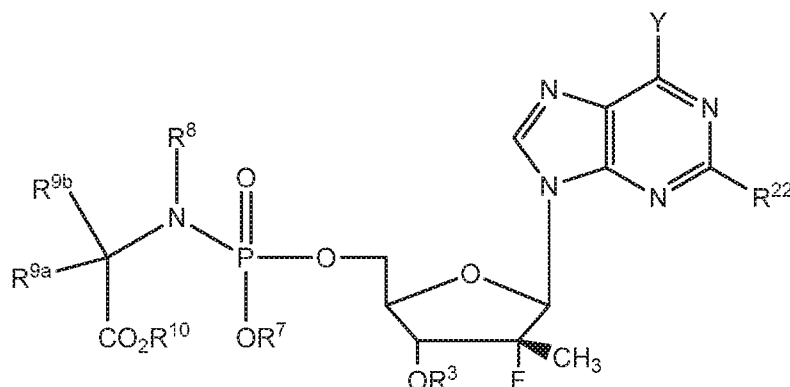


Formula III

wherein the variables Y, R³, R⁷, R⁸, R^{9a}, R^{9b}, R¹⁰, R¹² and R²² are described herein.

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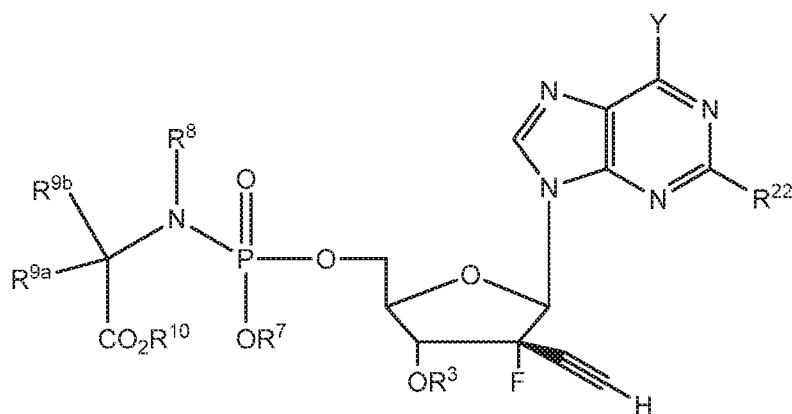
In one embodiment, compounds of Formula IV are disclosed:



Formula IV

wherein the variables Y, R³, R⁷, R⁸, R^{9a}, R^{9b}, R¹⁰ and R²² are described herein.

In one embodiment, compounds of Formula V are disclosed:

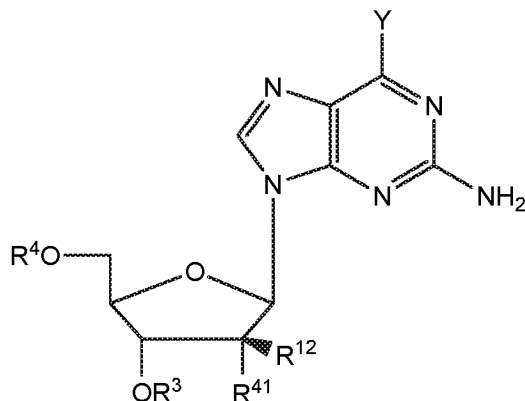


Formula V

wherein the variables Y, R³, R⁷, R⁸, R^{9a}, R^{9b}, R¹⁰ and R²² are described herein.

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In one embodiment, compounds of Formula VI are disclosed:

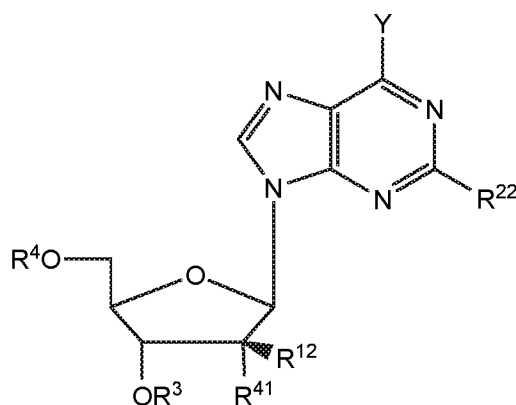


Formula VI

wherein:

- 5 R^{41} is halogen (in particular F or Cl), OR^3 , N_3 , NH_2 or CN ; and the variables Y, R^3 , R^4 , and R^{12} are described herein.

In one embodiment, compounds of Formula VII are disclosed:



Formula VII

- 10 Wherein the variables Y, R^3 , R^4 , R^{12} and R^{41} are described herein.

The phosphorus in any of the Formulas above may be chiral and thus can be provided as an R or S enantiomer or mixture thereof, including a racemic mixture.

- 15 Compound 5 was separated into the enantiomer compounds 5-1 and 5-2. Compound 5-2 was also prepared by chiral synthesis and assigned compound 24.

In one embodiment, compounds, methods, and compositions are provided for the treatment of a host infected with or exposed to hepatitis C described herein. The compounds of the invention can be administered in an effective amount alone or in combination with another

anti-HCV drug, to treat the infected host. In certain embodiments, it is useful to administer a combination of drugs that modulates the same or a different pathway or inhibits a different target in the virus. As the disclosed β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleotides are NS5B polymerase inhibitors, it may be useful to administer the compound to a host in combination with a protease inhibitor, such as an NS3/4A protease inhibitor (for example, telaprevir (Incivek®) boceprevir (Victrelis™) simeprevir (Olysio™), or paritaprevir, or an NS5A inhibitor (for example, Ombitasvir). The compounds of the invention can also be administered in combination with a structurally different NS5B polymerase inhibitor such as another compound described herein or below, including Gilead's Sovaldi®. The compounds of the invention can also be administered in combination with interferon alfa-2a, which may be pegylated or otherwise modified, and/or ribavirin.

The β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleotides of the invention are typically administered orally, for example in pill or tablet form, but may be administered via an other route which the attending physician considers appropriate, including via intravenous, transdermal, subcutaneous, topical, parenteral, or other suitable route.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a sample chromatogram of a semi-prep run illustrating the separation of the stereoisomers of Compound 5 using a Phenomenex Luna column as disclosed in Example 9. The y axis is shown in mAU and the x axis is measured in minutes.

Figure 2 is a graph of the HCV replication inhibition curves for Compound 5-2 (Table 7) and Sofosbuvir. Compound 5-2 has an EC₅₀ = 4 nM, a TC₅₀ greater than one hundred micromolar and a therapeutic index of greater than 25,000. Sofosbuvir has an EC₅₀ = 53 nM, a TC₅₀ greater than one hundred micromolar and a therapeutic index greater than 1,920. The y-axis is the percent of virus control and the x-axis is the concentration of drug in μ M.

Figure 3 is a graph of the HCV replication inhibition curves for Compound 25 (Table 7) and Sofosbuvir. As described in Example 27, Compound 25 has an EC₅₀ = 4 nM, a TC₅₀ of greater than 100 μ M, and a therapeutic index of greater than 25,000. Sofosbuvir has an EC₅₀ = 53 nM, a TC₅₀ greater than one hundred micromolar and a therapeutic index greater than 1,920. The y-axis is the percent of virus control and the x-axis is the concentration of drug in μ M.

Figure 4 is an intra-assay comparison of the anti-HCV activity for Compounds 5-2, 25, 27 (Table 7) and Sofosbuvir. The y-axis is the percent of virus control and the x-axis is the concentration of drug in μM . See, Example 27.

Figure 5 is a graph that shows the stability of compounds 5-2; the N²-acetate of compound 5-2, the N²-butyrate of compound 5-2; the N²-methyl derivative of compound 5-2; and the N²-n-pentylcarbamate of compound 5-2 in human blood. The x axis is incubation time measured in minutes and the y axis is the measurement of the percent of the parent compound remaining.

Figure 6 is a graph showing the in vitro time course dealkylation of 2'-deoxy-2'- α -fluoro-2'- β -methyl-N²-methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate to 2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate in the presence of a human liver S9 fraction. The x axis is measured in minutes and the y axis is the measurement of the concentration of the compound remaining in nM.

Figure 7 is a graph showing the stability of compounds 5-2; the N²-acetate of compound 5-2, the N²-butyrate of compound 5-2; the N²-methyl derivative of compound 5-2; and the N²-n-pentylcarbamate of compound 5-2 in the presence of a human liver S9 fraction. The x axis is measured in minutes and the y axis is the measurement of percent compound remaining.

Figure 8 shows the predominant Compound 25 metabolites generated in human hepatocytes. The x axis is incubation time in hours. The y axis is intracellular concentration in pmol/ 10^6 cells. See Example 33.

Figure 9 shows the predominant Compound 27 metabolites generated in human hepatocytes. The x axis is incubation time in hours. The y axis is intracellular concentration in pmol/ 10^6 cells. See Example 33.

Figure 10 shows the predominant Compound 5-2 metabolites generated in human hepatocytes. The x axis is incubation time in hours. The y axis is intracellular concentration in pmol/ 10^6 cells. See Example 33.

Figure 11 is a graph showing the activation pathways for Compounds 25, 27 and 5-2. As can be seen, Compounds 25, 27 and 5-2 are converted to their corresponding monophosphate analogs which are subsequently metabolized to a common MP analog; β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-guanine monophosphate. The monophosphate is then stepwise

phosphorylated to the active triphosphate: β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-guanine triphosphate. See Example 33.

DETAILED DESCRIPTION OF THE INVENTION

5 The invention disclosed herein is a compound, method, and composition for the treatment of infections in or exposure to humans and other host animals of the HCV virus that includes the administration of an effective amount of a compound of Formula I-VII as described herein or a pharmaceutically acceptable salt or prodrug thereof, optionally in a pharmaceutically acceptable carrier. The compounds of this invention either possess antiviral activity, or are metabolized to a
10 compound that exhibits such activity.

 The compounds and compositions can also be used to treat conditions related to or occurring as a result of a HCV viral exposure. For example, the active compound can be used to treat HCV antibody positive and HCV antigen positive conditions, viral-based chronic liver inflammation, liver cancer resulting from advanced hepatitis C, cirrhosis, acute hepatitis C,
15 fulminant hepatitis C, chronic persistent hepatitis C, and anti-HCV-based fatigue. In one embodiment, the compounds or formulations that include the compounds can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are HCV antibody or HCV antigen positive or who have been exposed to hepatitis C.

 In particular, it has been discovered that a 5'-stabilized phosphate prodrug or derivative
20 of β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diamino purine nucleotide, as well as β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-dimethyl-2,6-diamino purine nucleotide, and other β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2'-modified-N⁶-substituted purine nucleotides as described below, are highly active against HCV. This is surprising because the activity of the parent nucleoside β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diamino purine in a replicon
25 assay (EC_{50} = 15.7 micromolar) indicates that it is not suitable for use as a human drug due to insufficient activity, however, the stabilized phosphate prodrug (phosphoramidate) exhibits an EC_{50} = 26 nanomolar, in a replicon assay, which is at least an 870 fold increase in activity. Likewise, the activity of the parent nucleoside β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-dimethyl-2,6-diaminopurine in a replicon assay (EC_{50} = 10.7 micromolar, " μ M") indicates that it
30 is also not suitable for use as a human drug due to insufficient activity, however, the stabilized

phosphate prodrug (phosphoramidate) exhibits an $EC_{50} = 12$ nanomolar, ("nM"), in a replicon assay, which is more than a 1,300 fold increase in activity.

Despite the volume of antiviral nucleoside literature and patent filings, the 5'-stabilized phosphate derivative of 2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diamino purine nucleotide, 2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-dimethyl-2,6-diamino purine nucleotide, and other β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2'-modified-N⁶-substituted purine nucleotides have not been specifically disclosed.

Unless otherwise specified, the compounds described herein are provided in the β -D-configuration. In an alternative embodiment, the compounds can be provided in a β -L-configuration. Likewise, any substituent group that exhibits chirality can be provided in racemic, enantiomeric, diastereomeric form or any mixture thereof. Where a phosphoramidate, thiophosphoramidate or other stabilized phosphorus prodrug in which the phosphorus exhibits chirality is used as the R⁴ stabilized phosphate prodrug, it can be provided as an R or S chiral phosphorus derivative or a mixture thereof, including a racemic mixture. The amino acid of the phosphoramidate or thiophosphoramidate can be in the D- or L-configuration, or a mixture thereof, including a racemic mixture. All of the combinations of these stereo configurations are included in the invention described herein.

The present invention includes the following features:

(a) a compound of Formula I-VII as described herein, and pharmaceutically acceptable salts and prodrugs thereof;

(b) Formulas I-VII as described herein, and pharmaceutically acceptable salts and prodrugs thereof for use in the treatment or prophylaxis of a hepatitis C virus infection;

(c) use of Formulas I-VII, and pharmaceutically acceptable salts and prodrugs thereof in the manufacture of a medicament for treatment of a hepatitis C virus infection;

(d) a method for manufacturing a medicament intended for the therapeutic use for treating a hepatitis C virus infection, characterized in that a Formulas I-VII as described herein is used in the manufacture;

(e) a pharmaceutical formulation comprising an effective host-treating amount of the Formulas I-VII or a pharmaceutically acceptable salt or prodrug thereof together with a pharmaceutically acceptable carrier or diluent;

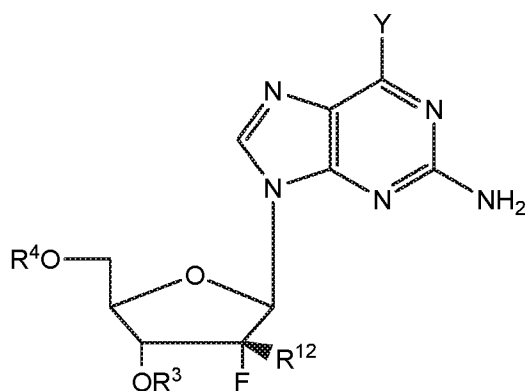
(f) Formulas I-VII as described herein substantially in the absence of stereoisomers of the described compound, or substantially isolated from other chemical entities; and,

(g) processes for the preparation of therapeutic products that contain an effective amount of a Formulas I-VII, as described herein.

5

I. 2'-Deoxy-2'- α -Fluoro-2'- β -C-Substituted-2-Modified-N⁶-Substituted Purine Nucleotides of the Invention

The active compounds of the invention are those depicted, for example, in Formula I, which can be provided in a pharmaceutically acceptable composition, salt or prodrug thereof:



10

Formula I

wherein:

Y is NR¹R²;

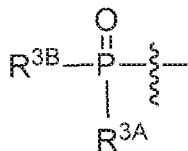
R¹ is C₁-C₅alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C₁-C₅haloalkyl (including CH₂F, CH₂Cl, CF₃, CH₂CF₃, CF₂CH₃ and CF₂CF₃), C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(heterocycle), -(C₀-C₂alkyl)(aryl), -(C₀-C₂alkyl)(heteroaryl), -OR²⁵, -C(O)R^{3C} (including -C(O)CH₃, -C(O)CH₂CH₃-C(O)CH(CH₃)₂, -C(O)OCH₃, -C(O)OC₂H₅, -C(O)OC₃H₇, -C(O)OC₄H₉, and -C(O)OC₅H₁₁), -C(S)R^{3D}, or -SO₂R²⁸ each of which can be optionally substituted;

20

R² is hydrogen, optionally substituted C₁-C₅alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C₁-C₅haloalkyl (including CHF₂, CH₂F, CF₃, CH₂CF₃ and CF₂CF₃), optionally substituted -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), optionally substituted -(C₀-C₂alkyl)(heterocycle), optionally substituted -(C₀-C₂alkyl)(aryl),

optionally substituted $-(C_0-C_2\text{alkyl})(\text{heteroaryl})$, $-C(O)R^{3C}$ (including $-C(O)CH_3$, $-C(O)CH_2CH_3$, $-C(O)CH(CH_3)_2$, $-C(O)OCH_3$, $-C(O)OC_2H_5$, $-C(O)OC_3H_7$, $-C(O)OC_4H_9$, and $-C(O)OC_5H_{11}$), $-C(S)R^{3D}$, or $-SO_2R^{28}$; and

wherein at least one of R^1 and R^2 is methyl, CH_2F , CHF_2 or CF_3 ;



5 R^3 is hydrogen, R^{3A} , diphosphate, triphosphate, an optionally substituted carbonyl linked amino acid, or $-C(O)R^{3C}$;

R^{3A} can be selected from O^- , OH , an $-O$ -optionally substituted aryl, an $-O$ -optionally substituted heteroaryl, or an optionally substituted heterocyclyl;

10 R^{3B} can be selected from O^- , OH , an optionally substituted N-linked amino acid or an optionally substituted N-linked amino acid ester;

R^{3C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$, $-O$ -alkyl, $-O$ -alkenyl, $-O$ -alkynyl, $-O-(C_0-C_2)(\text{cycloalkyl})$, $-O-(C_0-C_2)(\text{heterocyclo})$, $-O-(C_0-C_2)(\text{aryl})$, or $-O-(C_0-C_2)(\text{heteroaryl})$, each of which can be optionally substituted;

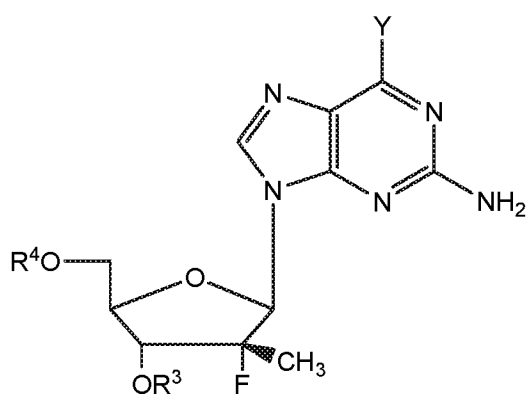
15 R^4 is a monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, including but not limited to a phosphoramidate, a thiophosphoramidate, or any other moiety that is metabolized to a monophosphate, diphosphate or triphosphate *in vivo* in the host human or animal; or

20 R^3 and R^4 together with the oxygens that they are bonded to can form a 3',5'-cyclic prodrug, including but not limited to, a 3',5'-cyclic phosphate prodrug;

R^{12} is CH_3 , CH_2F , CHF_2 , CF_3 , or ethynyl.

A stabilized phosphate prodrug is any moiety that can deliver a mono, di, or triphosphate.

In another embodiment, compounds of Formula Ia are disclosed:

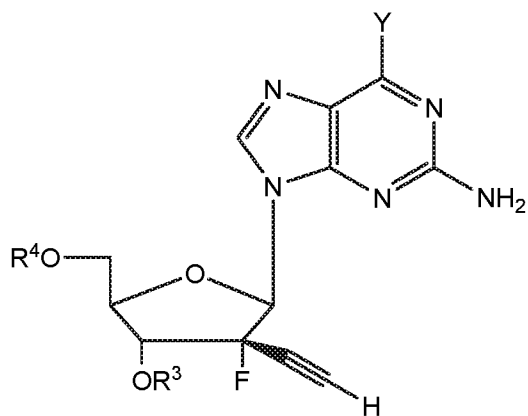


Formula Ia

wherein:

Y, R³ and R⁴ are as defined above.

5 In another embodiment, compounds of Formula Ib are disclosed:

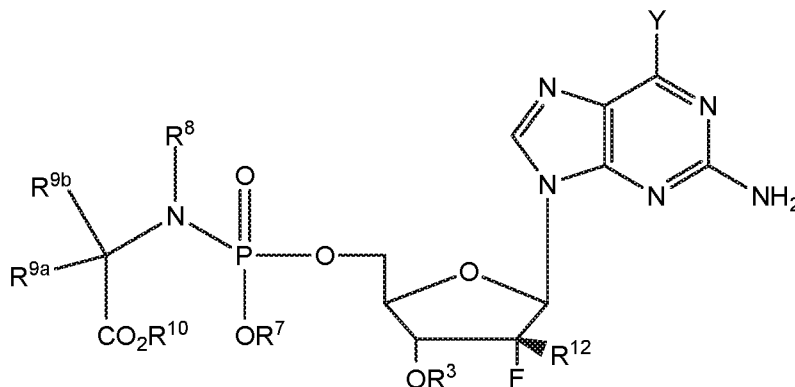


Formula Ib

wherein:

Y, R³ and R⁴ are as defined above.

In another embodiment, the compound is according to Formula Ic:



Formula Ic

wherein:

- 5 R^7 is hydrogen, C_{1-6} alkyl; C_{3-7} cycloalkyl; heteroaryl, heterocyclic, or aryl, which includes, but is not limited to, phenyl or naphthyl, where phenyl or naphthyl are optionally substituted with C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} alkoxy, F, Cl, Br, I, nitro, cyano, C_{1-6} haloalkyl, $-N(R^7)_2$, C_{1-6} acylamino, $NHSO_2C_{1-6}$ alkyl, $-SO_2N(R^7)_2$, $COR^{7'}$, and $-SO_2C_{1-6}$ alkyl; (R^7 is independently hydrogen or C_{1-6} alkyl; $R^{7'}$ is $-OR^{11}$ or $-N(R^7)_2$);
- 10 R^8 is hydrogen, C_{1-6} alkyl, or R^{9a} or R^{9b} and R^8 together are $(CH_2)_n$ so as to form a cyclic ring that includes the adjoining N and C atoms; where n is 2 to 4;
- R^{9a} and R^{9b} are (i) independently selected from hydrogen, C_{1-6} alkyl, cycloalkyl, $-(CH_2)_c(NR^9)_2$, C_{1-6} hydroxyalkyl, $-CH_2SH$, $-(CH_2)_2S(O)(Me)$, $-(CH_2)_3NHC(=NH)NH_2$, (1H-indol-3-yl)methyl, (1H-imidazol-4-yl)methyl, $-(CH_2)_cCOR^{9''}$, aryl and aryl(C_{1-3} alkyl)-, the aryl
- 15 groups can be optionally substituted with a group selected from hydroxyl, C_{1-6} alkyl, C_{1-6} alkoxy, halogen, nitro and cyano; (ii) R^{9a} and R^{9b} both are C_{1-6} alkyl; (iii) R^{9a} and R^{9b} together are $(CH_2)_r$ so as to form a spiro ring; (iv) R^{9a} is hydrogen and R^{9b} and R^8 together are $(CH_2)_n$ so as to form a cyclic ring that includes the adjoining N and C atoms (v) R^{9b} is hydrogen and R^{9a} and R^8 together are $(CH_2)_n$ so as to form a cyclic ring that includes the adjoining N and C atoms, where c is 1 to
- 20 6, n is 2 to 4, r is 2 to 5 and where R^9 is independently hydrogen or C_{1-6} alkyl and $R^{9''}$ is $-OR^{11}$ or $-N(R^{11})_2$); (vi) R^{9a} is hydrogen and R^{9b} is hydrogen, CH_3 , CH_2CH_3 , $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph , CH_2 -indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H , $CH_2C(O)NH_2$, CH_2CH_2COOH , $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, CH_2 -imidazol-4-yl, CH_2OH , $CH(OH)CH_3$, $CH_2((4'-OH)-Ph)$, CH_2SH , or lower cycloalkyl; or (vii) R^{9a}
- 25 is CH_3 , CH_2CH_3 , $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph , CH_2 -indol-3-yl,

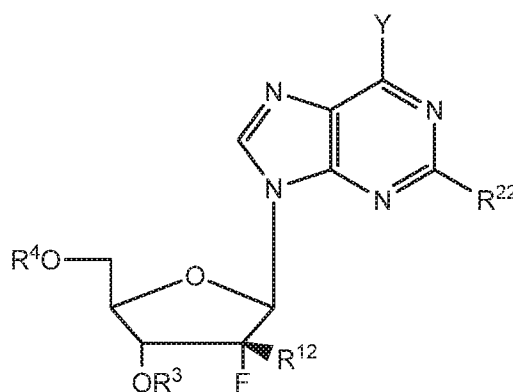
-CH₂CH₂SCH₃, CH₂CO₂H, CH₂C(O)NH₂, CH₂CH₂COOH, CH₂CH₂C(O)NH₂, CH₂CH₂CH₂CH₂NH₂, -CH₂CH₂CH₂NHC(NH)NH₂, CH₂-imidazol-4-yl, CH₂OH, CH(OH)CH₃, CH₂((4'-OH)-Ph), CH₂SH, or lower cycloalkyl and R^{9b} is hydrogen;

R¹⁰ is hydrogen, C₁₋₆alkyl optionally substituted with an alkoxy, di(lower alkyl)-amino, or halogen, C₁₋₆haloalkyl, C₃₋₇cycloalkyl, heterocycloalkyl, aminoacyl, aryl, such as phenyl, heteroaryl, such as, pyridinyl, substituted aryl, or substituted heteroaryl;

R¹¹ is an optionally substituted C₁₋₆alkyl, an optionally substituted cycloalkyl; an optionally substituted C₂₋₆alkynyl, an optionally substituted C₂₋₆alkenyl, or optionally substituted acyl, which includes but is not limited to C(O)(C₁₋₆ alkyl); and

Y, R³ and R¹² are as defined herein.

In one embodiment, compounds of Formula II are disclosed:



Formula II

wherein:

Y is NR¹R²;

R¹ is C₁₋₅alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C₁₋₅haloalkyl (including CH₂F, CHF₂, CF₃, CH₂CF₃, CF₂CH₃ and CF₂CF₃), C₂₋₆ alkenyl, C₂₋₆ alkynyl, -(C₀₋₂alkyl)(C₃₋₆cycloalkyl), -(C₀₋₂alkyl)(heterocycle), -(C₀₋₂alkyl)(aryl), -(C₀₋₂alkyl)(heteroaryl), -OR²⁵, -C(O)R^{3C} (including -C(O)CH₃, -C(O)CH₂CH₃-C(O)CH(CH₃)₂, -C(O)OCH₃, -C(O)OC₂H₅, -C(O)OC₃H₇, -C(O)OC₄H₉, and -C(O)OC₅H₁₁), -C(S)R^{3D}, or -SO₂R²⁸ each of which can be optionally substituted;

R² is hydrogen, optionally substituted C₁₋₅alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C₁₋₅haloalkyl (including CHF₂,

CHF₂, CF₃, CH₂CF₃ and CF₂CF₃), optionally substituted $-(C_0-C_2\text{alkyl})(C_3-C_6\text{cycloalkyl})$, optionally substituted $-(C_0-C_2\text{alkyl})(\text{heterocycle})$, optionally substituted $-(C_0-C_2\text{alkyl})(\text{aryl})$, optionally substituted $-(C_0-C_2\text{alkyl})(\text{heteroaryl})$, $-C(O)R^{3C}$ (including $-C(O)CH_3$, $-C(O)CH_2CH_3$, $-C(O)CH(CH_3)_2$, $-C(O)OCH_3$, $-C(O)OC_2H_5$, $-C(O)OC_3H_7$, $-C(O)OC_4H_9$, and $-C(O)OC_5H_{11}$), $-C(S)R^{3D}$, or $-SO_2R^{28}$; and
 wherein at least one of R¹ and R² is methyl, CH₂F, CHF₂ or CF₃;

$$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}^{3B}-\text{P}-\text{R}^{3A} \\ | \\ \text{R}^{3A} \end{array}$$

R³ is hydrogen, carbonyl linked amino acid, or $-C(O)R^{3C}$;

R^{3A} can be selected from O⁻, OH, an $-O$ -optionally substituted aryl, an $-O$ -optionally substituted heteroaryl, or an optionally substituted heterocyclyl;

R^{3B} can be selected from O⁻, OH, an optionally substituted N-linked amino acid or an optionally substituted N-linked amino acid ester;

R^{3C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$, $-O$ -alkyl, $-O$ -alkenyl, $-O$ -alkynyl, $-O-(C_0-C_2)(\text{cycloalkyl})$, $-O-(C_0-C_2)(\text{heterocyclo})$, $-O-(C_0-C_2)(\text{aryl})$, $-O-(C_0-C_2)(\text{heteroaryl})$, $-S$ -alkyl, $-S$ -alkenyl, $-S$ -alkynyl, $-S-(C_0-C_2)(\text{cycloalkyl})$, $-S-(C_0-C_2)(\text{heterocyclo})$, $-S-(C_0-C_2)(\text{aryl})$, or $-S-(C_0-C_2)(\text{heteroaryl})$ each of which can be optionally substituted;

R^{3D} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$, $-O$ -alkyl, $-O$ -alkenyl, $-O$ -alkynyl, $-O-(C_0-C_2)(\text{cycloalkyl})$, $-O-(C_0-C_2)(\text{heterocyclo})$, $-O-(C_0-C_2)(\text{aryl})$, or $-O-(C_0-C_2)(\text{heteroaryl})$, each of which can be optionally substituted;

R⁴ is a monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, including but not limited to a phosphoramidate, a thiophosphoramidate, or any other moiety that is metabolized to a monophosphate, diphosphate or triphosphate *in vivo* in the host human or animal; or

R³ and R⁴ together with the oxygens that they are bonded to can form a 3',5'-cyclic prodrug, including but not limited to, a 3',5'-cyclic phosphate prodrug;

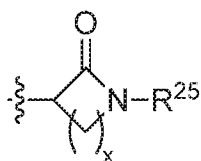
R⁵ is C₁-C₅alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C₁-C₅haloalkyl (including CHF₂, CH₂F, CF₃, CH₂CF₃ and CF₂CF₃), C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(heterocycle), -(C₀-C₂alkyl)(aryl), -(C₀-C₂alkyl)(heteroaryl), -OR²⁵, -C(O)R^{3C} (including -C(O)CH₃, -C(O)CH₂CH₃-C(O)CH(CH₃)₂, -C(O)OCH₃, -C(O)OC₂H₅, -C(O)OC₃H₇, -C(O)OC₄H₉, and -C(O)OC₅H₁₁), -C(S)R^{3D}, or -SO₂R²⁸ each of which can be optionally substituted;

R⁶ is hydrogen, optionally substituted C₁-C₅alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C₁-C₅haloalkyl (including CHF₂, CH₂F, CF₃, CH₂CF₃ and CF₂CF₃), optionally substituted -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), optionally substituted -(C₀-C₂alkyl)(heterocycle), optionally substituted -(C₀-C₂alkyl)(aryl), optionally substituted -(C₀-C₂alkyl)(heteroaryl), -C(O)R^{3C} (including -C(O)CH₃, -C(O)CH₂CH₃-C(O)CH(CH₃)₂, -C(O)OCH₃, -C(O)OC₂H₅, -C(O)OC₃H₇, -C(O)OC₄H₉, and -C(O)OC₅H₁₁), -C(S)R^{3D}, or -SO₂R²⁸; or

R⁵ and R⁶ together with the nitrogen that they are bonded to can form a heterocyclic ring;

R¹² is CH₃, CH₂F, CHF₂, CF₃, or ethynyl;

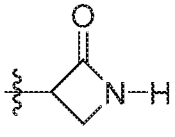
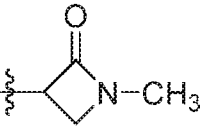
R²² is Cl, Br, F, CN, N₃, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₁-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl), -(C₀-C₂alkyl)(heteroaryl); -ONHC(=O)OR²³, -NHOR²⁴, -OR²⁵, -SR²⁵, -NH(CH₂)₁₋₄N(R²⁶)₂, -NHNHR²⁶, -N=NR²⁷, -NHC(O)NHNHR²⁷, -NHC(S)NHNHR²⁷, -C(O)NHNHR²⁷, -NR²⁷SO₂R²⁸, -SO₂NR²⁷R²⁹,



-C(O)NR²⁷R²⁹, -CO₂R²⁹, -SO₂R²⁹, -P(O)H(OR²⁹), -P(O)(OR²⁹)(OR³⁰), -P(O)(OR²⁹)(NR²⁹R³⁰) or -NR⁵R⁶;

for example including but not limited to the following embodiments, chloro, bromo, fluoro, cyano, azido, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and n-pentyl, 1,1-dimethylpropyl, 2,2-dimethylpropyl, 3-methylbutyl, 1-methylbutyl, 1-ethylpropyl, vinyl, allyl, 1-butyryl, 2-butyryl, acetylenyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, -(CH₂)-cyclopropyl, -(CH₂)-cyclobutyl, -(CH₂)-cyclopentyl, -(CH₂)-cyclohexyl, aziridine, oxirane, thiirane, azetidine, oxetane, thietane, pyrrolidine, tetrahydrofuran, thiolane, pyrazolidine, piperidine, oxane, thiane, -(CH₂)-aziridine, -(CH₂)-oxirane, -(CH₂)-thiirane, -(CH₂)-azetidine, -(CH₂)-oxetane, -(CH₂)-thietane, -(CH₂)-pyrrolidine, -(CH₂)-tetrahydrofuran, -

(CH₂)-thiolane, -(CH₂)-pyrazolidine, -(CH₂)-piperidine, -(CH₂)-oxane, -(CH₂)-thiane, phenyl, pyridyl, -ONHC(=O)OCH₃, -ONHC(=O)OCH₂CH₃, -NHOH, NHOCH₃, -OCH₃, OC₂H₅, -OPh, OCH₂Ph, -SCH₃, -SC₂H₅, -SPh, SCH₂Ph, -NH(CH₂)₂NH₂, -NH(CH₂)₂N(CH₃)₂, -NHNH₂, -NHNHCH₃, -N=NH, -N=NCH₃, -N=NCH₂CH₃, -NHC(O)NHNH₂, -NHC(S)NHNH₂,
 5 -C(O)NHNH₂, -NH₂SO₂CH₃, -NH₂SO₂CH₂CH₃, -SO₂NHCH₃, -SO₂N(CH₃)₂, -C(O)NH₂, -C(O)NHCH₃, -C(O)N(CH₃)₂, -CO₂CH₃, -CO₂CH₂CH₃, -CO₂Ph, -CO₂CH₂Ph, -SO₂CH₃,

-SO₂CH₂CH₃, -SO₂Ph, -SO₂CH₂Ph, , , -P(O)H(OH), -P(O)H(OCH₃), -P(O)(OH)(OH), -P(O)(OH)(OCH₃), -P(O)(OCH₃)(OCH₃), -P(O)(OH)(NH₂), -P(O)(OH)(NHCH₃), -P(O)(OH)N(CH₃)₂, -NHC(O)CH₃, -NHC(O)CH₂CH₃, -NHC(O)CH(CH₃)₂,
 10 -NHC(O)OCH₃, -NHC(O)OCH₂CH₃, -NHC(O)OCH(CH₃)₂, -NHC(O)OCH₂CH₂CH₃, -NHC(O)OCH₂CH₂CH₂CH₃ and -NHC(O)OCH₂CH₂CH₂CH₂CH₃;

R²³ is C₁-C₅alkyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(heterocycle)-(C₀-2alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) each of which can be optionally substituted;

R²⁴ is hydrogen, C₁-C₆ alkyl, -(C₁-C₂alkyl)(C₃-C₆cycloalkyl),
 15 -(C₁-C₂alkyl)(C₃-C₆heterocycle) -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted;

R²⁵ is hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted;

20 R²⁶ is independently selected from hydrogen, C₁-C₆alkyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(heterocycle), -(C₀-C₂alkyl)(aryl), or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted;

R²⁷ hydrogen or optionally substituted C₁-C₆ alkyl;

R²⁸ is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl),
 25 -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) each of which can be optionally substituted;

R²⁹ is hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted; or

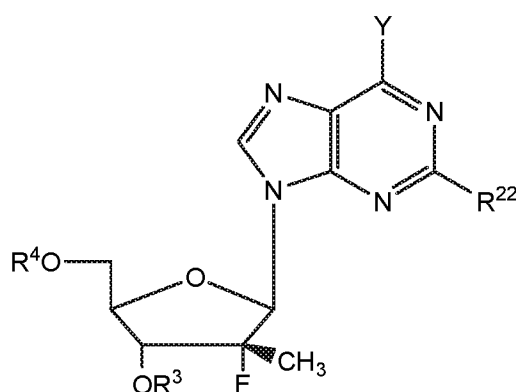
R^{27} and R^{29} together with the nitrogen that they are bonded to can form a heterocyclic ring;

R^{30} is hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, $-(C_0$ - C_2 alkyl)(C_3 - C_6 cycloalkyl), $-(C_0$ - C_2 alkyl)(C_3 - C_6 heterocycle), $-(C_0$ - C_2 alkyl)(aryl) or $-(C_0$ - C_2 alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted; or

R^{29} and R^{30} can be bonded together to form a heterocyclic ring;

x is 1, 2 or 3.

In another embodiment, compounds of Formula IIa are disclosed:

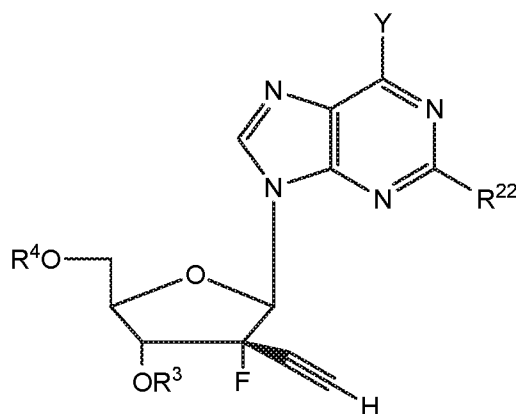


Formula IIa

wherein:

Y , R^3 , R^4 and R^{22} are as defined above.

In another embodiment, compounds of Formula IIb are disclosed:



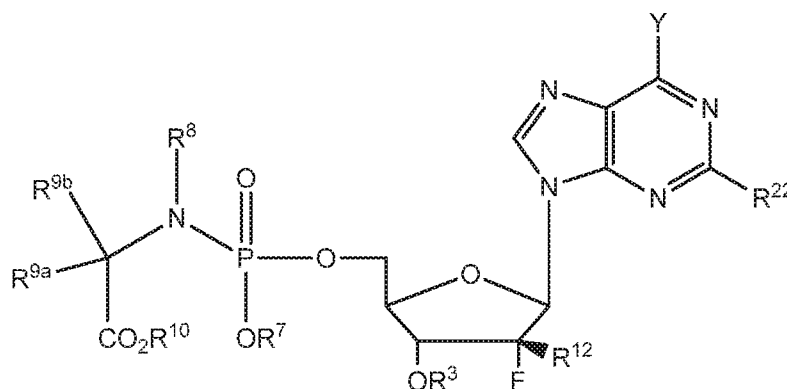
Formula IIb

wherein:

Y, R³, R⁴ and R²² are as defined above.

In a typical embodiment, the compound is a β-D isomer with reference to the corresponding nucleoside (i.e., in the naturally occurring configuration). In an alternative configuration, the compound is provided as a β-L isomer. The compound is typically at least 90% free of the opposite enantiomer, and can be at least 98%, 99% or even 100% free of the opposite enantiomer. Unless described otherwise, the compound is at least 90% free of the opposite enantiomer.

In another embodiment, the compound is according to Formula III:



Formula III

wherein:

R⁷ is hydrogen, C₁₋₆alkyl; C₃₋₇cycloalkyl; heteroaryl, heterocyclic, or aryl, which includes, but is not limited to, phenyl or naphthyl, where phenyl or naphthyl are optionally substituted with C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₁₋₆alkoxy, F, Cl, Br, I, nitro, cyano, C₁₋₆haloalkyl, -N(R^{7'})₂, C₁₋₆acylamino, NHSO₂C₁₋₆alkyl, -SO₂N(R^{7'})₂, COR^{7''}, and -SO₂C₁₋₆alkyl; (R^{7'} is independently hydrogen or C₁₋₆alkyl; R^{7''} is -OR¹¹ or -N(R⁷)₂);

R⁸ is hydrogen, C₁₋₆alkyl, or R^{9a} or R^{9b} and R⁸ together are (CH₂)_n so as to form a cyclic ring that includes the adjoining N and C atoms; where n is 2 to 4;

R^{9a} and R^{9b} are (i) independently selected from hydrogen, C₁₋₆alkyl, cycloalkyl, -(CH₂)_c(NR^{9'})₂, C₁₋₆hydroxyalkyl, -CH₂SH, -(CH₂)₂S(O)(Me), -(CH₂)₃NHC(=NH)NH₂, (1H-indol-3-yl)methyl, (1H-imidazol-4-yl)methyl, -(CH₂)_cCOR^{9''}, aryl and aryl(C₁₋₃alkyl)-, the aryl groups can be optionally substituted with a group selected from hydroxyl, C₁₋₆alkyl, C₁₋₆alkoxy, halogen, nitro and cyano; (ii) R^{9a} and R^{9b} both are C₁₋₆alkyl; (iii) R^{9a} and R^{9b} together are (CH₂)_r so as to form a spiro ring; (iv) R^{9a} is hydrogen and R^{9b} and R⁸ together are (CH₂)_n so as to form a cyclic ring that includes the adjoining N and C atoms (v) R^{9b} is hydrogen and R^{9a} and R⁸ together

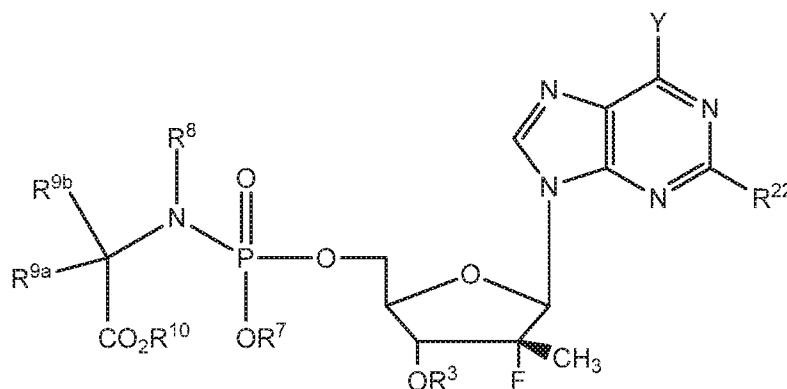
are $(\text{CH}_2)_n$ so as to form a cyclic ring that includes the adjoining N and C atoms, where c is 1 to 6, n is 2 to 4, r is 2 to 5 and where R^9 is independently hydrogen or C_{1-6} alkyl and $\text{R}^{9''}$ is $-\text{OR}^{11}$ or $-\text{N}(\text{R}^{11'})_2$; (vi) R^{9a} is hydrogen and R^{9b} is hydrogen, CH_3 , CH_2CH_3 , $\text{CH}(\text{CH}_3)_2$, $\text{CH}_2\text{CH}(\text{CH}_3)_2$, $\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$, CH_2Ph , CH_2 -indol-3-yl, $-\text{CH}_2\text{CH}_2\text{SCH}_3$, $\text{CH}_2\text{CO}_2\text{H}$, $\text{CH}_2\text{C}(\text{O})\text{NH}_2$, $\text{CH}_2\text{CH}_2\text{COOH}$, $\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}_2$, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{NHC}(\text{NH})\text{NH}_2$, CH_2 -imidazol-4-yl, CH_2OH , $\text{CH}(\text{OH})\text{CH}_3$, $\text{CH}_2((4'\text{-OH})\text{-Ph})$, CH_2SH , or lower cycloalkyl; or (vii) R^{9a} is CH_3 , CH_2CH_3 , $\text{CH}(\text{CH}_3)_2$, $\text{CH}_2\text{CH}(\text{CH}_3)_2$, $\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$, CH_2Ph , CH_2 -indol-3-yl, $-\text{CH}_2\text{CH}_2\text{SCH}_3$, $\text{CH}_2\text{CO}_2\text{H}$, $\text{CH}_2\text{C}(\text{O})\text{NH}_2$, $\text{CH}_2\text{CH}_2\text{COOH}$, $\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}_2$, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{NHC}(\text{NH})\text{NH}_2$, CH_2 -imidazol-4-yl, CH_2OH , $\text{CH}(\text{OH})\text{CH}_3$, $\text{CH}_2((4'\text{-OH})\text{-Ph})$, CH_2SH , or lower cycloalkyl and R^{9b} is hydrogen;

R^{10} is hydrogen, C_{1-6} alkyl optionally substituted with an alkoxy, di(lower alkyl)-amino, or halogen, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, heterocycloalkyl, aminoacyl, aryl, such as phenyl, heteroaryl, such as, pyridinyl, substituted aryl, or substituted heteroaryl;

R^{11} is an optionally substituted C_{1-6} alkyl, an optionally substituted cycloalkyl; an optionally substituted C_{2-6} alkynyl, an optionally substituted C_{2-6} alkenyl, or optionally substituted acyl, which includes but is not limited to $\text{C}(\text{O})(\text{C}_{1-6} \text{ alkyl})$; and

Y , R^3 , R^{12} and R^{22} are as defined above.

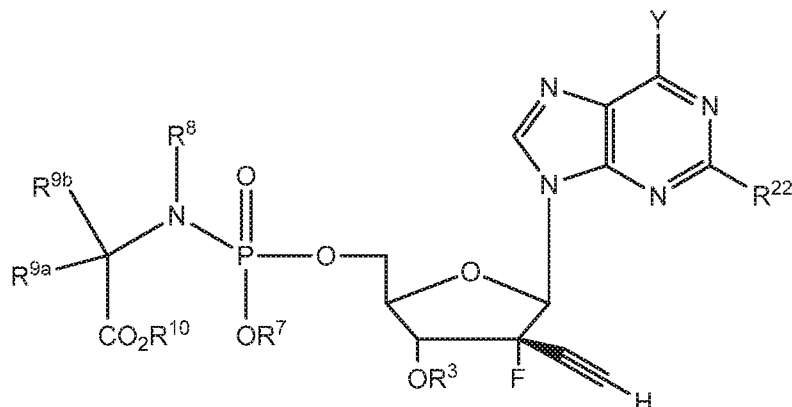
In one embodiment, compounds of Formula IV are disclosed:



Formula IV

wherein the variables Y , R^3 , R^7 , R^8 , R^{9a} , R^{9b} , R^{10} and R^{22} are described herein.

In one embodiment, compounds of Formula V are disclosed:

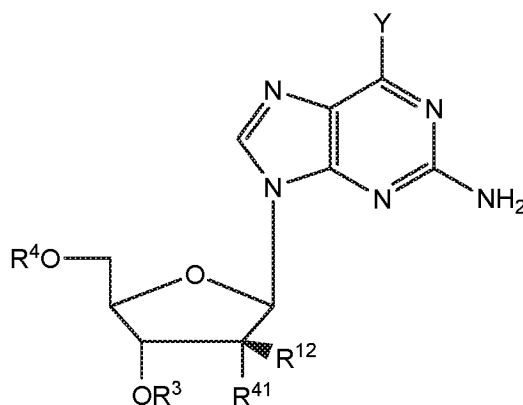


Formula V

wherein the variables Y, R³, R⁷, R⁸, R^{9a}, R^{9b}, R¹⁰ and R²² are described herein.

- 5 In an alternative embodiment, compounds, methods, and compositions are provided for the treatment of a host infected with or exposed to hepatitis C.

In one embodiment, compounds of Formula VI are disclosed:

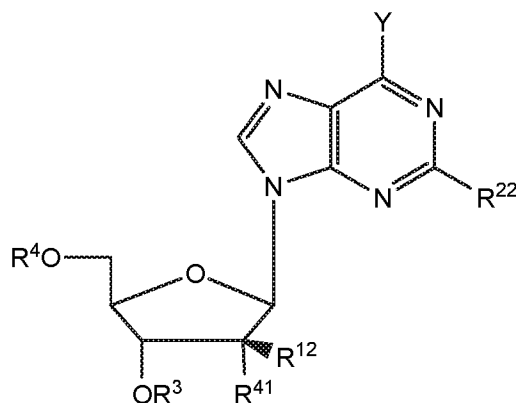


Formula VI

wherein:

R⁴¹ is halogen (in particular F or Cl), OR³ (including OH), N₃, NH₂ or CN; and the variables Y, R³, R⁴, and R¹² are described herein.

In one embodiment, compounds of Formula VII are disclosed:



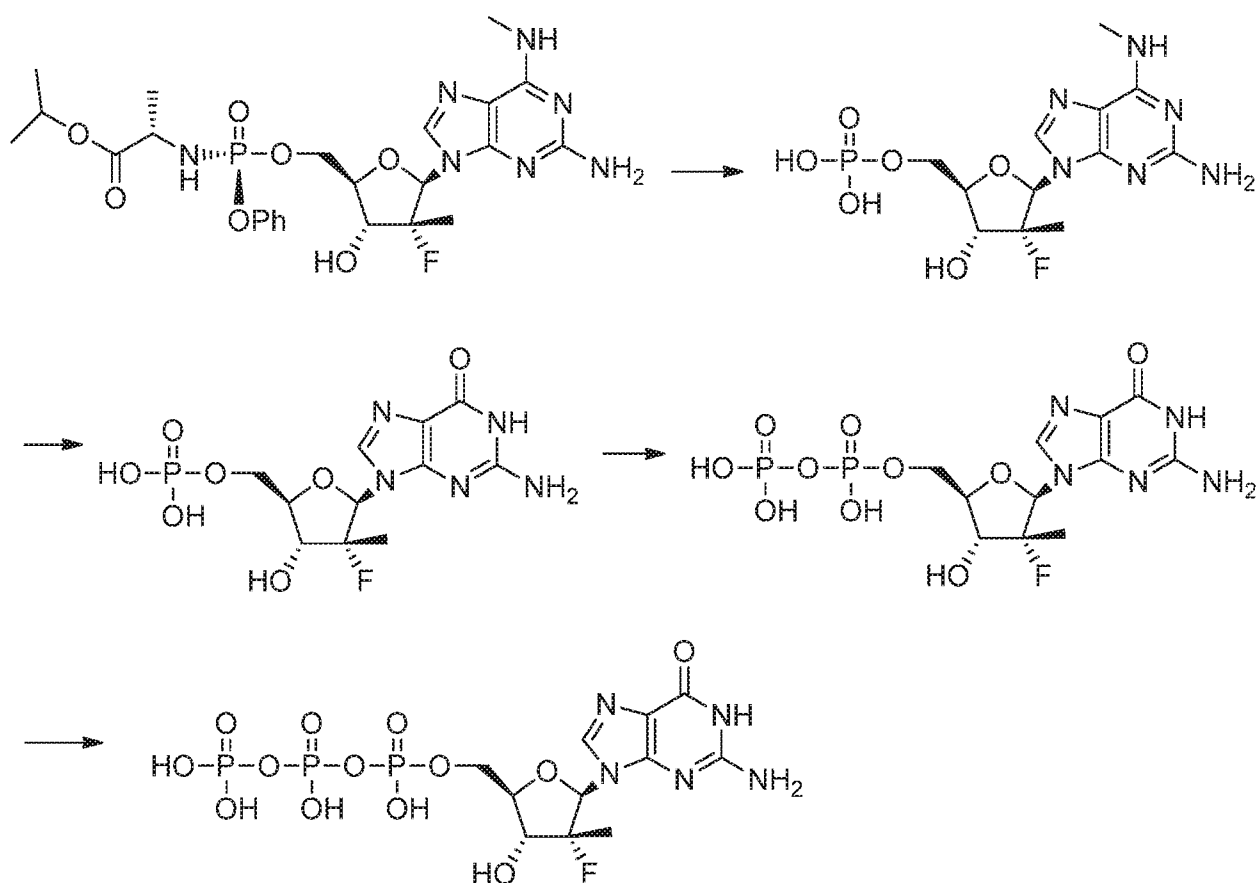
Formula VII

Wherein the variables Y, R³, R⁴, R¹² and R⁴¹ are described herein.

5

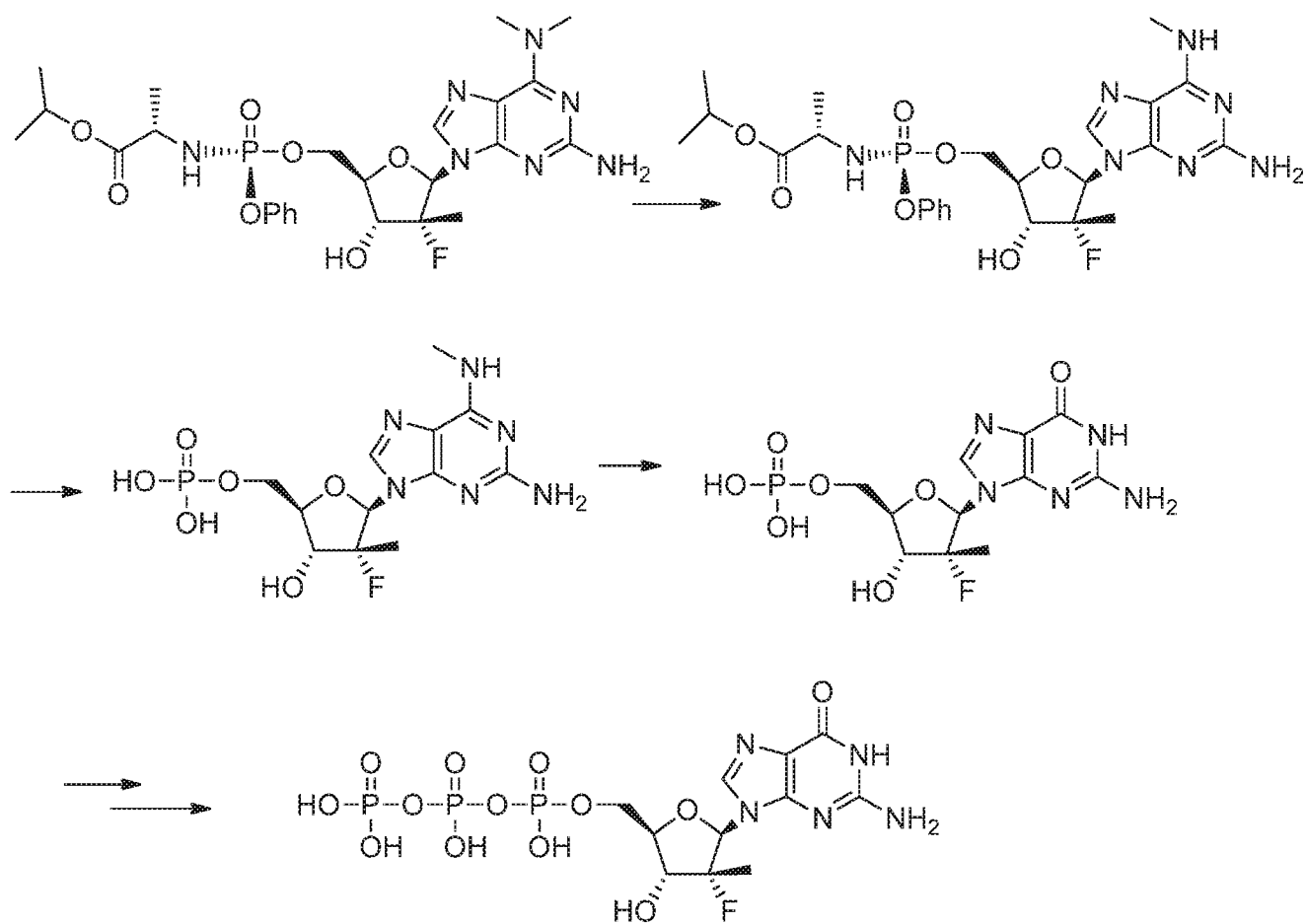
Metabolism of β -D-2'-deoxy-2'- α -fluoro-2'- β -C-substituted-N⁶-substituted-2,6-diaminopurine nucleotides

The metabolism of the β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate involves the production of a 5'-monophosphate and the subsequent anabolism of the N⁶-methyl-2,6-diaminopurine base to generate the β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-guanine nucleoside as the 5'-monophosphate. The monophosphate is then further anabolized to the active species; the 5'-triphosphate. The β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-guanine triphosphate has an IC₅₀ = 0.15 μ M against the HCV genotype 1b NS5B polymerase. The metabolic pathway for the β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate is illustrated in Scheme 1 below.

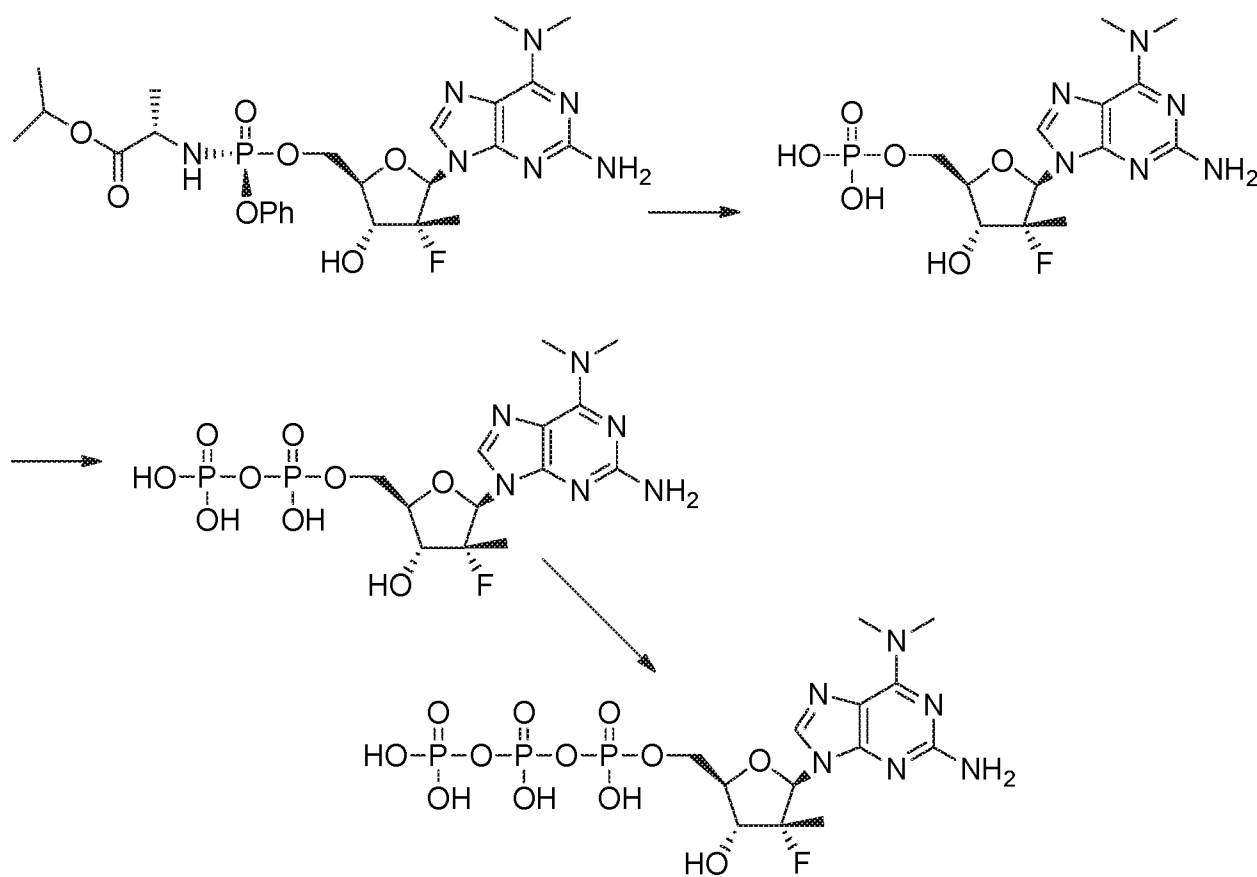


Scheme 1

- 5 The metabolism of the β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-dimethyl-2,6-diaminopurine nucleotide involves both the formation of the β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-dimethyl-2,6-diaminopurine nucleoside triphosphate as well as the generation of the corresponding guanine nucleoside triphosphate. These metabolic pathways are illustrated in Schemes 2 and 3 below.



Scheme 2



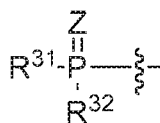
Scheme 3

5 Stabilized Phosphate Prodrugs

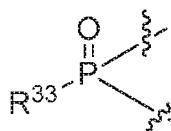
Stabilized phosphate prodrugs are moieties that can deliver a mono, di, or triphosphate *in vivo*. For example, McGuigan has disclosed phosphoramidates in US Patent Nos.: 8,933,053; 8,759,318; 8,658,616; 8,263,575; 8,119,779; 7,951,787 and 7,115,590. Alios has disclosed thiophosphoramidates in US 8,895,723 and 8,871,737 incorporated by reference herein. Alios has also disclosed cyclic nucleotides in US Patent No. 8,772,474 incorporated by reference herein. Idenix has disclosed cyclic phosphoramidates and phosphoramidate/SATE derivatives in WO 2013/177219 incorporated by reference herein. Idenix has also disclosed substituted carbonyloxymethylphosphoramidate compounds in WO 2013/039920 incorporated by reference herein. Hostetler has disclosed lipid phosphate prodrugs, see, for example, US 7,517,858. Hostetler has also disclosed lipid conjugates of phosphonate prodrugs, see, for example, US 8,889,658; 8,846,643; 8,710,030; 8,309,565; 8,008,308; and 7,790,703. Emory University has

disclosed nucleotide sphingoid and lipid derivatives in WO 2014/124430. RFS Pharma has disclosed purine nucleoside monophosphate prodrugs in WO 2010/091386. Cocrystal Pharma Inc. has also disclosed purine nucleoside monophosphate prodrugs in US Patent No.: 9,173,893 incorporated by reference herein. HepDirect™ technology is disclosed in the article "Design,
 5 Synthesis, and Characterization of a Series of Cytochrome P(450) 3A-Activated Prodrugs (HepDirect Prodrugs) Useful for Targeting Phosph(on)ate-Based Drugs to the Liver," (J. Am. Chem. Soc. 126, 5154-5163 (2004). Additional phosphate prodrugs include, but are not limited to phosphate esters, 3',5'-cyclic phosphates including CycloSAL, SATE derivatives (S-acyl-2thioesters) and DTE (dithiodiethyl) prodrugs. For literature reviews that disclose non-limiting
 10 examples see: A. Ray and K. Hostetler, "Application of kinase bypass strategies to nucleoside antivirals," Antiviral Research (2011) 277-291; M. Sofia, "Nucleotide prodrugs for HCV therapy," Antiviral Chemistry and Chemotherapy 2011; 22-23-49; and S. Peyrottes et al., "SATE Pronucleotide Approaches: An Overview," Mini Reviews in Medicinal Chemistry 2004, 4, 395. In one embodiment, a 5'-prodrug described in any of these patent filings or literature can be used
 15 in the R⁴ position of the presented compounds.

In one alternative embodiment, the stabilized phosphate prodrugs, include, but are not limited to those described in U.S. Patent No. 9,173,893 and U.S. Patent No. 8,609,627, incorporated by reference herein, including for processes of preparation. For example, 5'-prodrugs of Formula I-V can be represented by the group:



In an alternate embodiment, 3',5'-prodrugs of Formula I-V can be represented by the group:



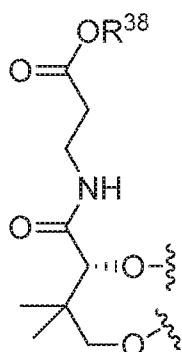
wherein:

when chirality exists at the phosphorous center it may be wholly or partially R_p or S_p or any mixture thereof.

Z is O or S;

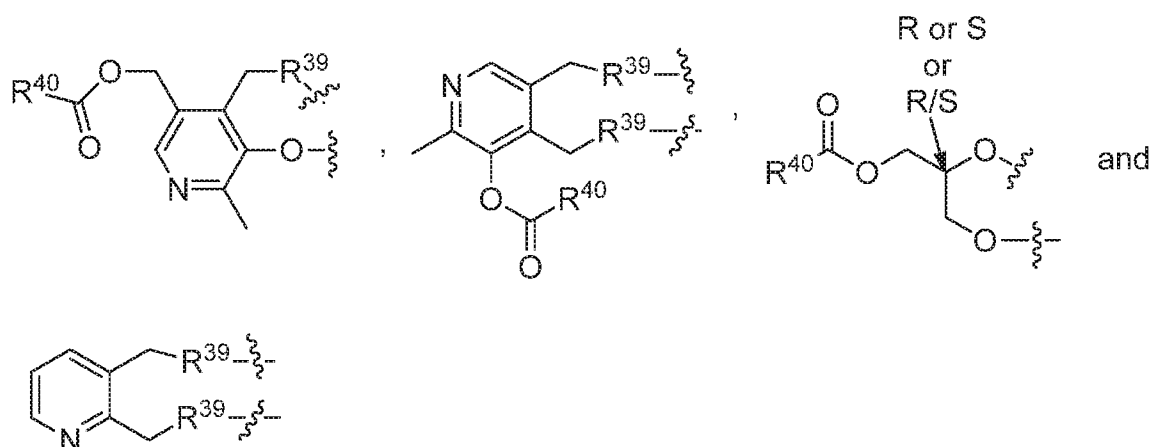
R^{35} is H, C_{1-20} alkyl, the carbon chain derived from a fatty alcohol (such as oleyl alcohol, octacosanol, triacontanol, linoleyl alcohol, and etc) or C_{1-20} alkyl substituted with a lower alkyl, alkoxy, di(lower alkyl)-amino, fluoro, C_{3-10} cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as, pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a lower alkyl, alkoxy, di(lower alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or cycloalkyl;

(d) R^{31} and R^{32} can come together to form a ring



where R^{38} is H, C_{1-20} alkyl, C_{1-20} alkenyl, the carbon chain derived from a fatty alcohol (such as oleyl alcohol, octacosanol, triacontanol, linoleyl alcohol, etc) or C_{1-20} alkyl substituted with a lower alkyl, alkoxy, di(lower alkyl)-amino, fluoro, C_{3-10} cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as, pyridinyl, substituted aryl, or substituted heteroaryl; wherein the substituents are C_{1-5} alkyl, or C_{1-5} alkyl substituted with a lower alkyl, alkoxy, di(lower alkyl)-amino, fluoro, C_{3-10} cycloalkyl, or cycloalkyl;

(e) R^{31} and R^{32} can come together to form a ring selected from



where R^{39} is O or NH and

R⁴⁰ is selected from H, C₁₋₂₀ alkyl, C₁₋₂₀ alkenyl, the carbon chain derived from a fatty acid (such as oleic acid, linoleic acid, and the like), and C₁₋₂₀ alkyl substituted with a lower alkyl, alkoxy, di(lower alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, cycloalkyl alkyl, cycloheteroalkyl, aryl, such as phenyl, heteroaryl, such as pyridinyl, substituted aryl, or substituted heteroaryl; wherein the
 5 substituents are C₁₋₅ alkyl, or C₁₋₅ alkyl substituted with a lower alkyl, alkoxy, di(lower alkyl)-amino, fluoro, C₃₋₁₀ cycloalkyl, or cycloalkyl.

The compounds can be prepared, for example, by preparing the 5'-OH analogs, then converting these to the monophosphate analogs.

10 Embodiments

In particular embodiments:

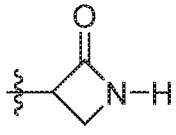
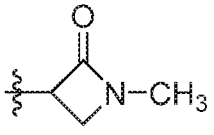
- (i) in Formula Ia, Y is NR¹R², R¹ is methyl, R² is hydrogen, R³ is hydrogen, R⁴ is a stabilized phosphate prodrug;
- (ii) in Formula Ia, Y is NR¹R², R¹ is methyl, R² is hydrogen, R³ is hydrogen, and R⁴ is
 15 a stabilized thiophosphate prodrug;
- (iii) in Formula Ia, Y is NR¹R², R¹ is methyl, R² is hydrogen, R³ is hydrogen, and R⁴ is a phosphoramidate;
- (iv) in Formula Ia, Y is NR¹R², R¹ is methyl, R² is hydrogen, R³ is hydrogen, and R⁴ is a thiophosphoramidate;
- (v) in Formula Ia, Y is NR¹R², R¹ is methyl, R² is hydrogen, R³ is hydrogen, and R⁴ is
 20 a monophosphate;
- (vi) in Formula Ia, Y is NR¹R², R¹ is methyl, R² is hydrogen, R³ is hydrogen, and R⁴ is a diphosphate;
- (vii) in Formula Ia, Y is NR¹R², R¹ is methyl, R² is hydrogen, R³ is hydrogen, and R⁴ is
 25 a triphosphate;
- (viii) in Formula Ia, Y is NR¹R², R¹ is methyl, R² is methyl, R³ is hydrogen, R⁴ is a stabilized phosphate prodrug;
- (ix) in Formula Ia, Y is NR¹R², R¹ is methyl, R² is methyl, R³ is hydrogen, and R⁴ is a stabilized thiophosphate prodrug;
- (x) in Formula Ia, Y is NR¹R², R¹ is methyl, R² is methyl, R³ is hydrogen, and R⁴ is
 30 a phosphoramidate;

- (xi) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, and R^4 is a thiophosphoramidate;
- (xii) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, and R^4 is a monophosphate;
- 5 (xiii) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, and R^4 is a diphosphate;
- (xiv) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, and R^4 is a triphosphate;
- (xv) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, R^4 is a stabilized phosphate prodrug;
- 10 (xvi) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, and R^4 is a stabilized thiophosphate prodrug;
- (xvii) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, and R^4 is a phosphoramidate;
- 15 (xviii) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, and R^4 is a thiophosphoramidate;
- (xix) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, and R^4 is a monophosphate;
- (xx) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is methyl, and R^4 is a diphosphate;
- 20 (xxi) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, and R^4 is a triphosphate;
- (xxii) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is propyl, R^3 is hydrogen, R^4 is a stabilized phosphate prodrug;
- 25 (xxiii) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is propyl, R^3 is hydrogen, and R^4 is a stabilized thiophosphate prodrug;
- (xxiv) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is propyl, R^3 is hydrogen, and R^4 is a phosphoramidate;
- (xxv) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is propyl, R^3 is hydrogen, and R^4 is a thiophosphoramidate;
- 30

- (xxvi) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is propyl, R^3 is hydrogen, and R^4 is a monophosphate;
- (xxvii) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is propyl, R^3 is hydrogen, and R^4 is a diphosphate;
- 5 (xxviii) in Formula Ia, Y is NR^1R^2 , Y is NR^1R^2 , R^1 is methyl, R^2 is propyl, R^3 is hydrogen, and R^4 is a triphosphate;
- (xxix) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is ethyl, R^3 is hydrogen, R^4 is a stabilized phosphate prodrug;
- (xxx) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is ethyl, R^3 is hydrogen, and R^4 is a stabilized thiophosphate prodrug;
- 10 (xxxi) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is ethyl, R^3 is hydrogen, and R^4 is a phosphoramidate;
- (xxxii) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is ethyl, R^3 is hydrogen, and R^4 is a thiophosphoramidate:
- 15 (xxxiii) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is ethyl, R^3 is hydrogen, and R^4 is a monophosphate;
- (xxxiv) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is ethyl, R^3 is hydrogen, and R^4 is a diphosphate;
- (xxxv) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is ethyl, R^3 is hydrogen, and R^4 is a triphosphate;
- 20 (xxxvi) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, R^4 is a stabilized phosphate prodrug;
- (xxxvii) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, and R^4 is a stabilized thiophosphate prodrug;
- 25 (xxxviii) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, and R^4 is a phosphoramidate;
- (xxxix) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, and R^4 is a thiophosphoramidate:
- (xl) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, and R^4 is a monophosphate;
- 30

- (xli) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, and R^4 is a diphosphate;
- (xlii) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is methyl, R^3 is hydrogen, and R^4 is a triphosphate;
- 5 (xliii) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is hydrogen, R^3 is hydrogen, R^4 is a stabilized phosphate prodrug;
- (xliv) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is hydrogen, R^3 is hydrogen, and R^4 is a stabilized thiophosphate prodrug;
- (xlv) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is hydrogen, R^3 is hydrogen, and R^4 is a phosphoramidate;
- 10 (xlvi) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is hydrogen, R^3 is hydrogen, and R^4 is a thiophosphoramidate;
- (xlvii) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is hydrogen, R^3 is hydrogen, and R^4 is a monophosphate;
- 15 (xlviii) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is hydrogen, R^3 is hydrogen, and R^4 is a diphosphate;
- (xlix) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is hydrogen, R^3 is hydrogen, and R^4 is a triphosphate;
- (l) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, R^4 is a stabilized phosphate prodrug;
- 20 (li) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, and R^4 is a stabilized thiophosphate prodrug;
- (lii) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, and R^4 is a phosphoramidate;
- 25 (liii) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, and R^4 is a thiophosphoramidate;
- (liv) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, and R^4 is a monophosphate;
- (lv) in Formula Ib, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is methyl, and R^4 is a diphosphate;
- 30

(lvi) in Formula Ia, Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl, R^3 is hydrogen, and R^4 is a triphosphate.

In alternative embodiments of any of the above, the compound has an R^{22} substituent. In some of these specific embodiments, the R^{22} is F, amide or carbamate. In other specific aspects of the embodiments above, R^{22} is chloro, bromo, cyano, azido, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and n-pentyl, 1,1-dimethylpropyl, 2,2-dimethylpropyl, 3-methylbutyl, 1-methylbutyl, 1-ethylpropyl, vinyl, allyl, 1-butyne, 2-butyne, acetylenyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, $-(\text{CH}_2)\text{-cyclopropyl}$, $-(\text{CH}_2)\text{-cyclobutyl}$, $-(\text{CH}_2)\text{-cyclopentyl}$, $-(\text{CH}_2)\text{-cyclohexyl}$, aziridine, oxirane, thiirane, azetidine, oxetane, thietane, pyrrolidine, tetrahydrofuran, thiolane, pyrazolidine, piperidine, oxane, thiane, $-(\text{CH}_2)\text{-aziridine}$, $-(\text{CH}_2)\text{-oxirane}$, $-(\text{CH}_2)\text{-thiirane}$, $-(\text{CH}_2)\text{-azetidine}$, $-(\text{CH}_2)\text{-oxetane}$, $-(\text{CH}_2)\text{-thietane}$, $-(\text{CH}_2)\text{-pyrrolidine}$, $-(\text{CH}_2)\text{-tetrahydrofuran}$, $-(\text{CH}_2)\text{-thiolane}$, $-(\text{CH}_2)\text{-pyrazolidine}$, $-(\text{CH}_2)\text{-piperidine}$, $-(\text{CH}_2)\text{-oxane}$, $-(\text{CH}_2)\text{-thiane}$, phenyl, pyridyl, $-\text{ONHC}(=\text{O})\text{OCH}_3$, $-\text{ONHC}(=\text{O})\text{OCH}_2\text{CH}_3$, $-\text{NHOH}$, $-\text{NHOCH}_3$, $-\text{OCH}_3$, $-\text{OC}_2\text{H}_5$, $-\text{OPh}$, $-\text{OCH}_2\text{Ph}$, $-\text{SCH}_3$, $-\text{SC}_2\text{H}_5$, $-\text{SPh}$, $-\text{SCH}_2\text{Ph}$, $-\text{NH}(\text{CH}_2)_2\text{NH}_2$, $-\text{NH}(\text{CH}_2)_2\text{N}(\text{CH}_3)_2$, $-\text{NHNH}_2$, $-\text{NHNHCH}_3$, $-\text{N}=\text{NH}$, $-\text{N}=\text{NCH}_3$, $-\text{N}=\text{NCH}_2\text{CH}_3$, $-\text{NHC}(\text{O})\text{NHNH}_2$, $-\text{NHC}(\text{S})\text{NHNH}_2$, $-\text{C}(\text{O})\text{NHNH}_2$, $-\text{NHSO}_2\text{CH}_3$, $-\text{NHSO}_2\text{CH}_2\text{CH}_3$, $-\text{SO}_2\text{NHCH}_3$, $-\text{SO}_2\text{N}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{C}(\text{O})\text{NHCH}_3$, $-\text{C}(\text{O})\text{N}(\text{CH}_3)_2$, $-\text{CO}_2\text{CH}_3$, $-\text{CO}_2\text{CH}_2\text{CH}_3$, $-\text{CO}_2\text{Ph}$, $-\text{CO}_2\text{CH}_2\text{Ph}$, $-\text{SO}_2\text{CH}_3$, $-\text{SO}_2\text{CH}_2\text{CH}_3$, $-\text{SO}_2\text{Ph}$, $-\text{SO}_2\text{CH}_2\text{Ph}$, , , $-\text{P}(\text{O})\text{H}(\text{OH})$, $-\text{P}(\text{O})\text{H}(\text{OCH}_3)$, $-\text{P}(\text{O})(\text{OH})(\text{OH})$, $-\text{P}(\text{O})(\text{OH})(\text{OCH}_3)$, $-\text{P}(\text{O})(\text{OCH}_3)(\text{OCH}_3)$, $-\text{P}(\text{O})(\text{OH})(\text{NH}_2)$, $-\text{P}(\text{O})(\text{OH})(\text{NHCH}_3)$, $-\text{P}(\text{O})(\text{OH})\text{N}(\text{CH}_3)_2$, $-\text{NHC}(\text{O})\text{CH}_3$, $-\text{NHC}(\text{O})\text{CH}_2\text{CH}_3$, $-\text{NHC}(\text{O})\text{CH}(\text{CH}_3)_2$, $-\text{NHC}(\text{O})\text{OCH}_3$, $-\text{NHC}(\text{O})\text{OCH}_2\text{CH}_3$, $-\text{NHC}(\text{O})\text{OCH}(\text{CH}_3)_2$, $-\text{NHC}(\text{O})\text{OCH}_2\text{CH}_2\text{CH}_3$, $-\text{NHC}(\text{O})\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ and $-\text{NHC}(\text{O})\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$;

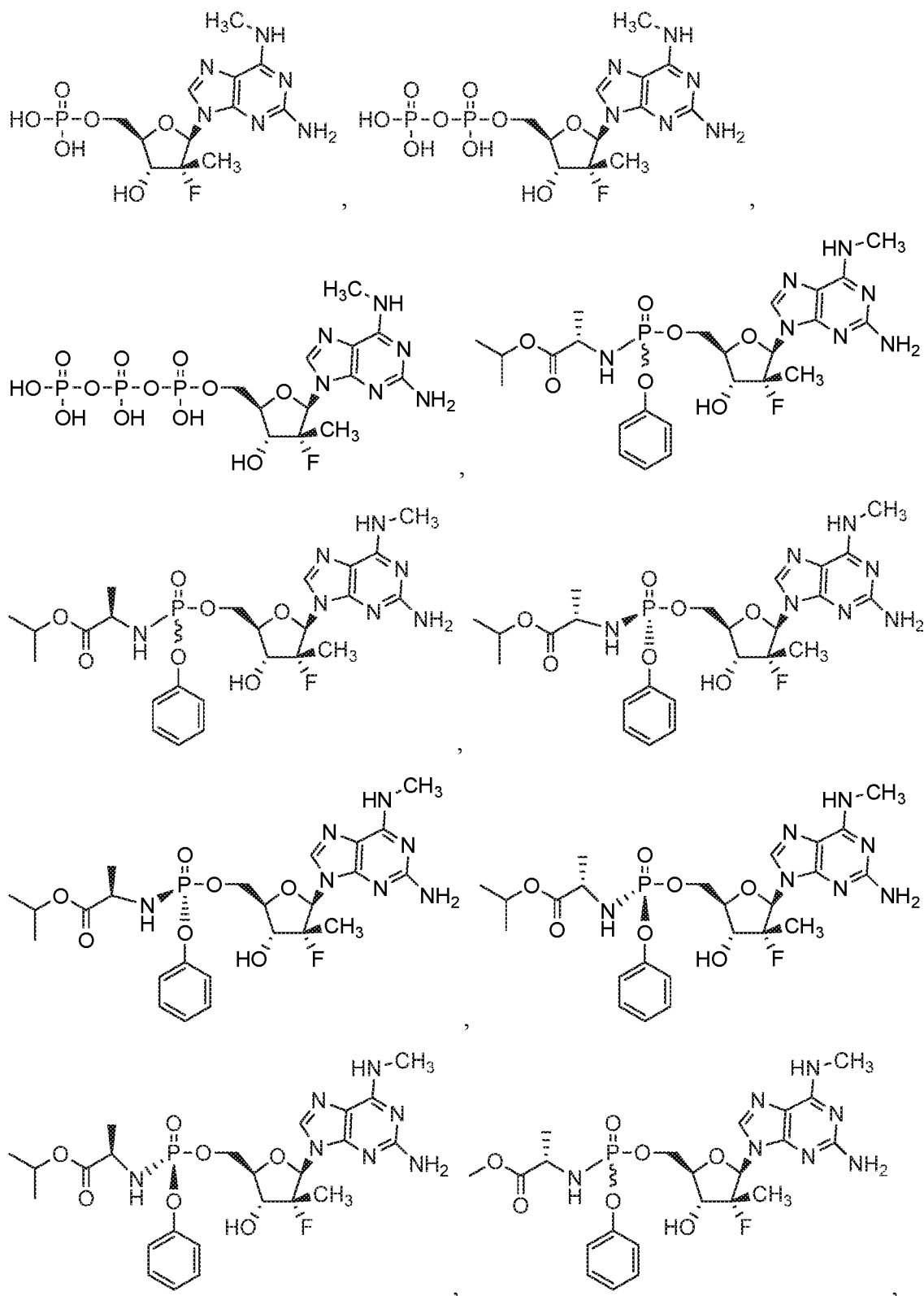
In alternative embodiments of compounds (i) through (lvi), an L-nucleoside is used in Formula I-VII.

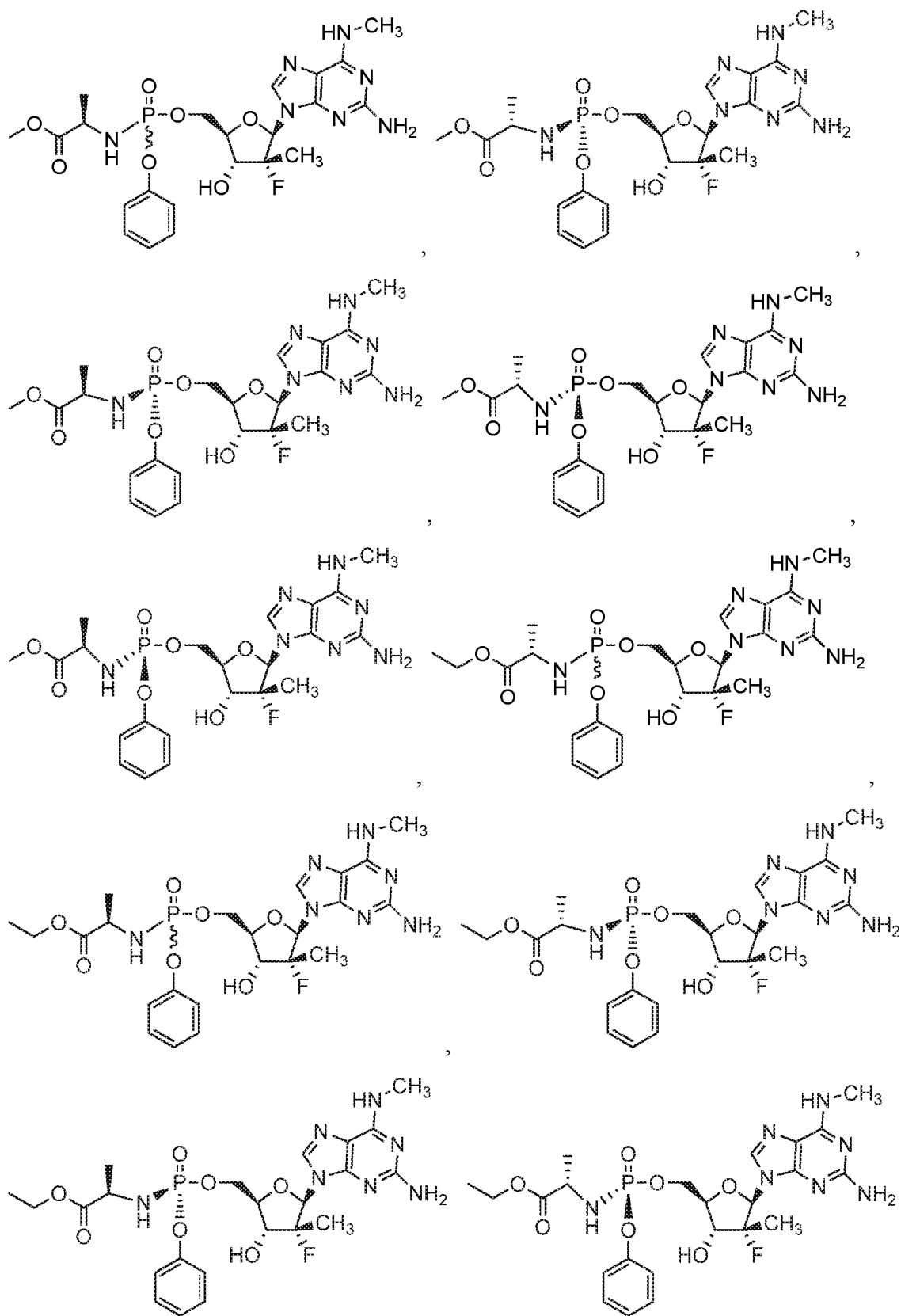
In an alternate embodiment, the Formula I R^{12} variable is CH_2F .

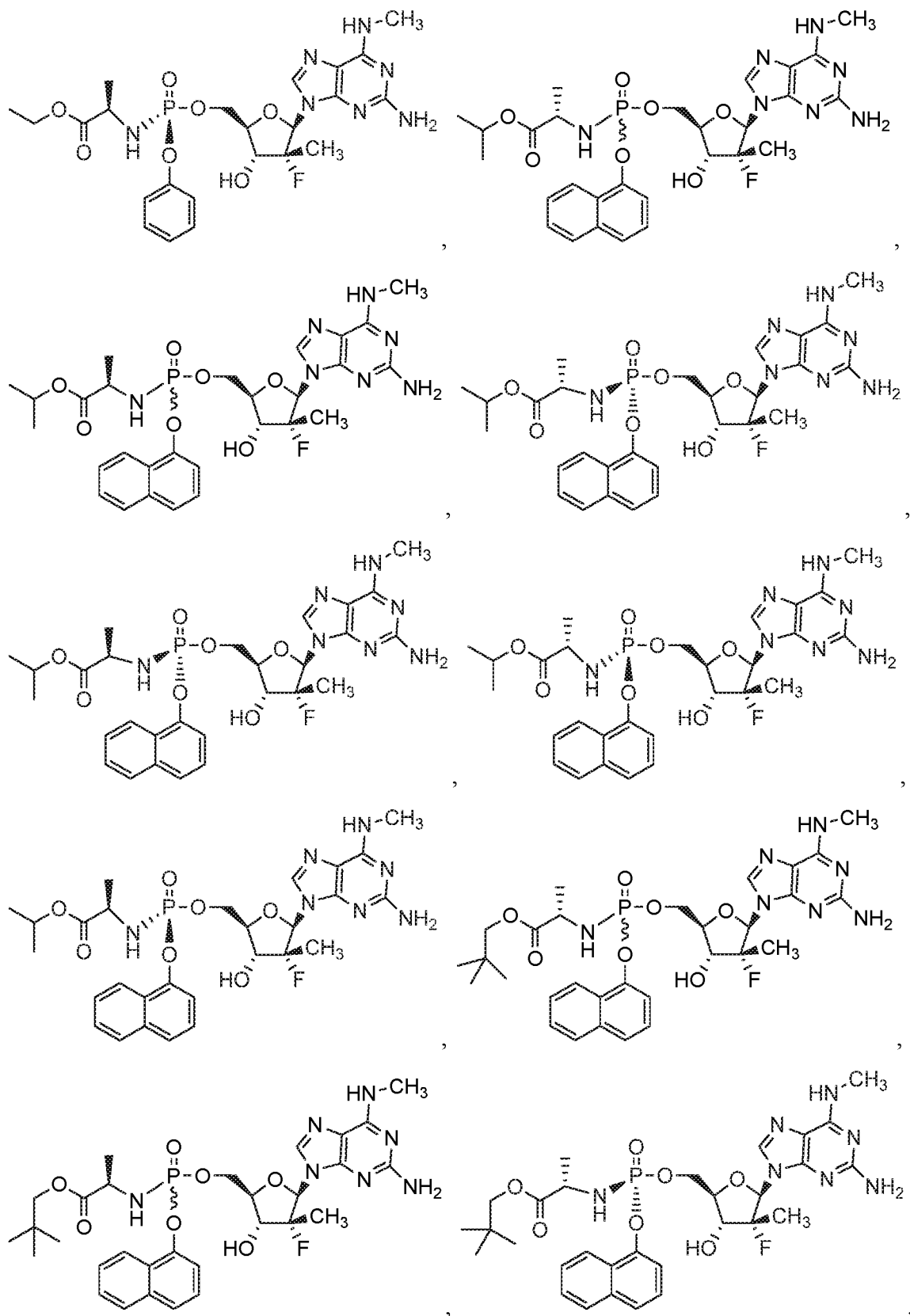
In an alternate embodiment, the Formula I R^{12} variable is CHF_2 .

In an alternate embodiment, the Formula I R^{12} variable is CF_3 .

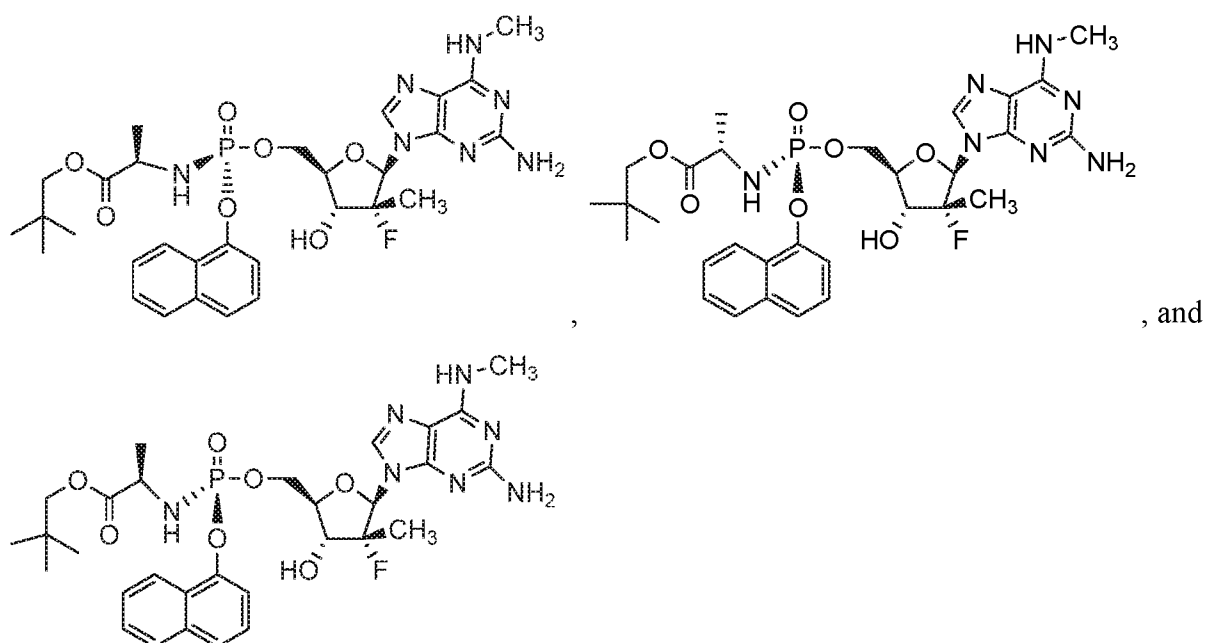
In one embodiment, a compound of Formula Ia is provided. Non-limiting examples of compounds of Formula Ia include:



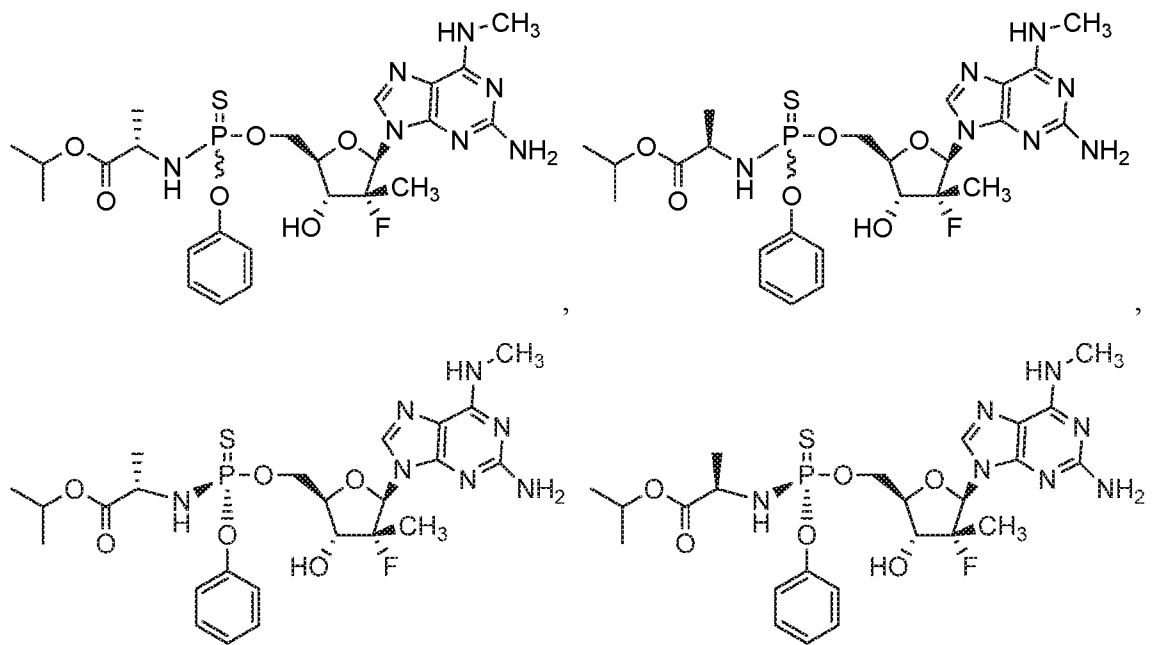


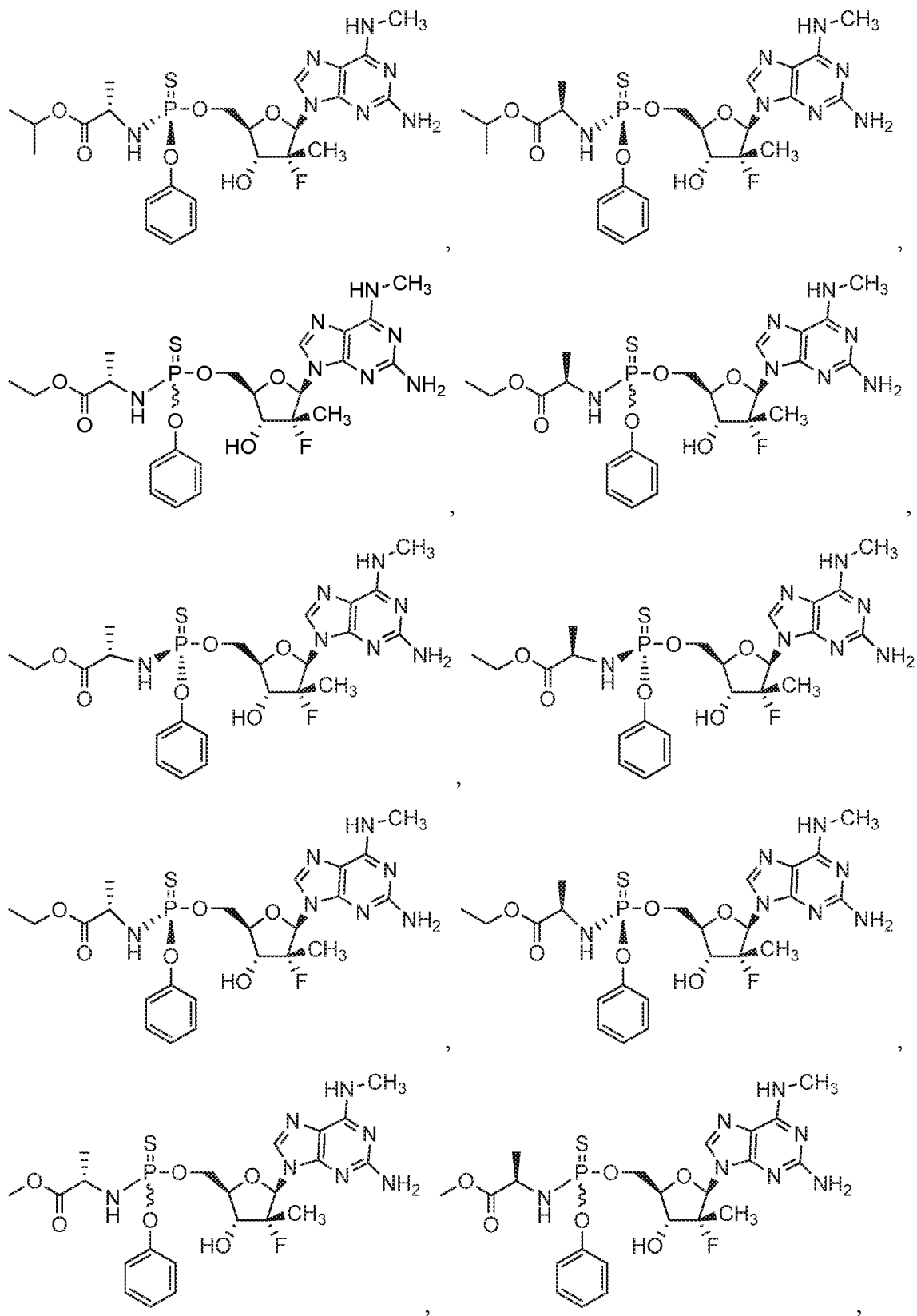


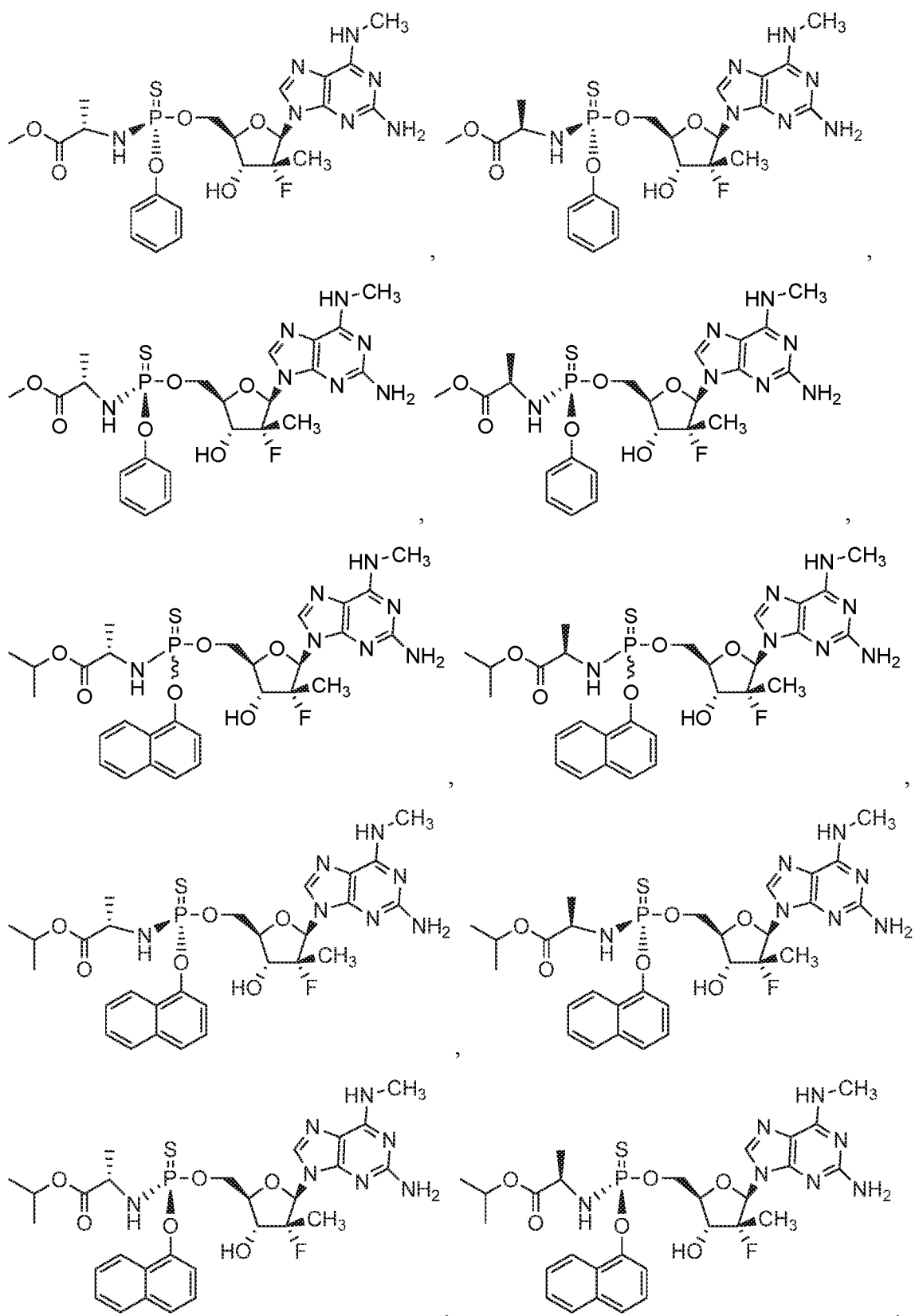
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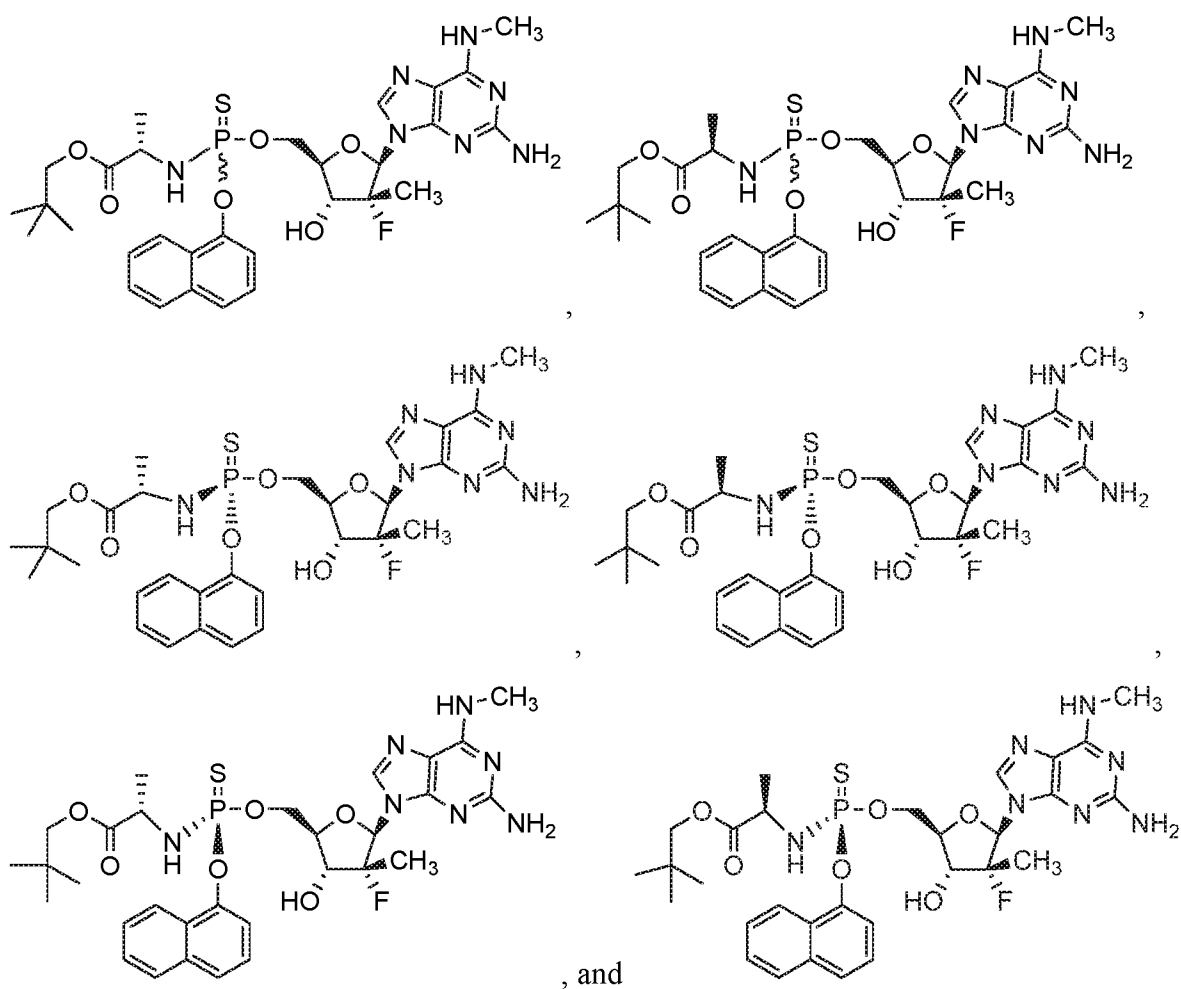


In one embodiment, a thiophosphoramidate of Formula Ia is provided. Non-limiting
 5 examples of thiophosphoramidates of Formula Ia include, but are not limited to:

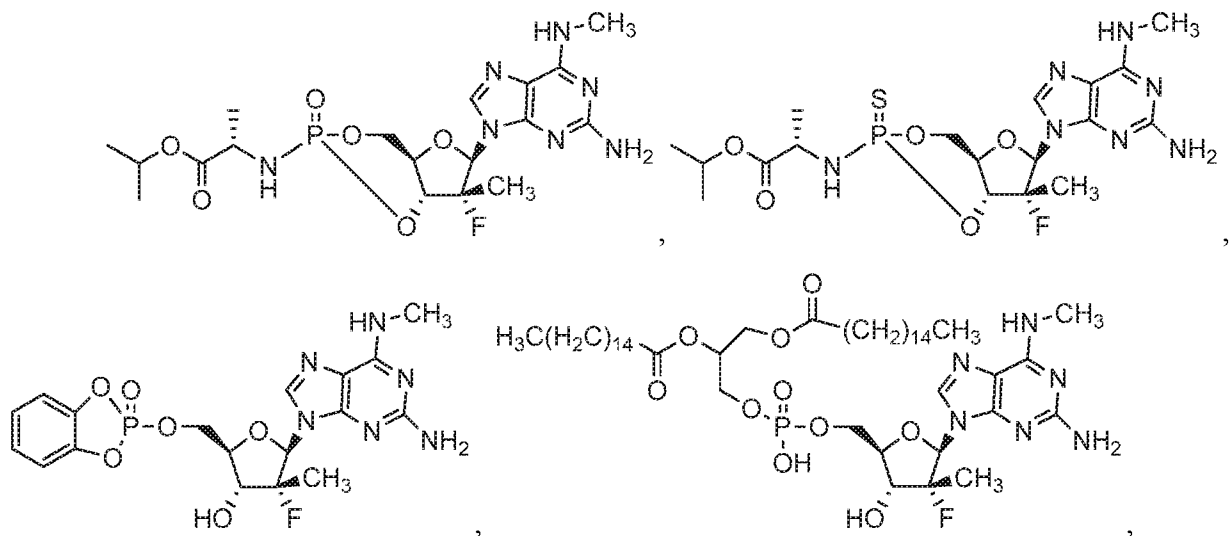


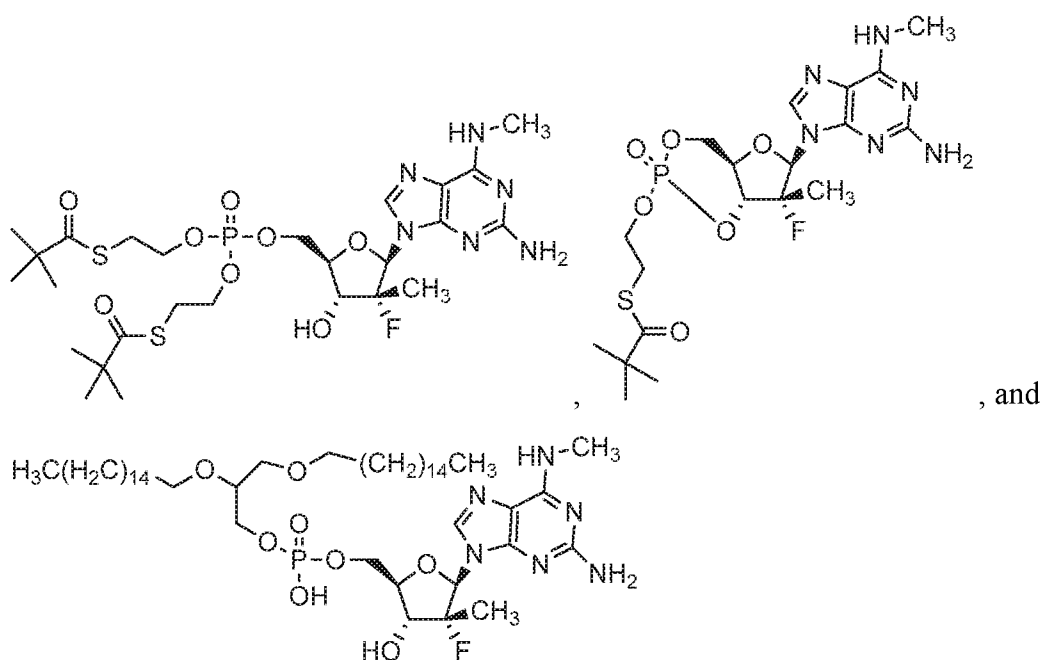






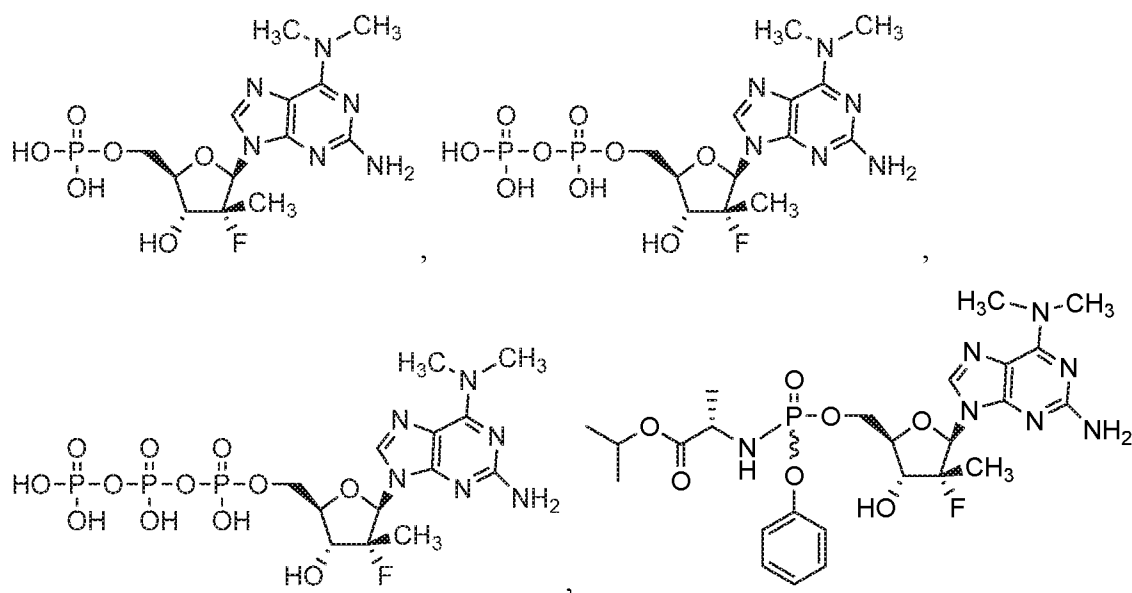
- 5 In one embodiment, a stabilized phosphate prodrug of Formula Ia is provided. Non-limiting examples of stabilized phosphate prodrugs of Formula Ia are illustrated below:

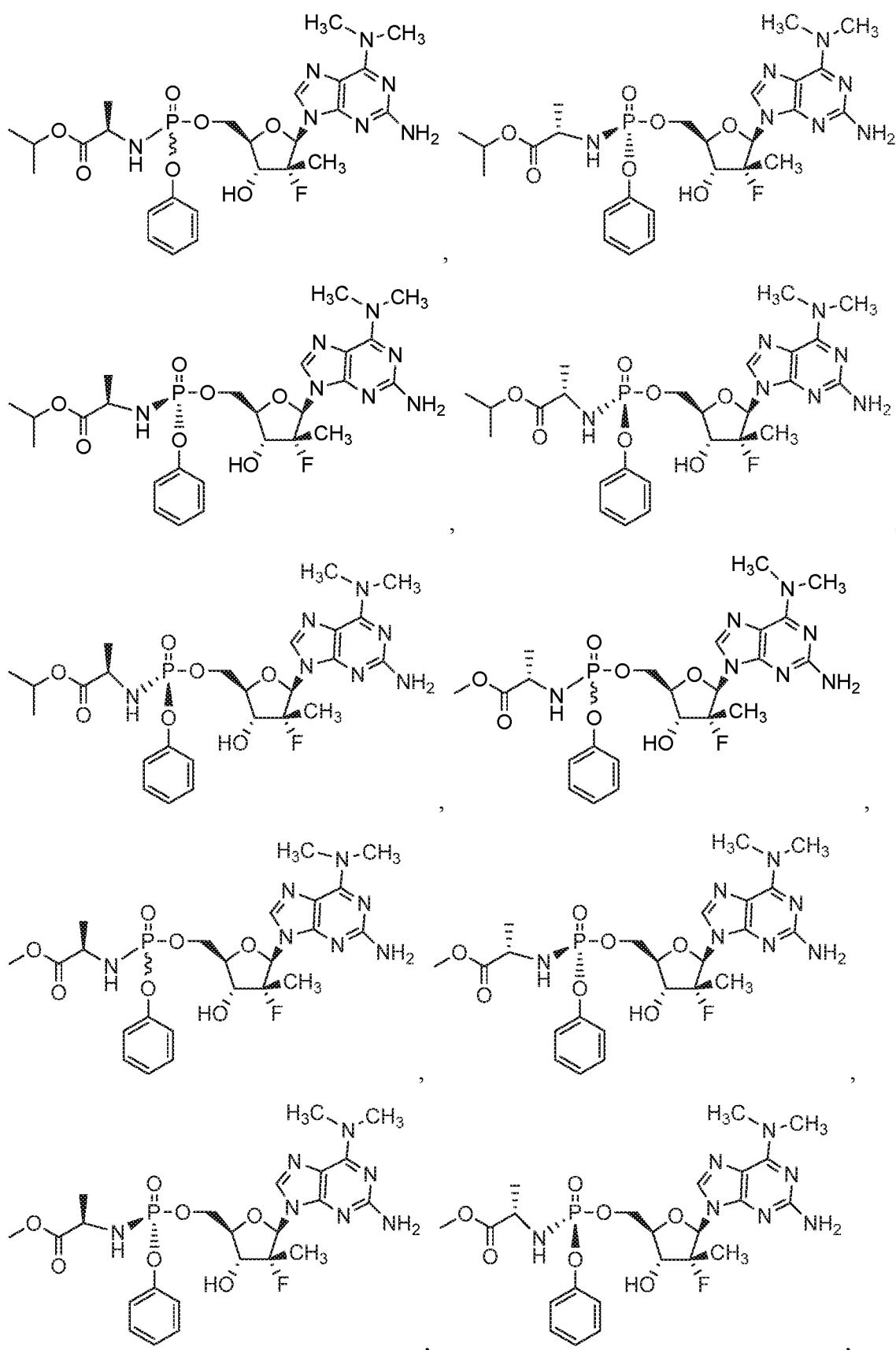




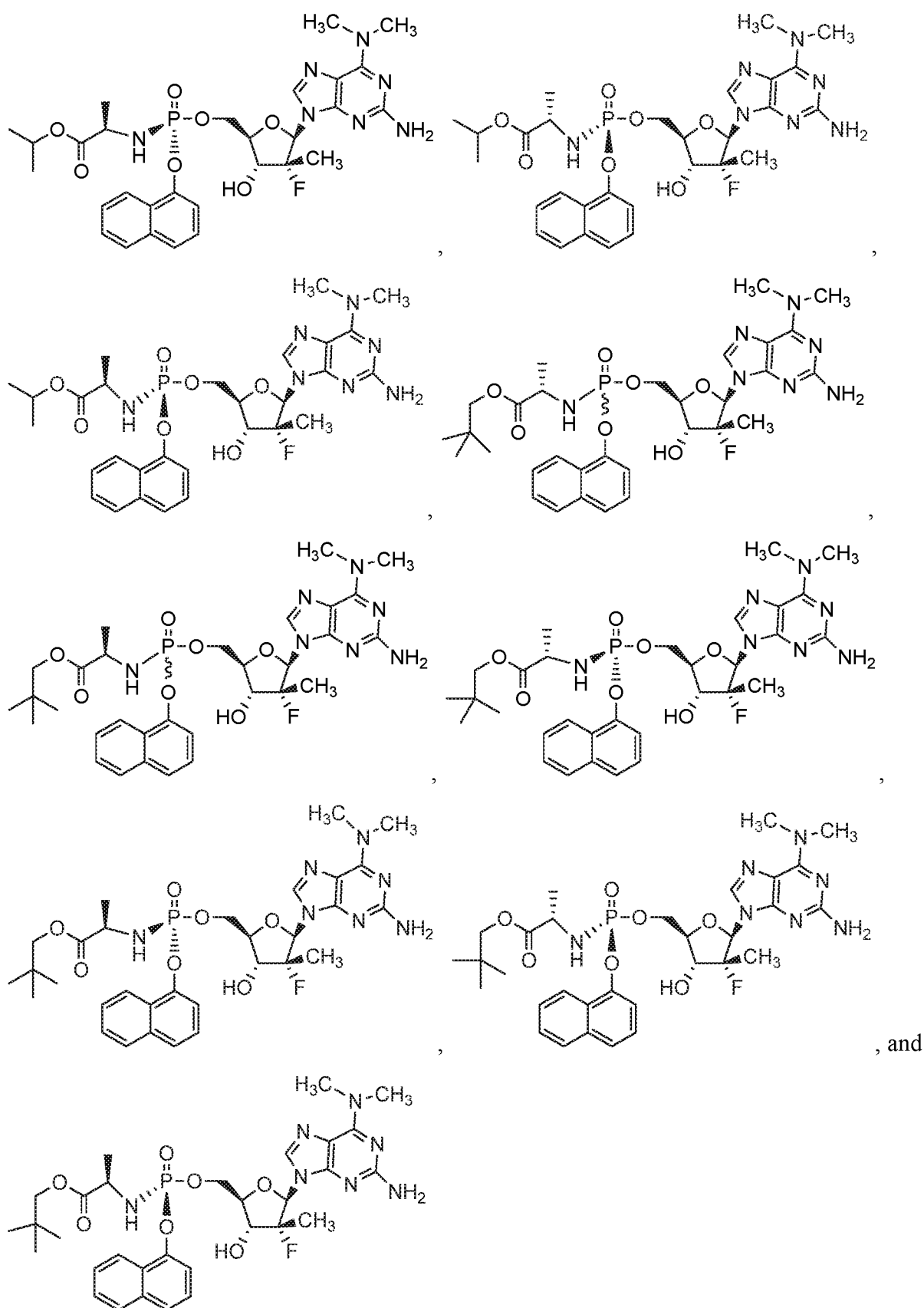
In another embodiment, a compound of Formula Ia is provided. Non-limiting examples of compounds of Formula Ia include:

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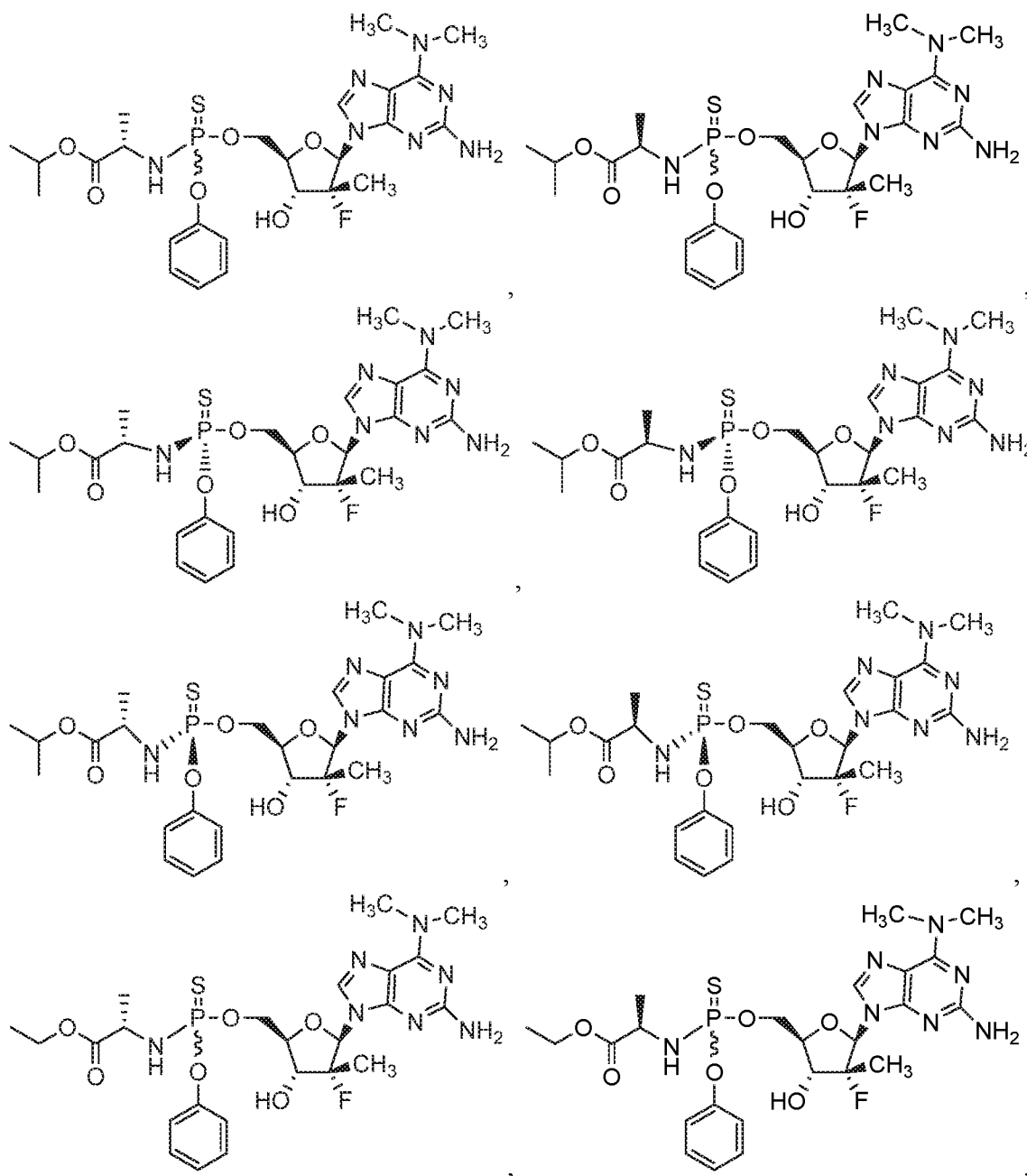


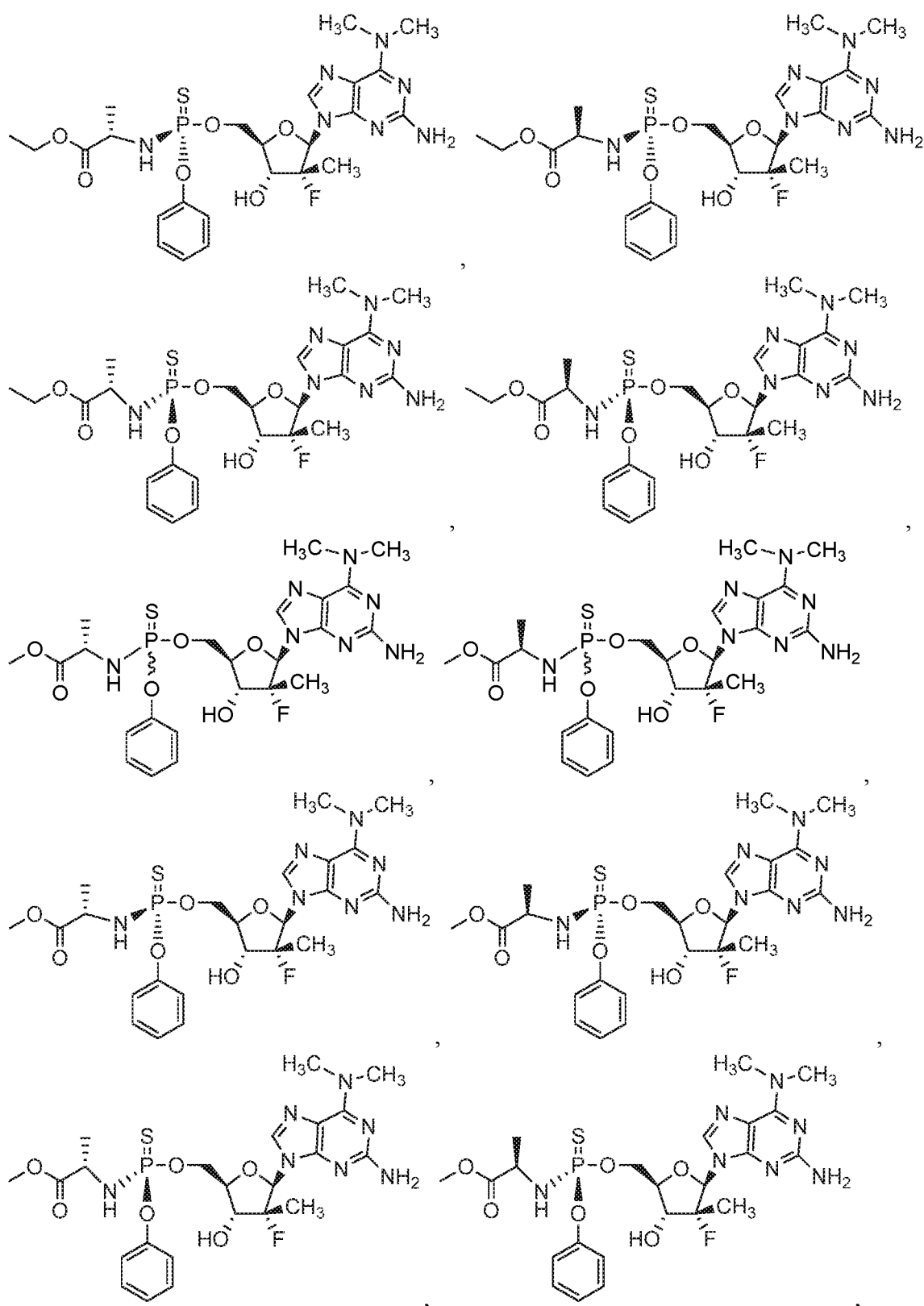


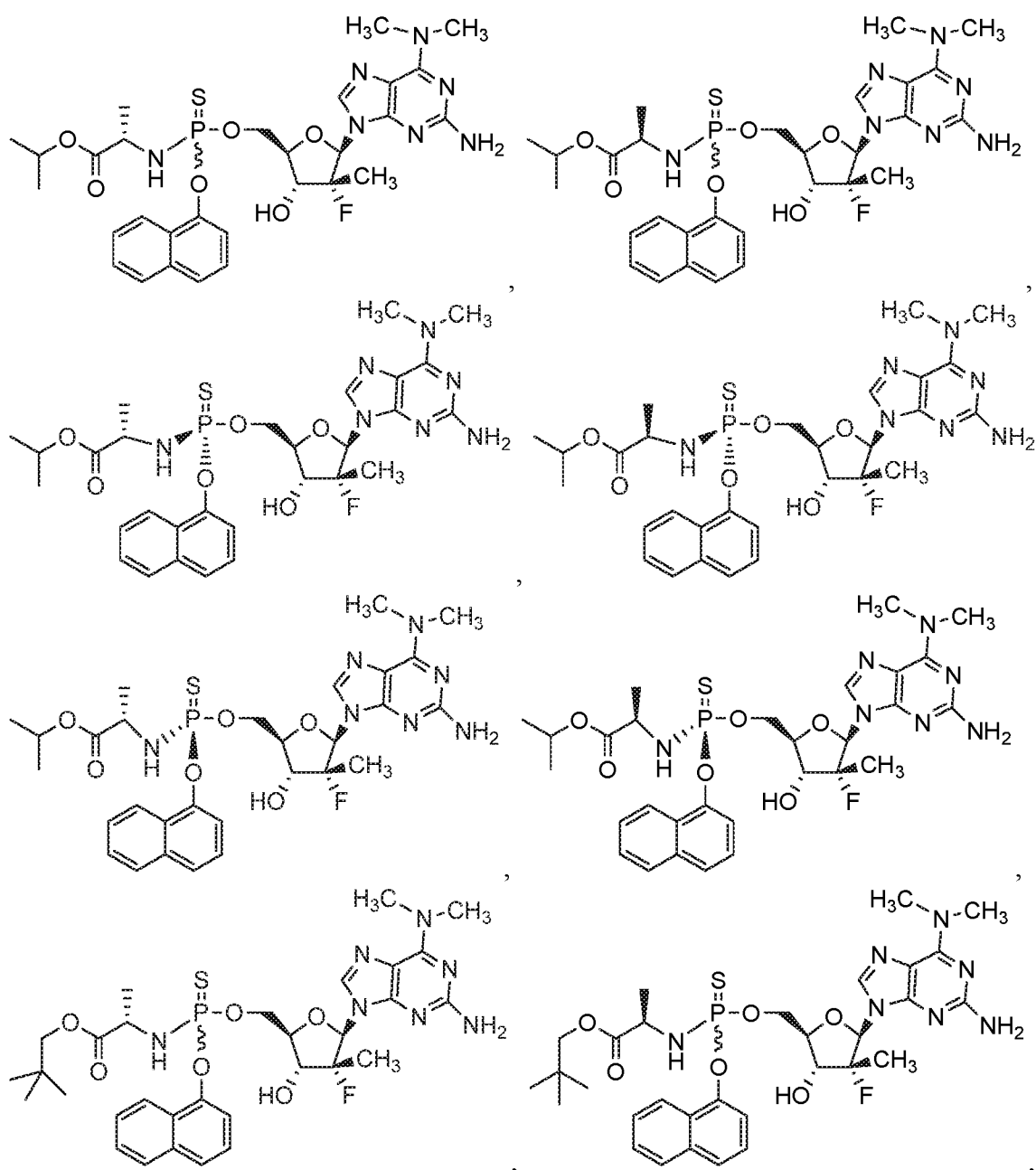


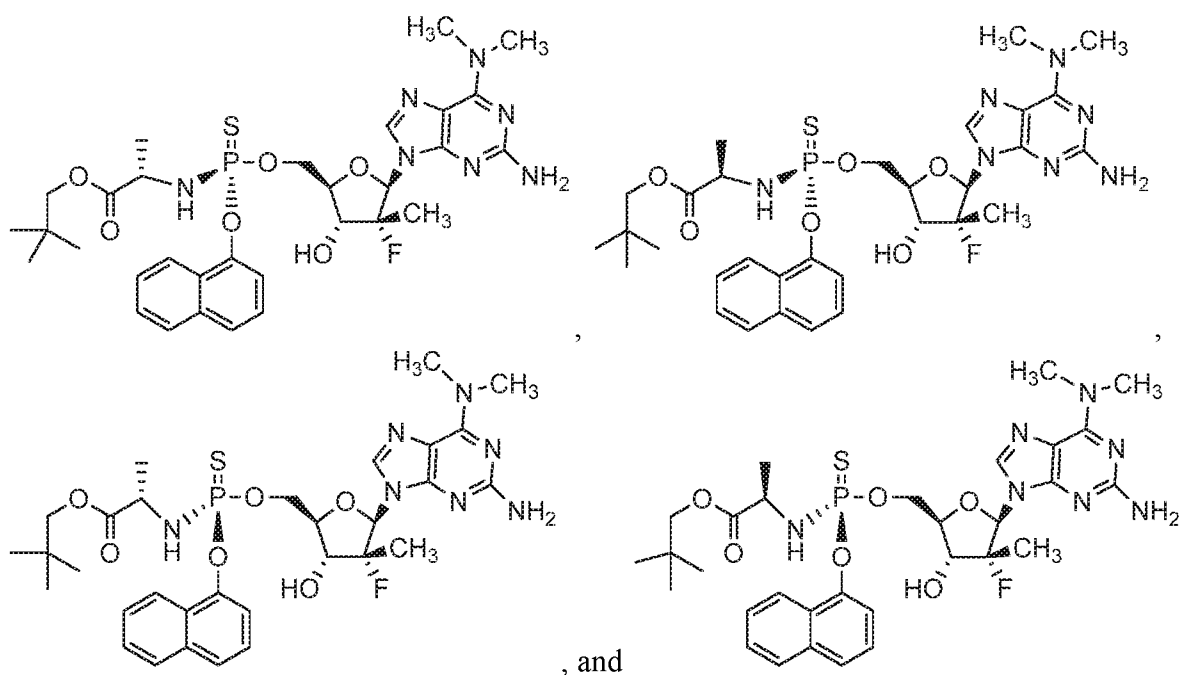


In one embodiment, a thiophosphoramidate of Formula Ia is provided. Non-limiting examples of thiophosphoramidates of Formula Ia include, but are not limited to:

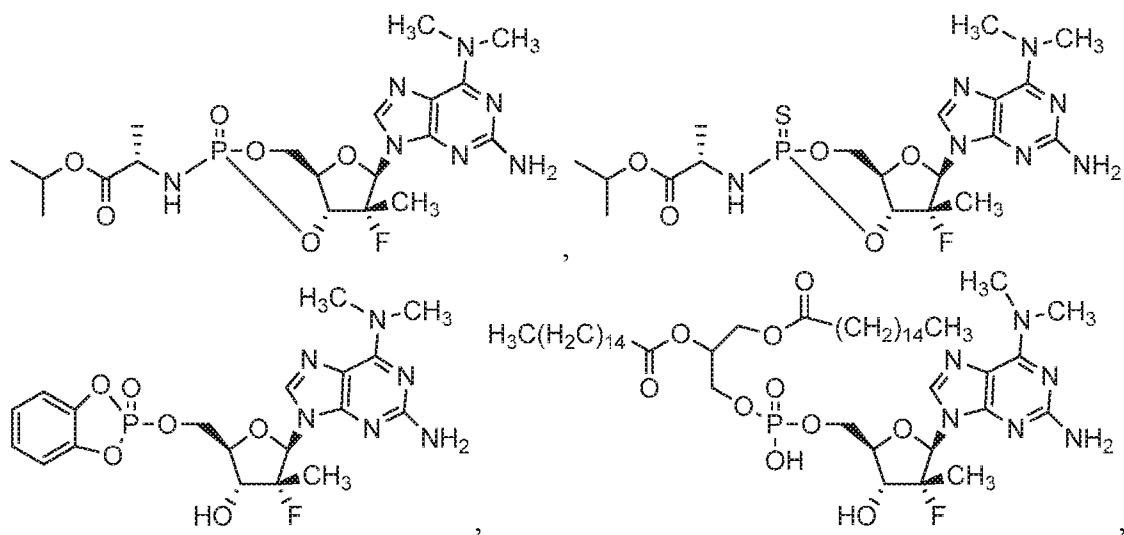


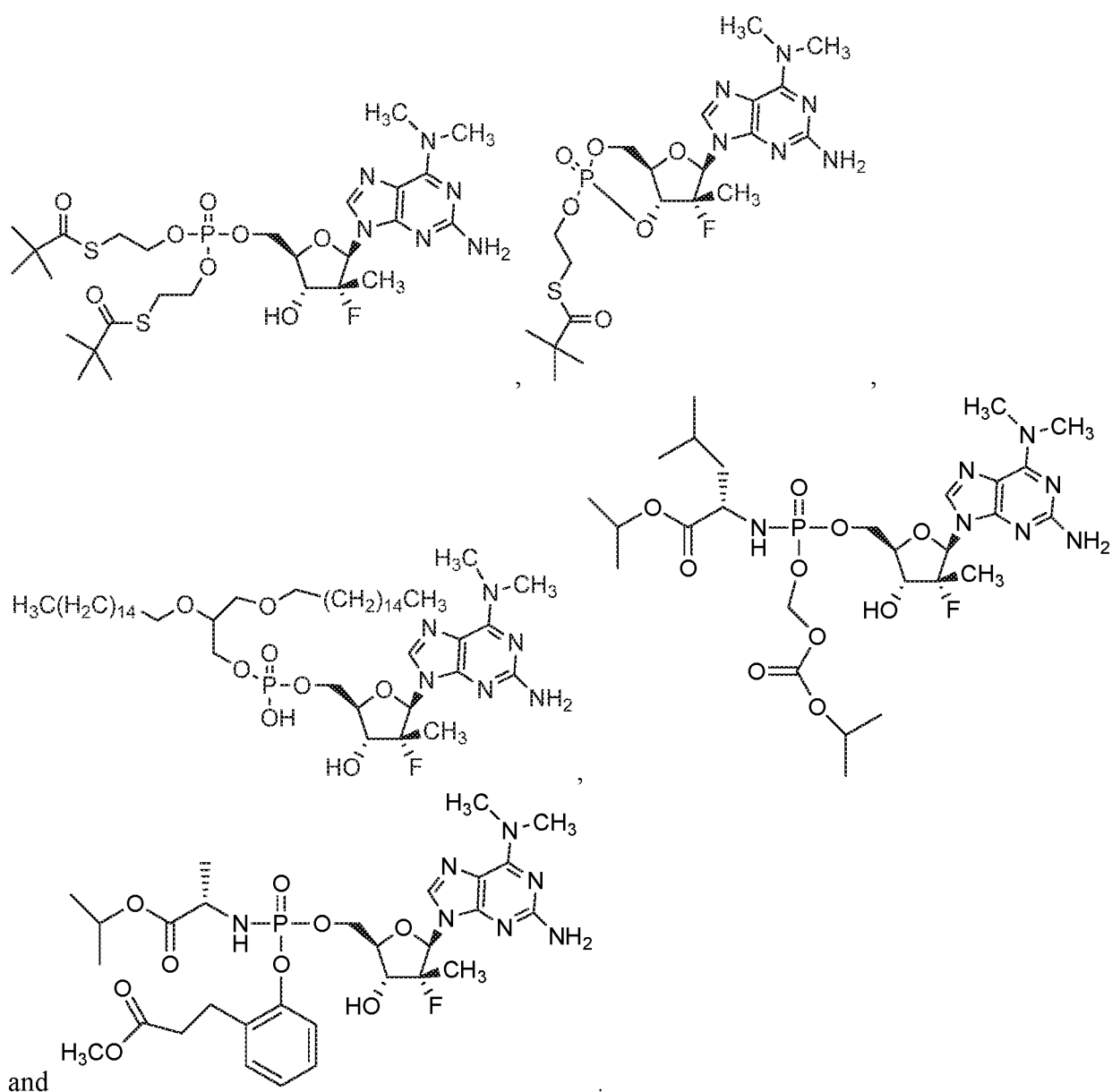




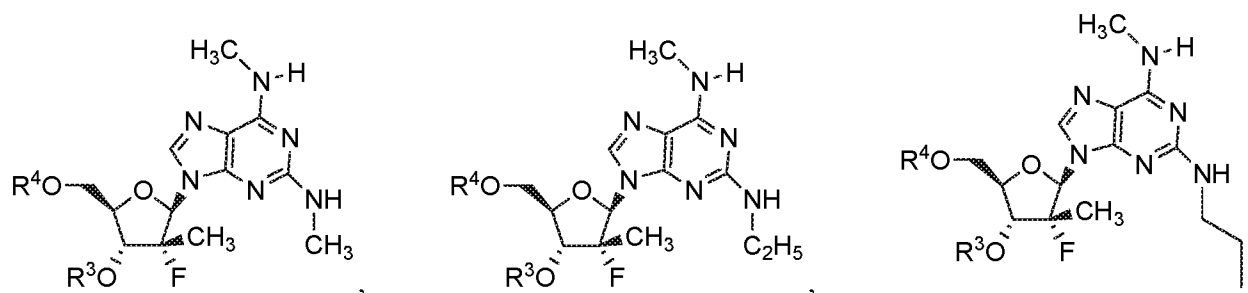


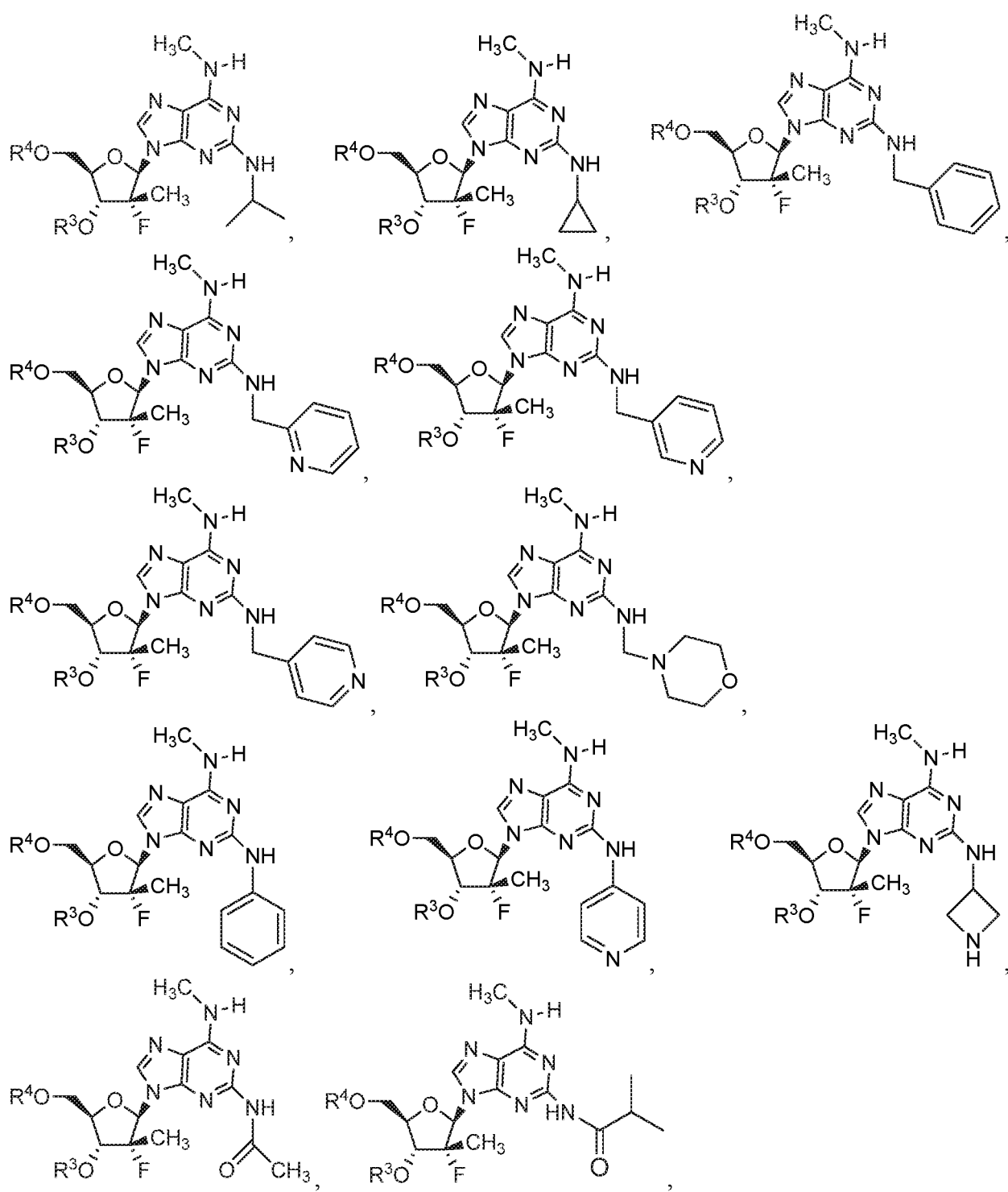
In one embodiment, a stabilized phosphate prodrug of Formula Ia is provided. Non-limiting examples of stabilized phosphate prodrugs of Formula Ia are illustrated below:



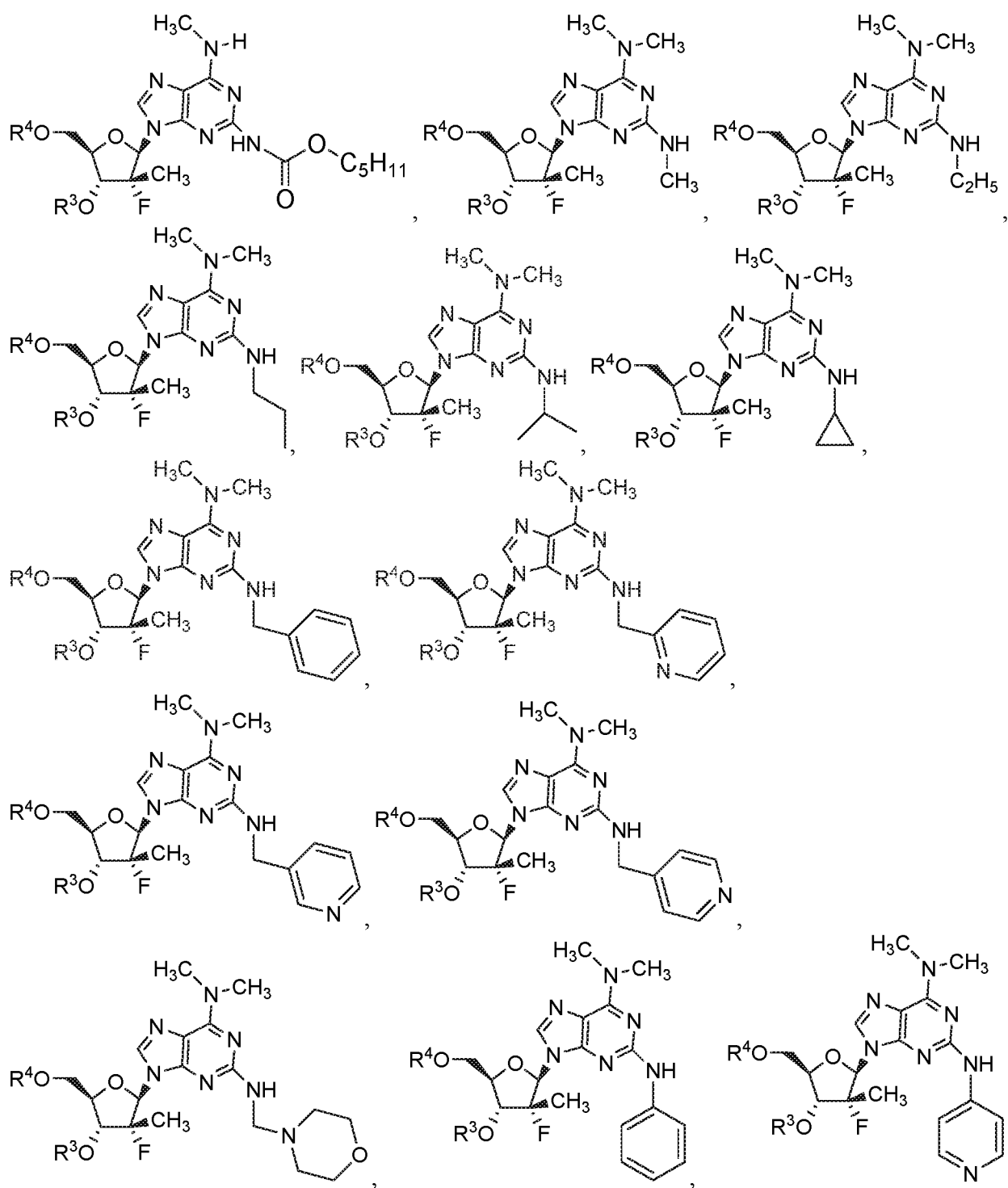


- 5 In one embodiment, a compound of Formula II is provided. Non-limiting examples of compounds of Formula II include:

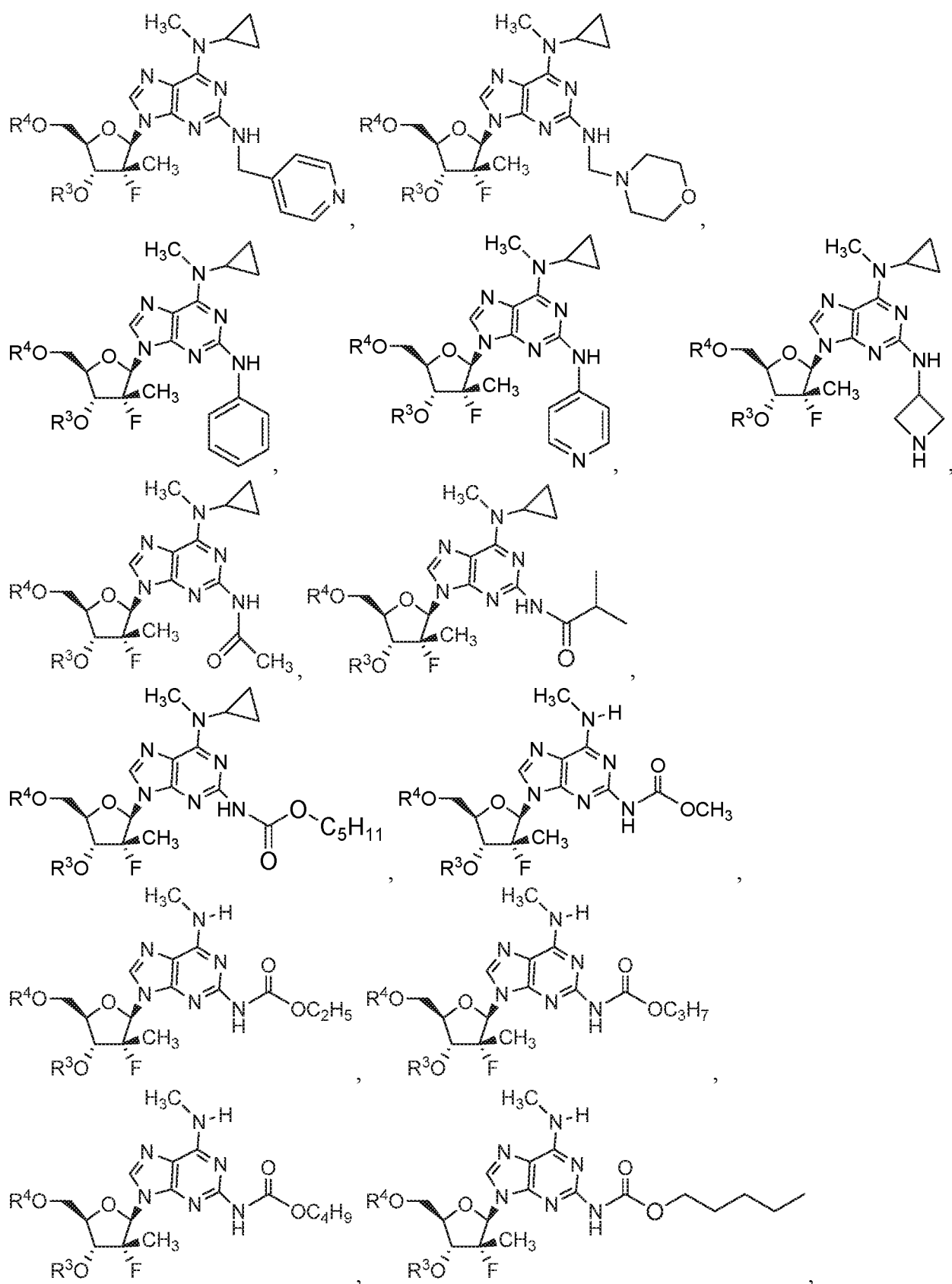




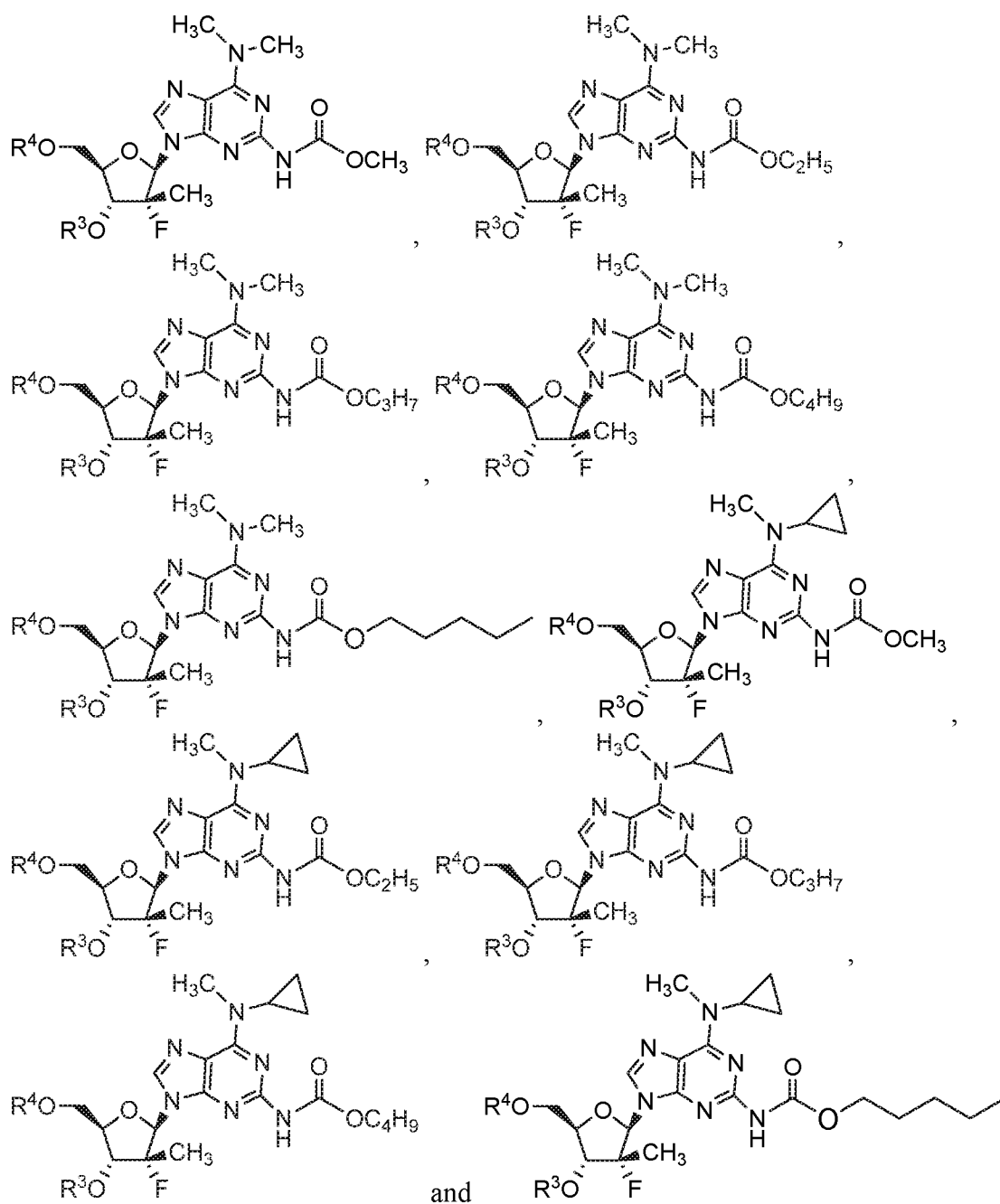
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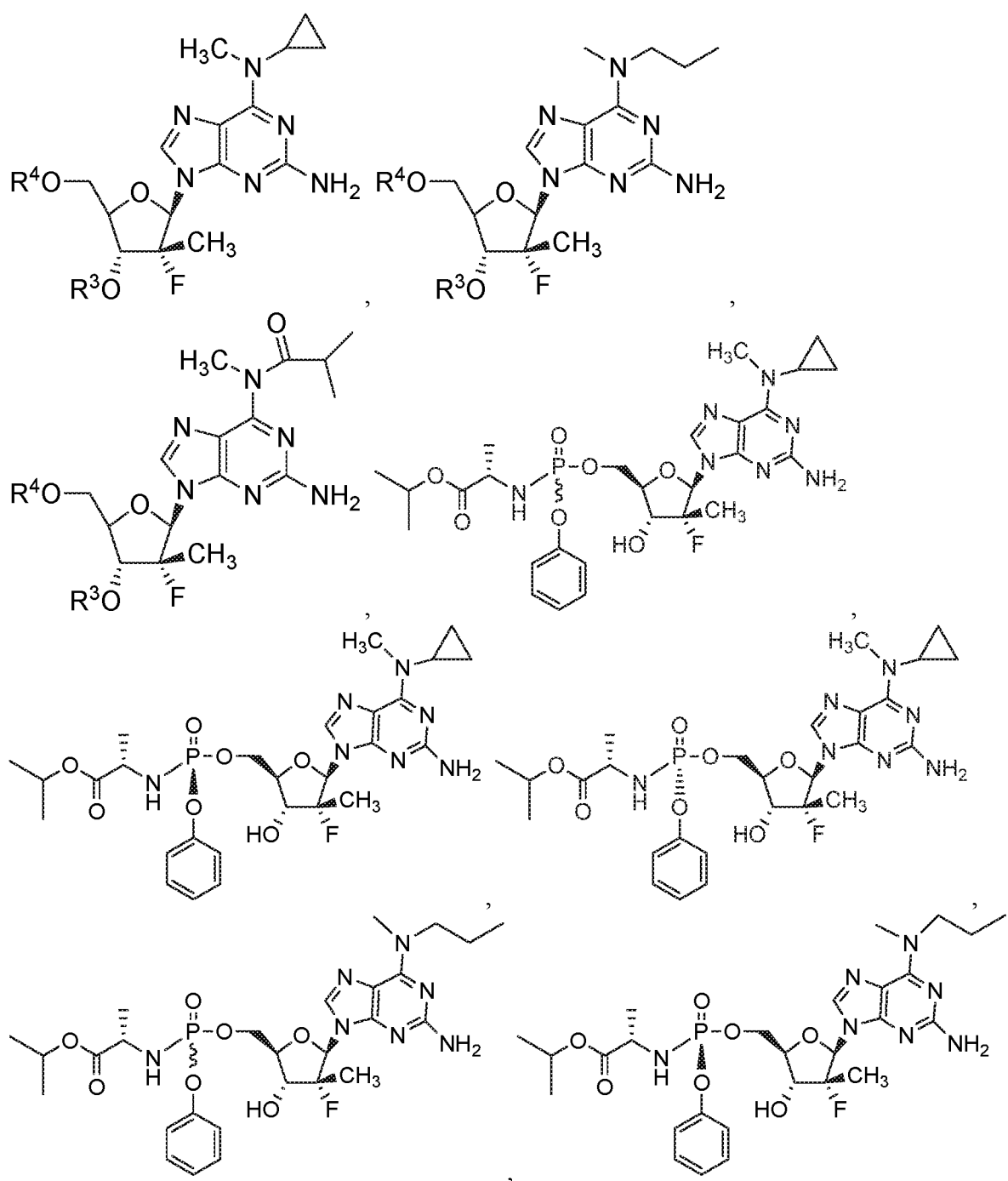


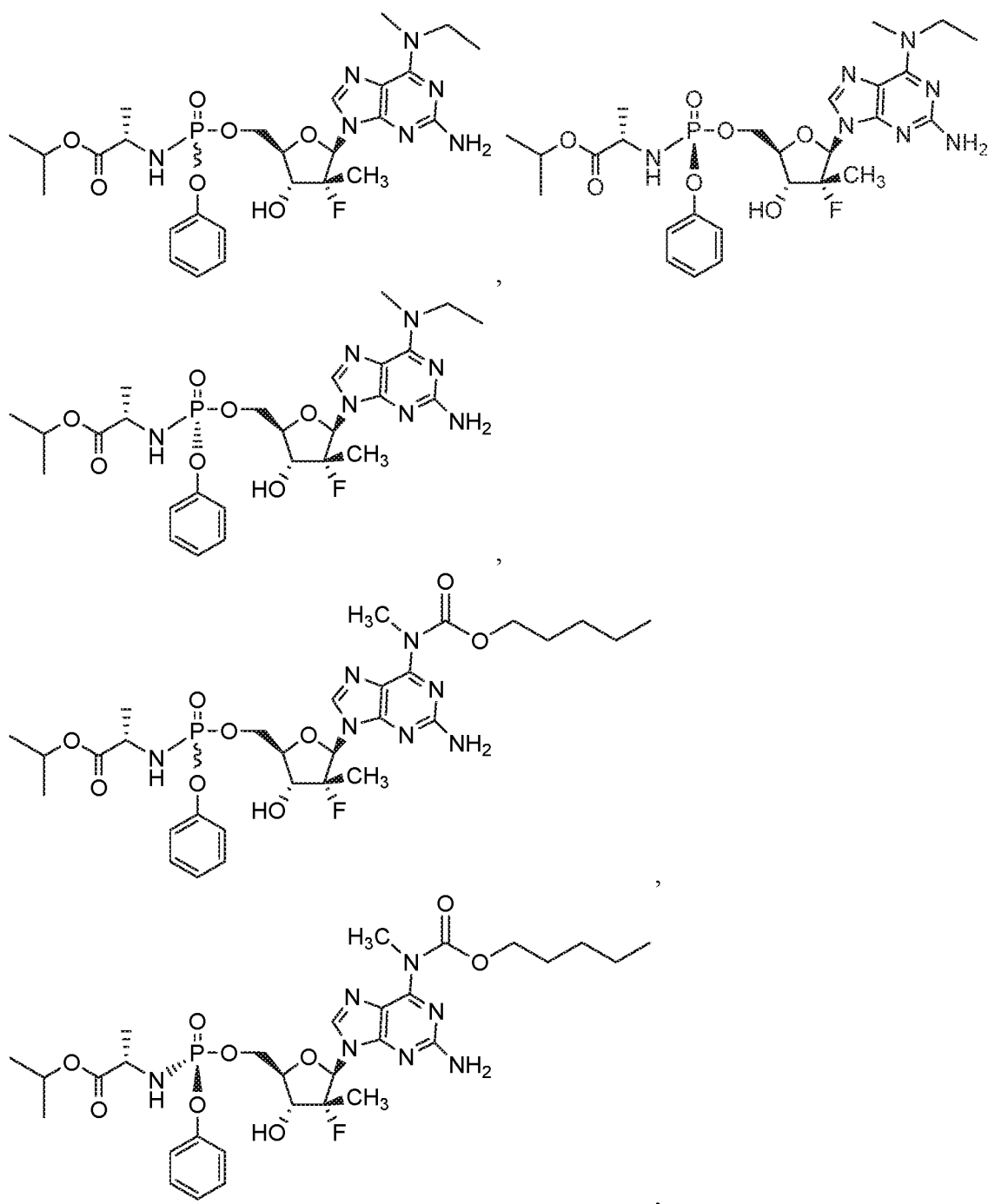


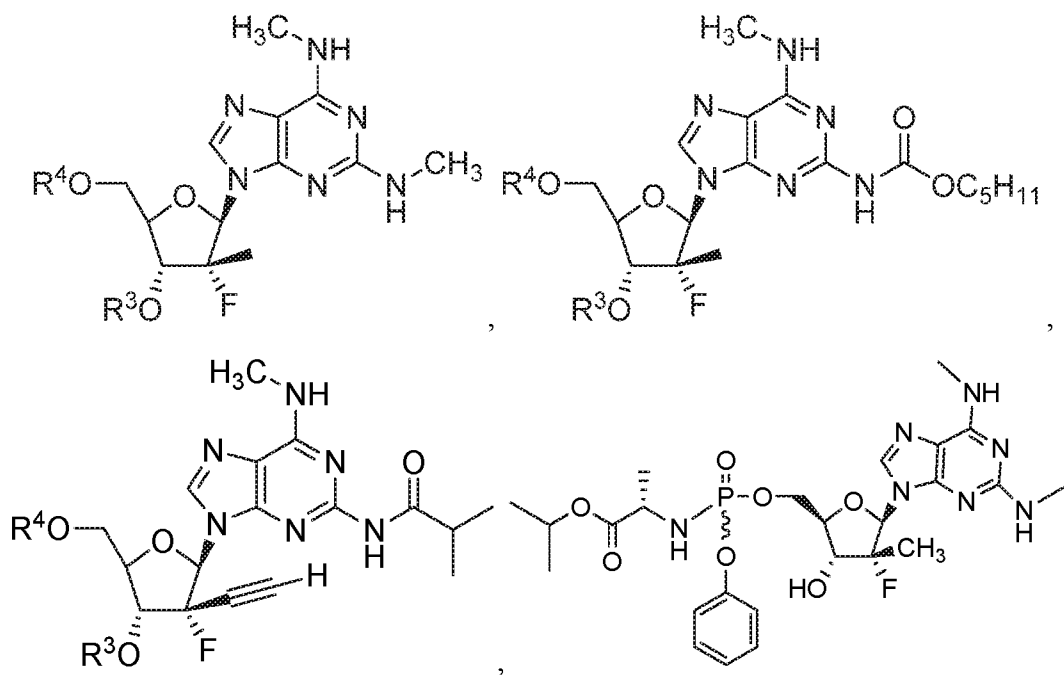
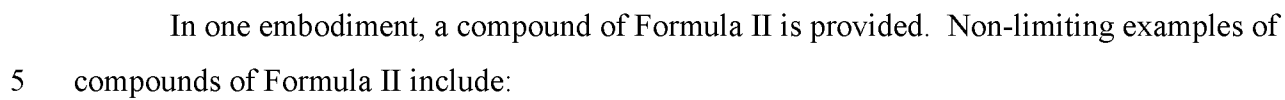
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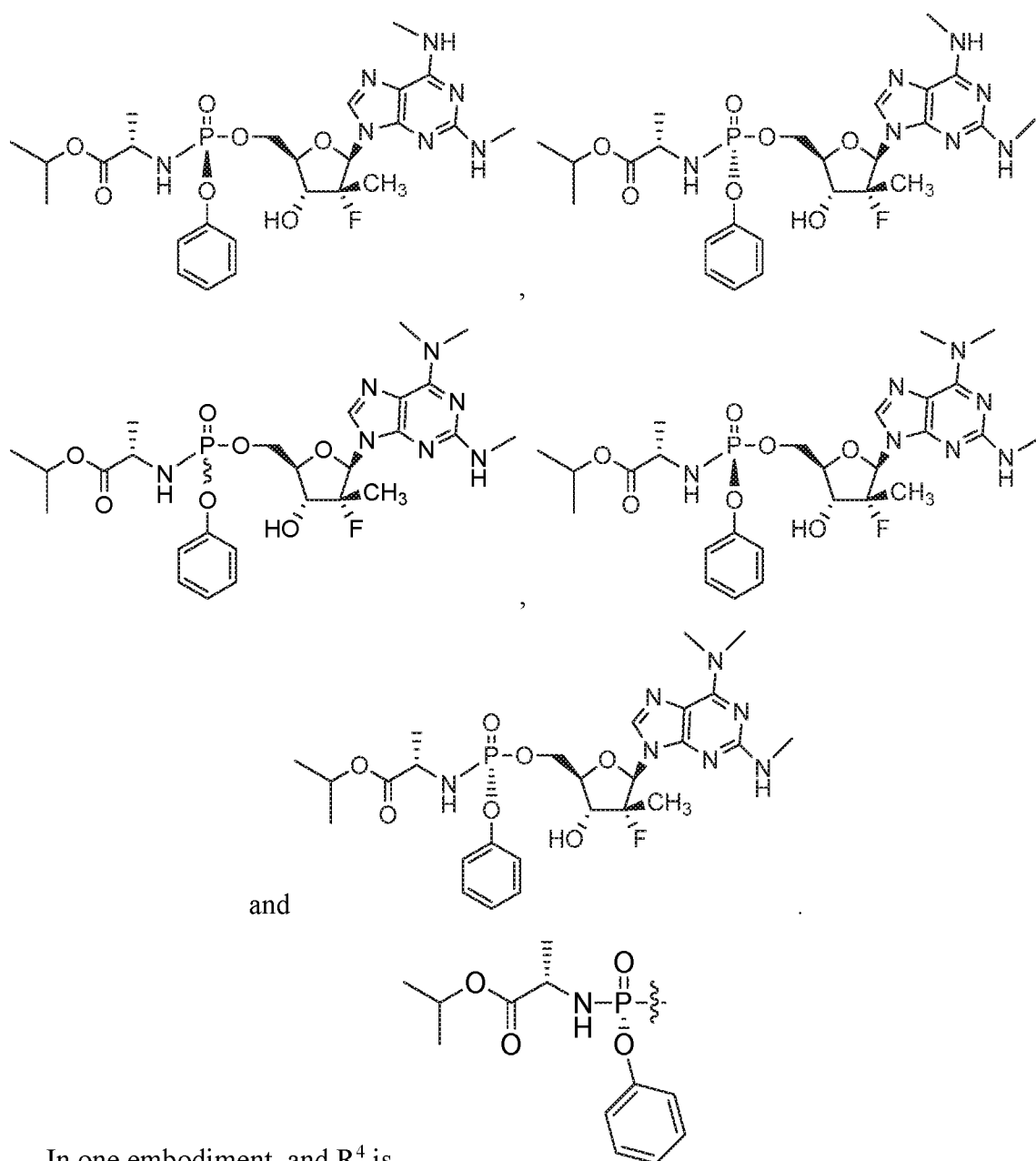


In one embodiment, a compound of Formula I is provided. Non-limiting examples of compounds of Formula I include:



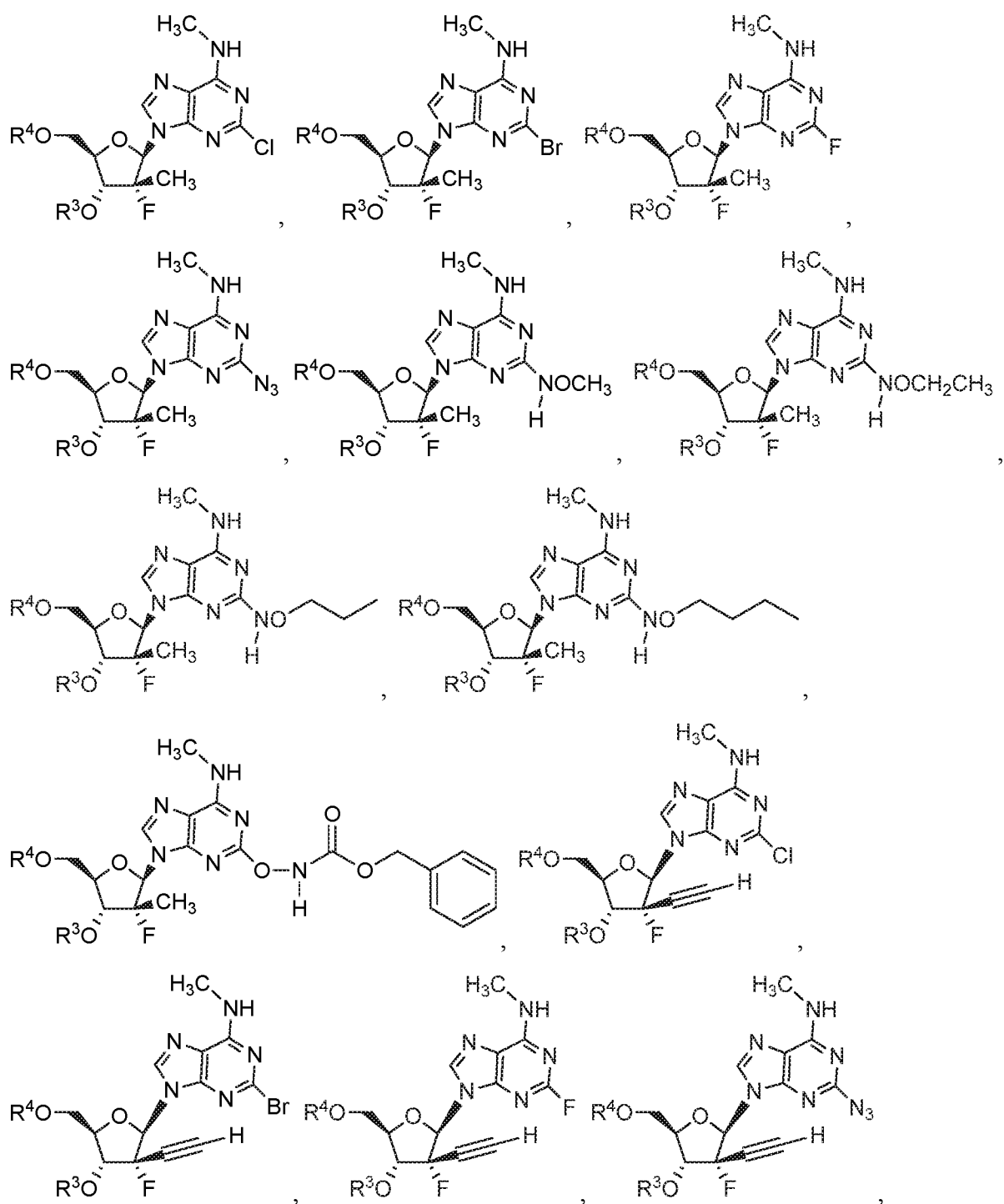






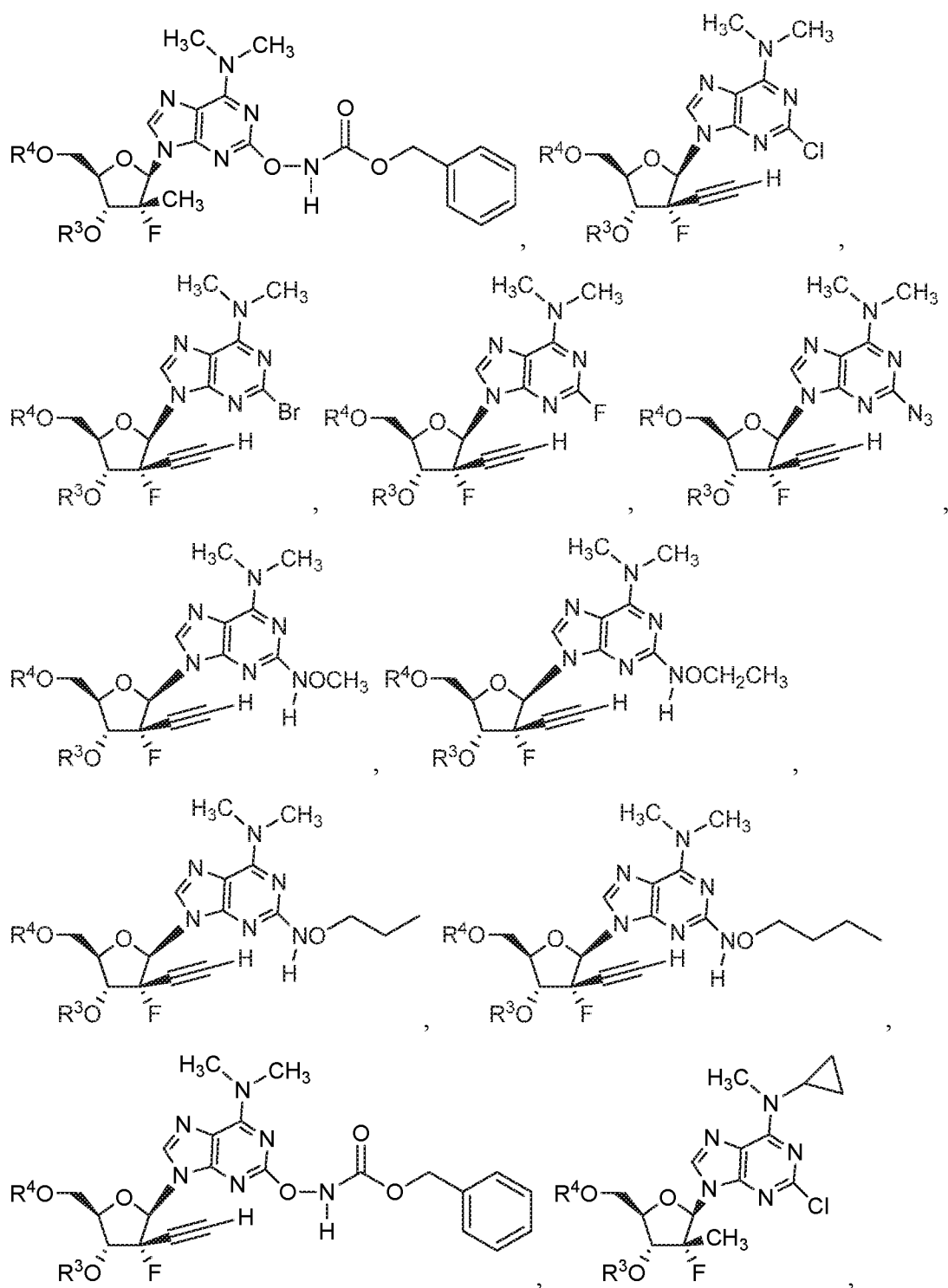
In one embodiment, and R^4 is

- 5 In one embodiment, a compound of Formula II is provided. Non-limiting examples of compounds of Formula II include:

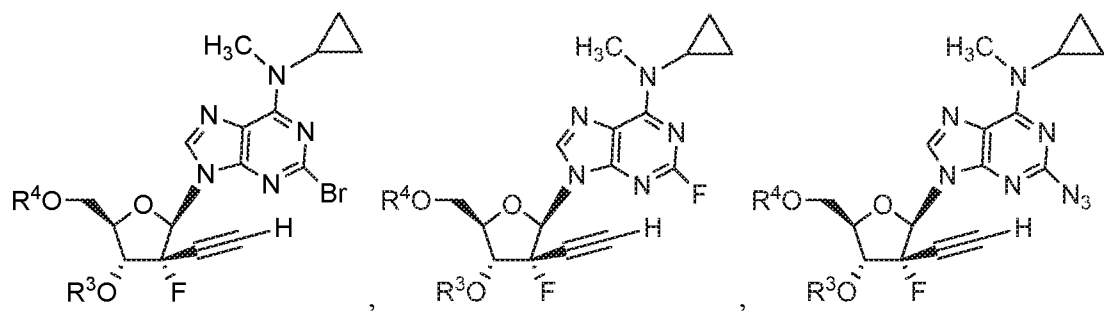
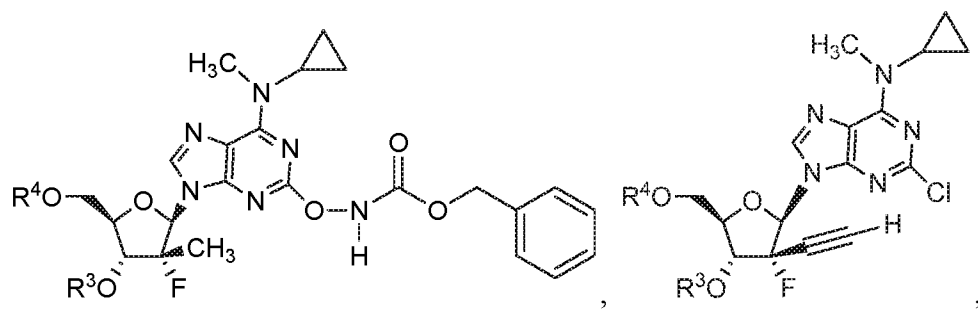
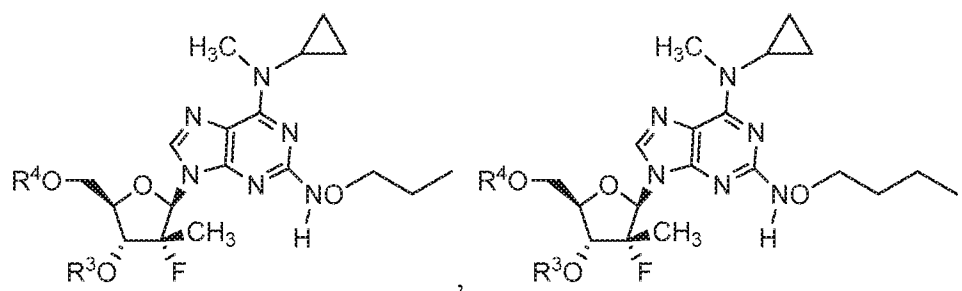
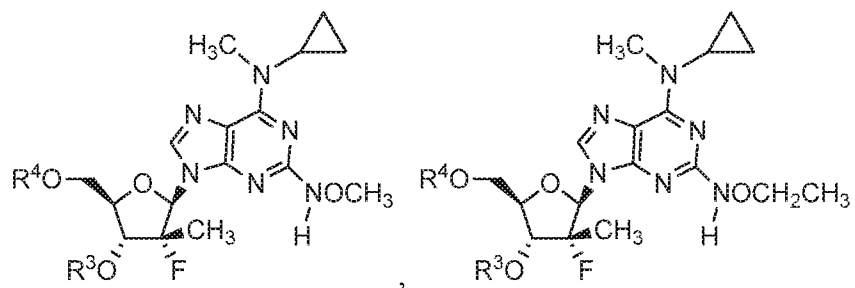
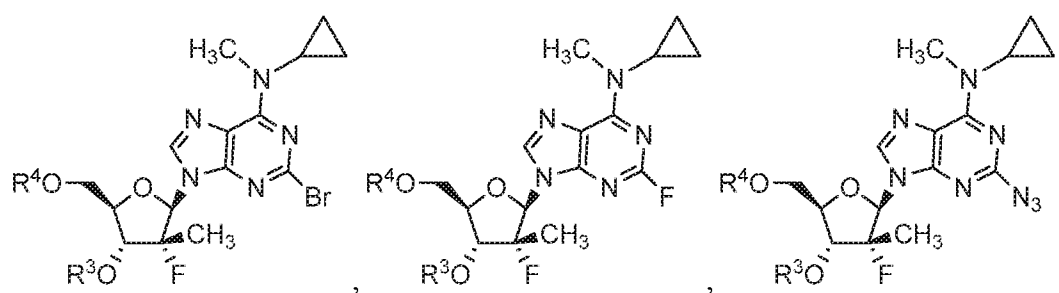


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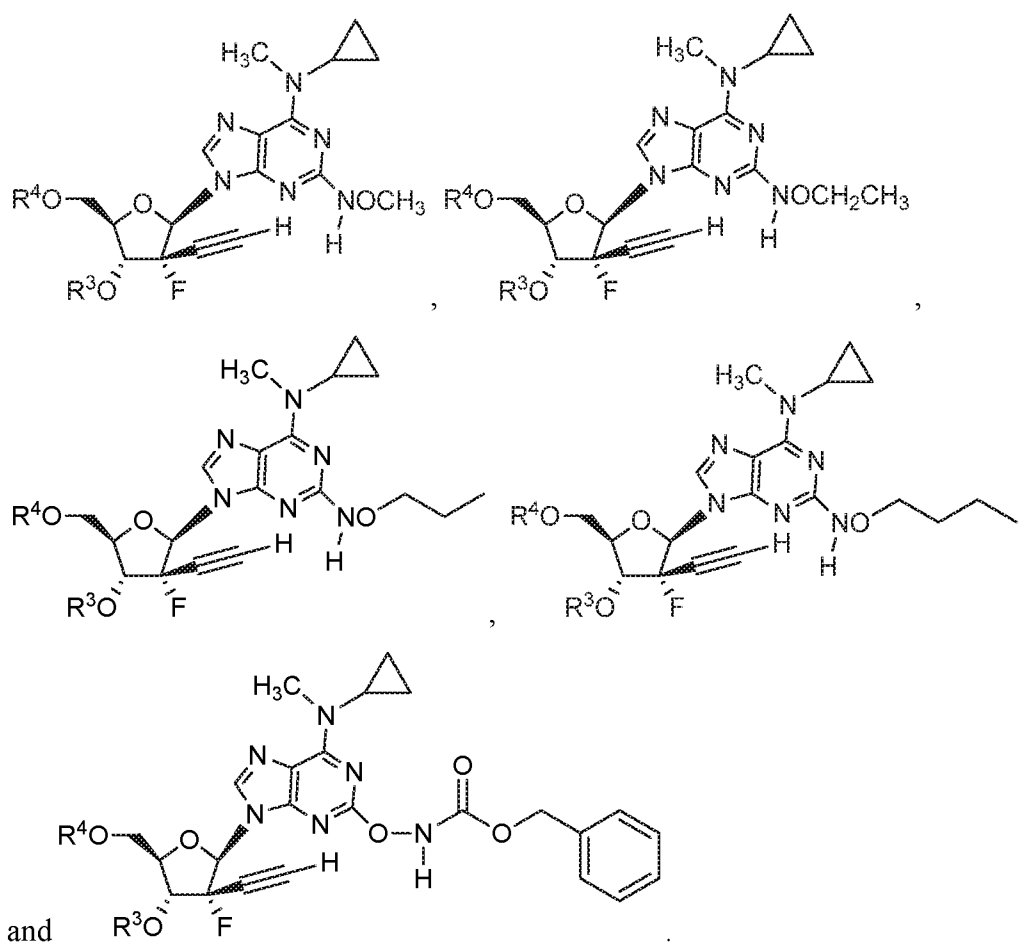




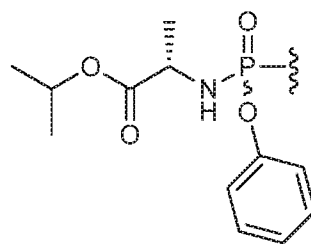
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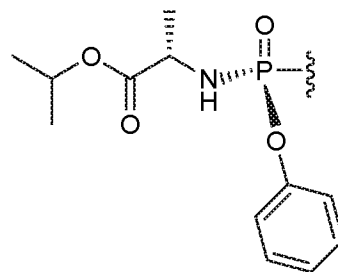
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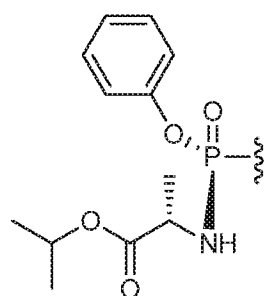


5 In some embodiments, R³ is H and R⁴ is



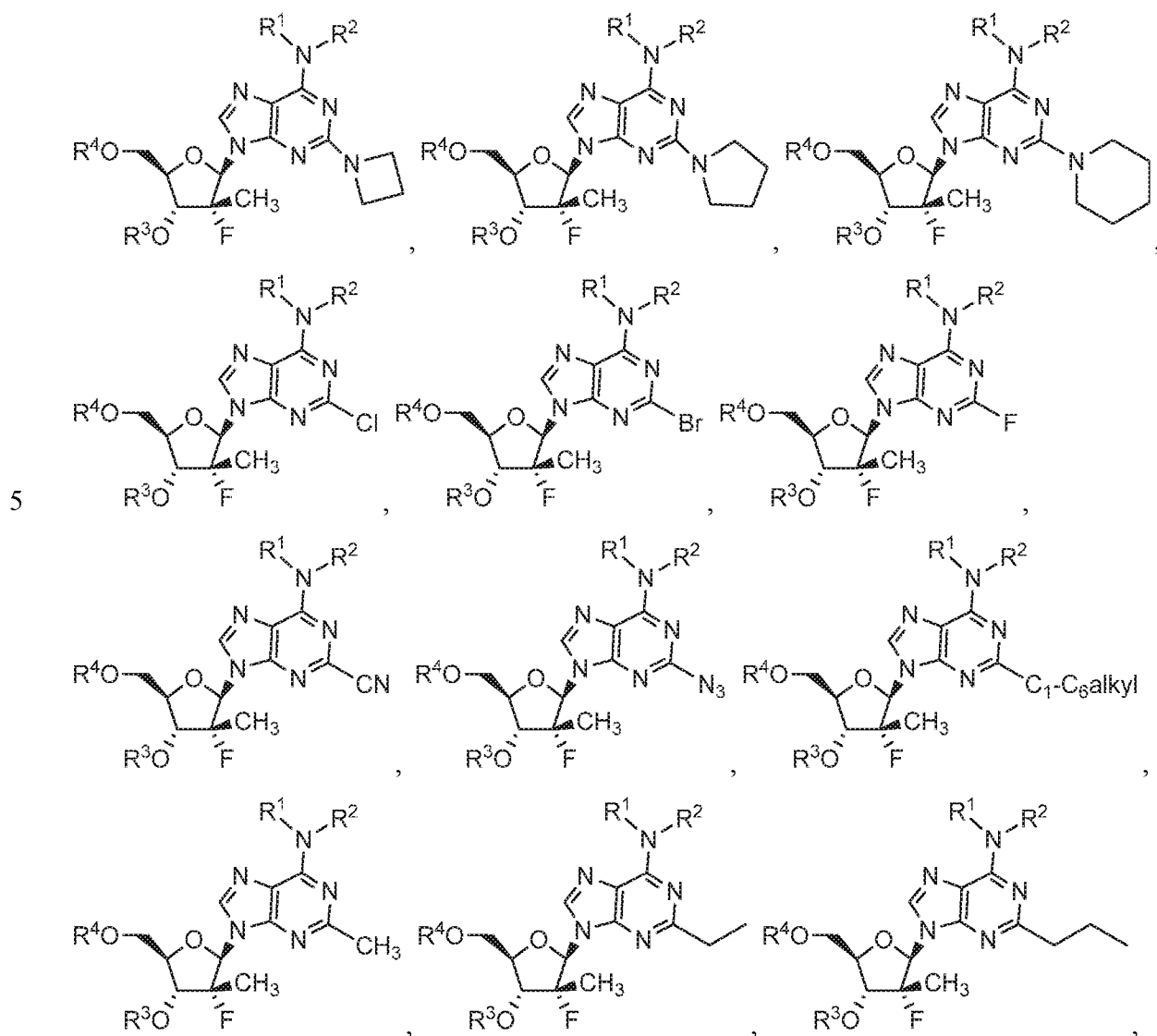
In some embodiments, R³ is H and R⁴ is

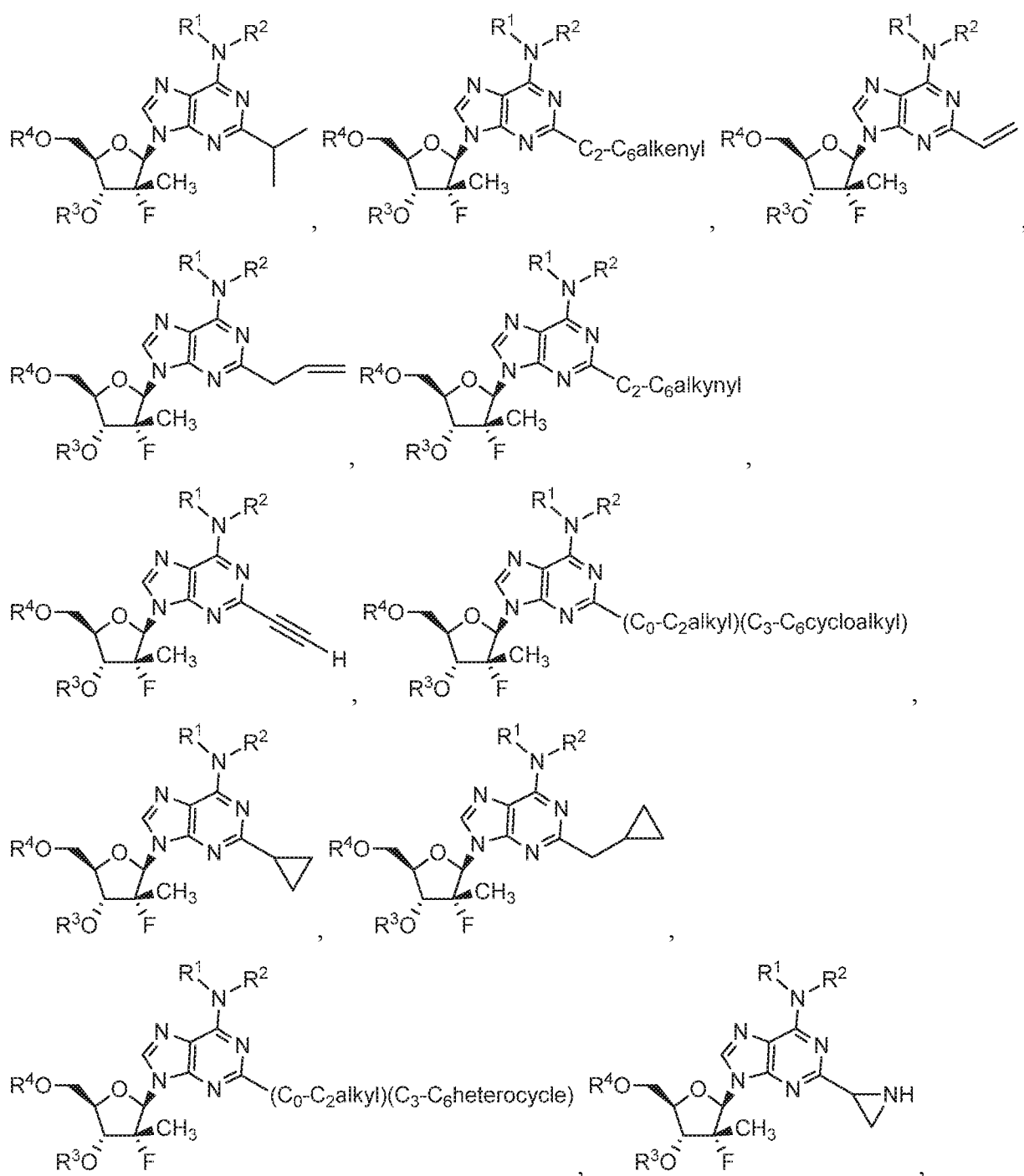




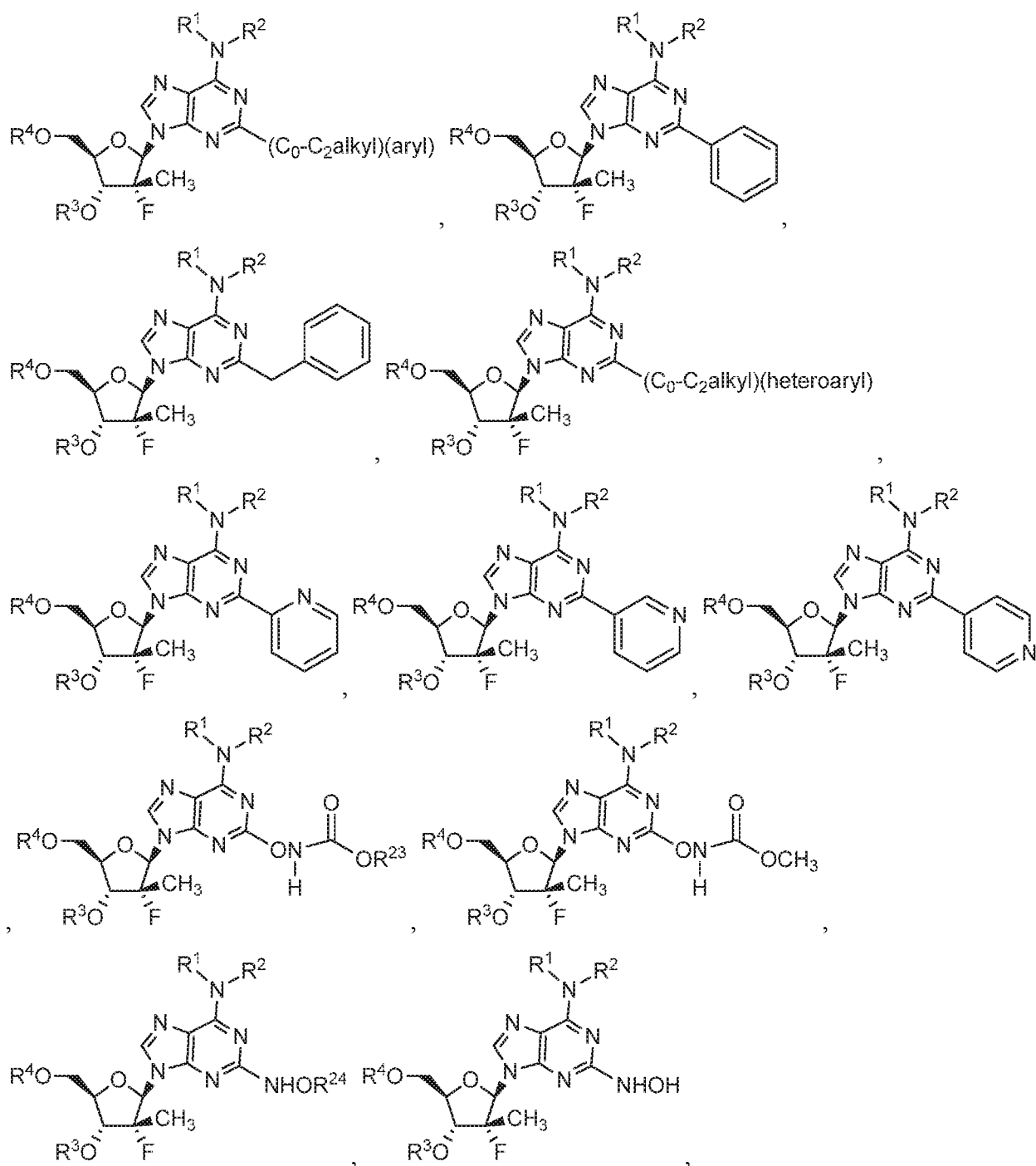
In some embodiments, R^3 is H and R^4 is

In one embodiment, a compound of Formula II is provided. Non-limiting examples of compounds of Formula II include:



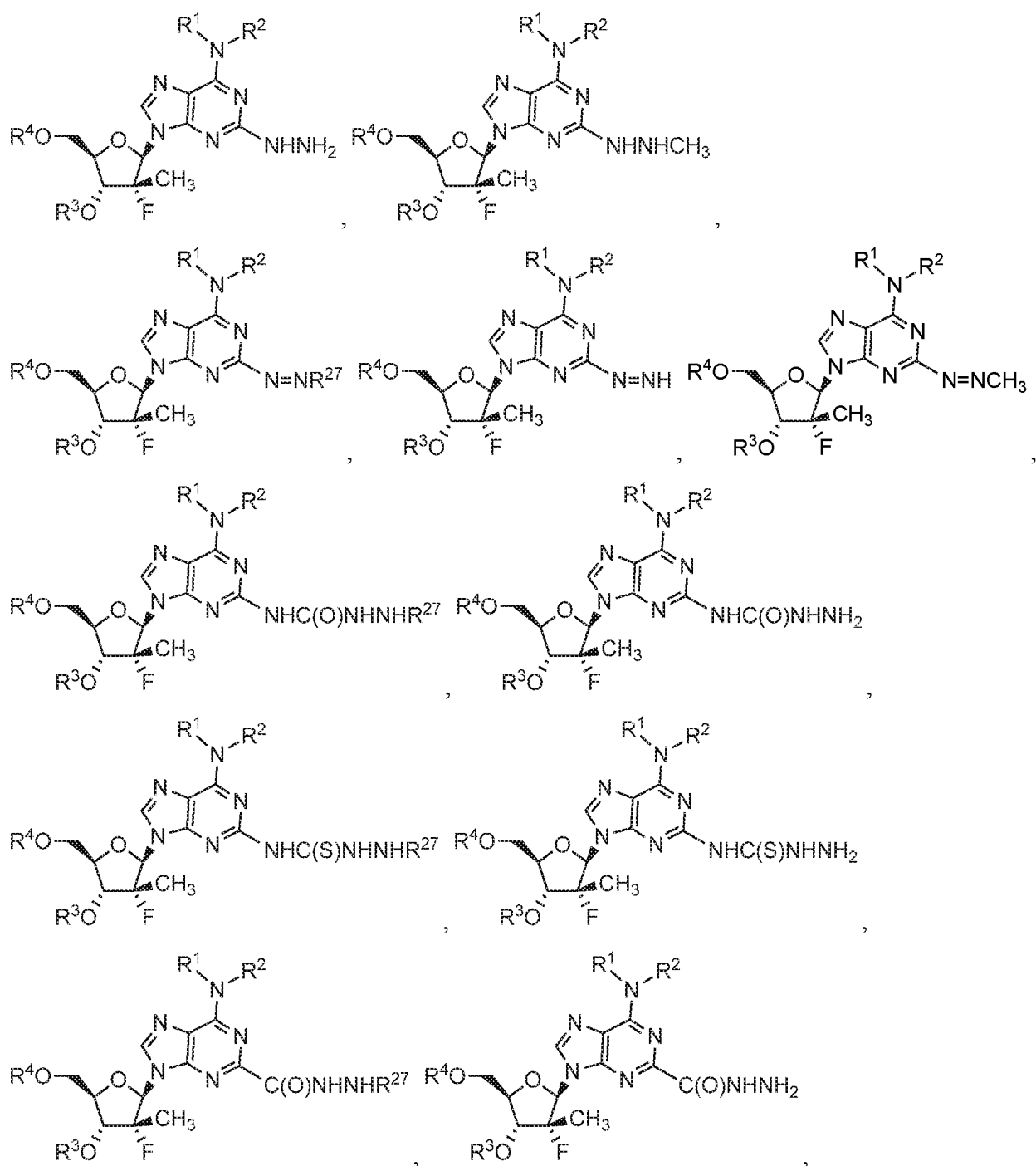


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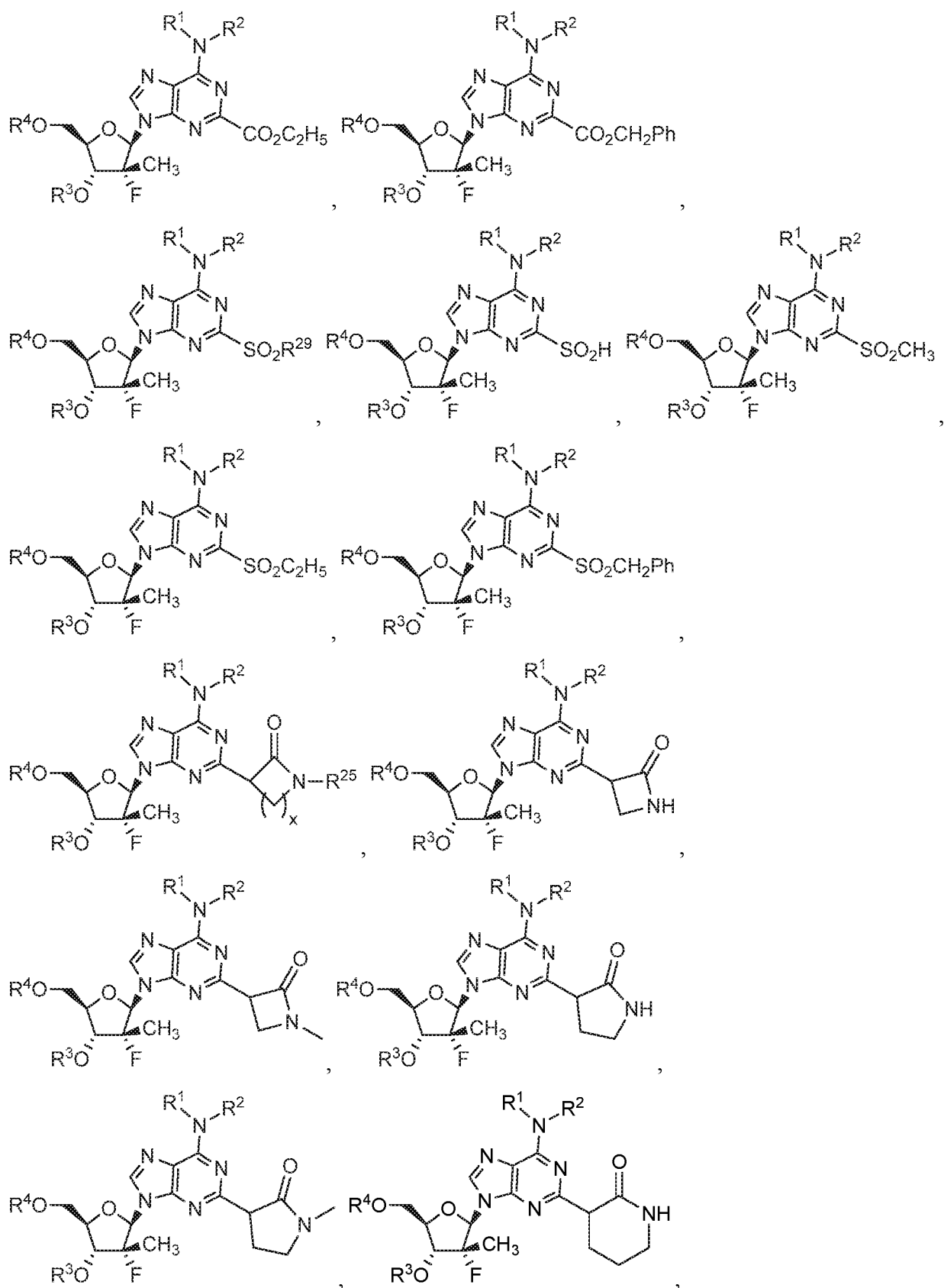
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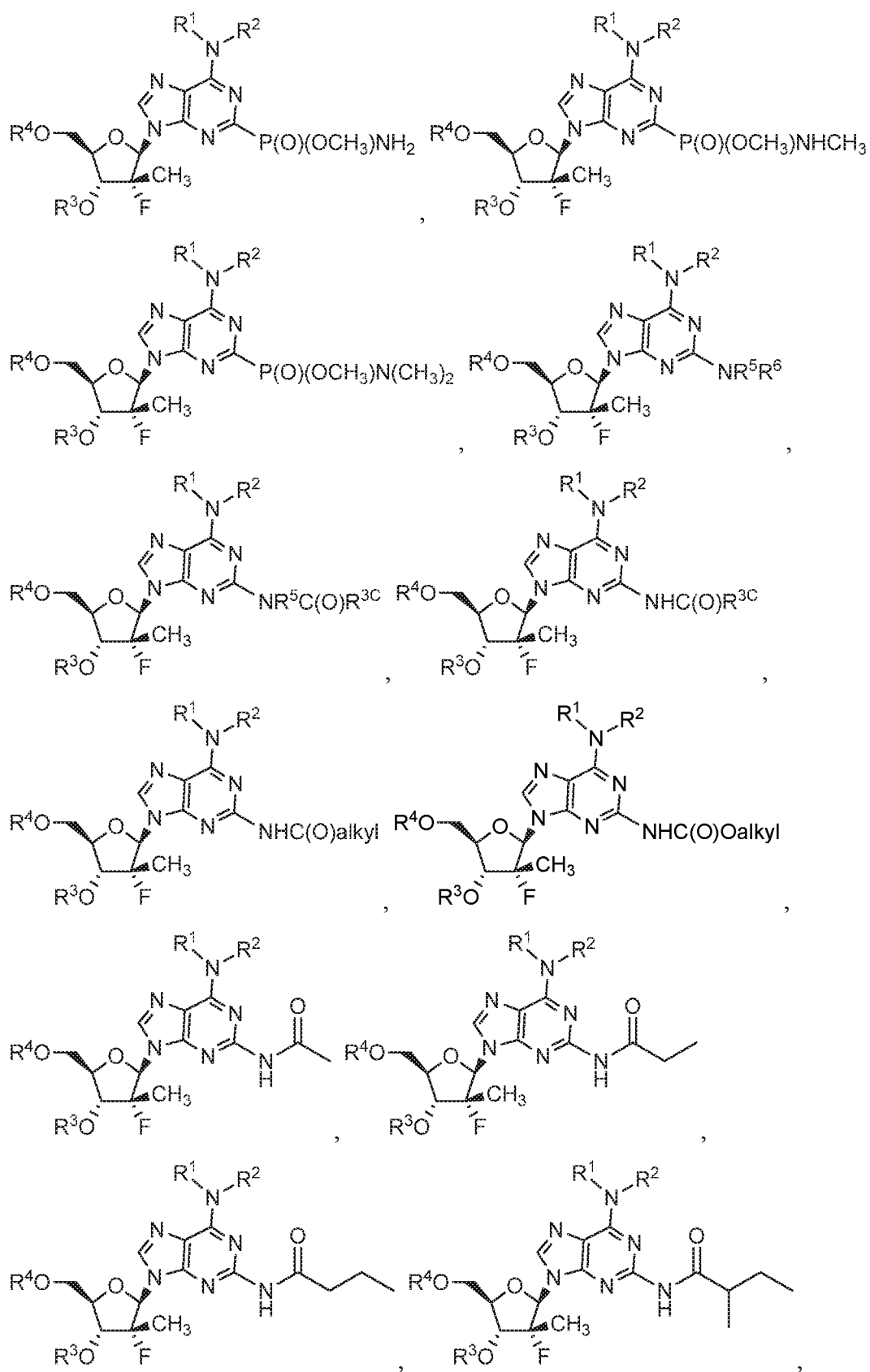


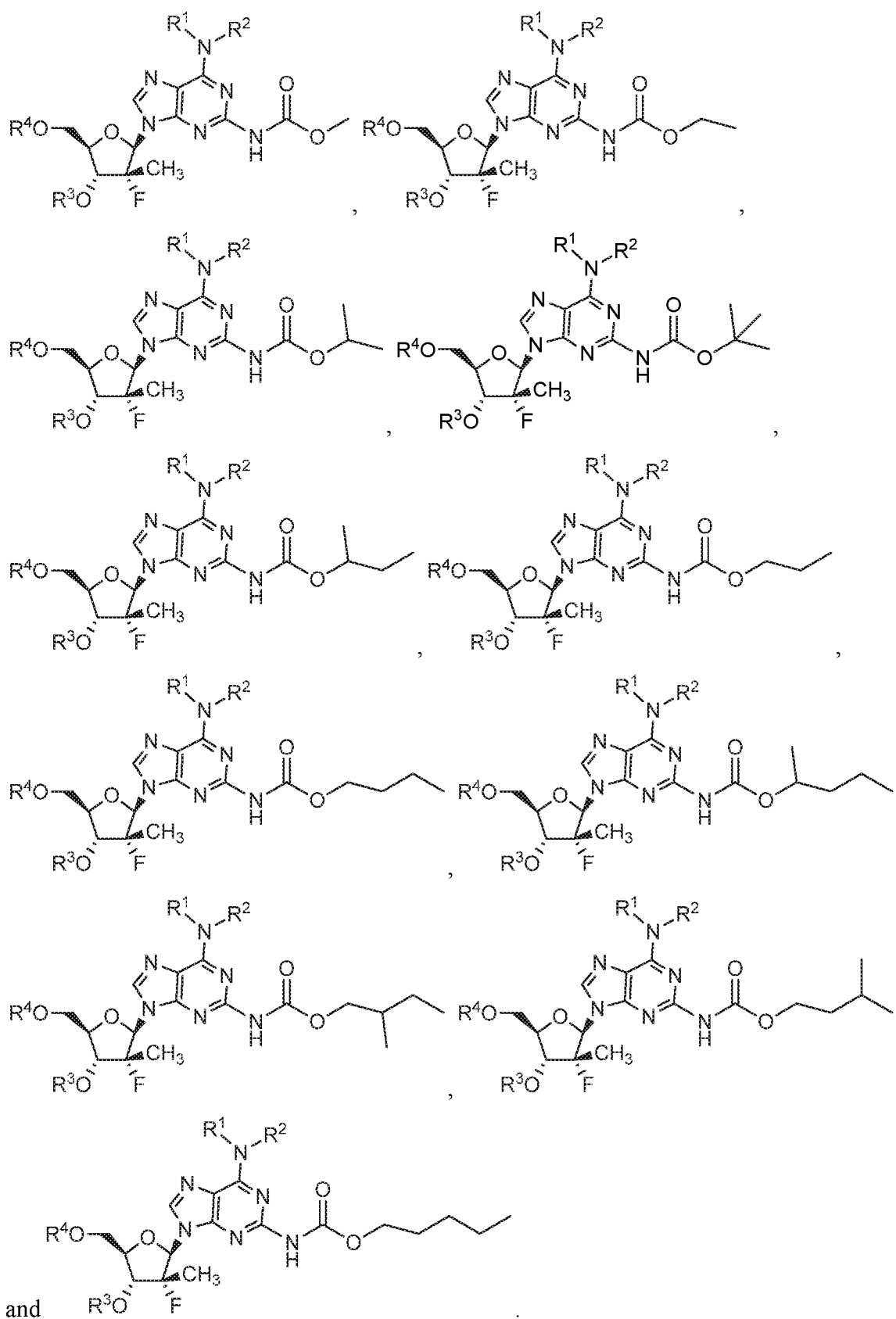
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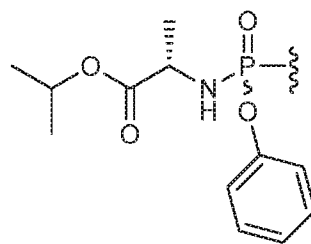




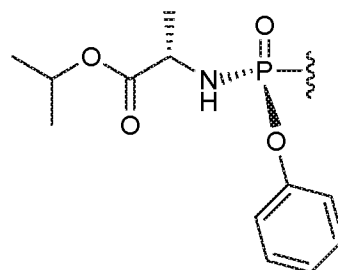




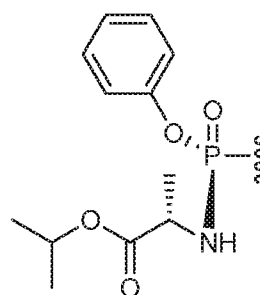




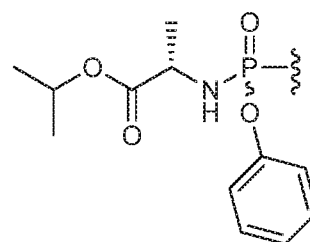
In some embodiments, R^3 is H and R^4 is



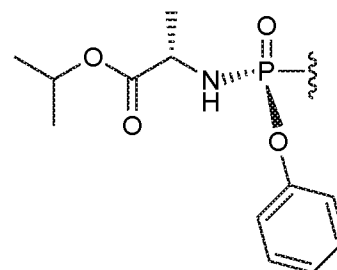
In some embodiments, R^3 is H and R^4 is



In some embodiments, R^3 is H and R^4 is

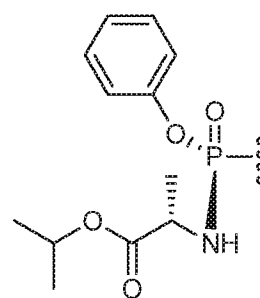


In some embodiments, R^1 is CH_3 , R^2 is H, R^3 is H and R^4 is

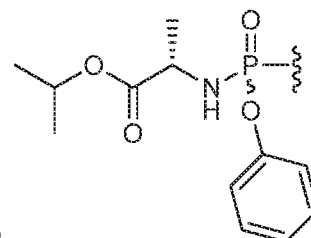


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In some embodiments, R^1 is CH_3 , R^2 is H, R^3 is H and R^4 is

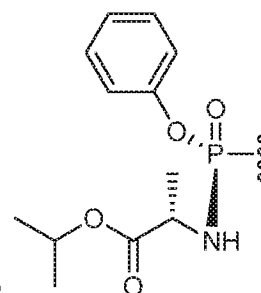
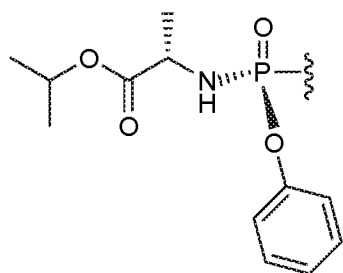


In some embodiments, R^1 is CH_3 , R^2 is H, R^3 is H and R^4 is



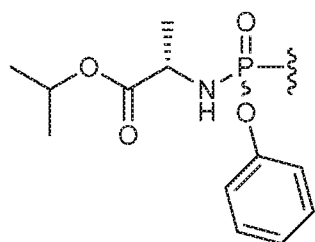
In some embodiments, R^1 is CH_3 , R^2 is CH_3 , R^3 is H and R^4 is

In some embodiments, R^1 is CH_3 , R^2 is CH_3 , R^3 is H and R^4 is

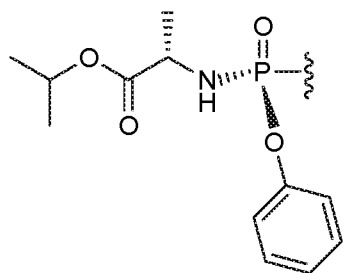


5 In some embodiments, R^1 is CH_3 , R^2 is CH_3 , R^3 is H and R^4 is

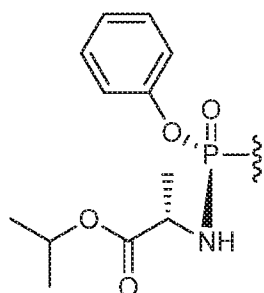
In some embodiments, R^1 is cyclopropyl, R^2 is CH_3 , R^3 is H and R^4 is



In some embodiments, R¹ is cyclopropyl, R² is CH₃, R³ is H and R⁴ is



In some embodiments, R¹ is cyclopropyl, R² is CH₃, R³ is H and R⁴ is



5

II. Definitions

The following terms are used to describe the present invention. In instances where a term is not specifically defined herein, that term is given an art-recognized meaning by those of ordinary skill applying that term in context to its use in describing the present invention.

10 The term "alkyl" shall mean within its context, a linear, or branch-chained fully saturated hydrocarbon radical or alkyl group which can be optionally substituted (for example, with halogen, including F). For example, an alkyl group can have 1, 2, 3, 4, 5, 6, 7 or 8 carbon atoms (i.e., C₁-C₈ alkyl), 1, 2, 3, 4, 5 or 6 carbon atoms (i.e., C₁-C₆ alkyl) or 1 to 4 carbon atoms (i.e., C₁-C₄ alkyl). Examples of suitable alkyl groups include, but are not limited to, methyl, ethyl, n-
15 propyl, iso-propyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, tert-pentyl, neopentyl, hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl and 2,3-dimethylbutyl.

The term "alkenyl" refers to a non-aromatic hydrocarbon group which contains at least one double bond between adjacent carbon atoms and a similar structure to an alkyl group as otherwise described herein. For example, an alkenyl group can have 2 to 8 carbon atoms (i.e.,
20 C₂-C₈ alkenyl), or 2 to 4 carbon atoms (i.e., C₂-C₄ alkenyl). Examples of suitable alkenyl groups include, but are not limited to, ethenyl or vinyl (-CH=CH₂), allyl (-CH₂CH=CH₂), 1-butenyl (-

C=CH-CH₂CH₃) and 2-butenyl (-CH₂CH=CHCH₂). The alkenyl group can be optionally substituted as described herein.

The term "alkynyl" refers to a non-aromatic hydrocarbon group containing at least one triple bond between adjacent carbon atoms and a similar structure to an alkyl group as otherwise described herein. For example, an alkynyl group can have 2 to 8 carbon atoms (i.e., C₂-C₈ alkyne), or 2 to 4 carbon atoms (i.e., C₂-C₄ alkynyl). Examples of alkynyl groups include, but are not limited to, acetylenic or ethynyl and propargyl. The alkynyl group can be optionally substituted as described herein.

The term "acyl" refers to the moiety -C(O)R in which the carbonyl moiety is bonded to R, for example, -C(O)alkyl. R can be selected from alkoxy, alkyl, cycloalkyl, lower alkyl (i.e., C₁-C₄); alkoxyalkyl, including methoxymethyl; aralkyl- including benzyl, aryloxyalkyl- such as phenoxymethyl; aryl including phenyl optionally substituted with halogen, C₁ to C₄ alkyl or C₁ to C₄ alkoxy. In one embodiment, the term "acyl" refers to a mono, di or triphosphate.

The term "lower acyl" refers to an acyl group in which the carbonyl moiety is lower alkyl (i.e., C₁-C₄).

The term "alkoxy" refers to the group -OR' where -OR' is -O-alkyl, -O-alkenyl, -O-alkynyl, -O-(C₀-C₂)(cycloalkyl), -O-(C₀-C₂)(heterocyclo), -O-(C₀-C₂)(aryl), or -O-(C₀-C₂)(heteroaryl), each of which can be optionally substituted.

The term "amino" refers to the group -NH₂.

The term "amino acid" or "amino acid residue" refers to a D- or L- natural or non-naturally occurring amino acid. Representative amino acids include, but are not limited to, alanine, β-alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, phenylalanine, histidine, isoleucine, lysine, leucine, methionine, proline, serine, threonine, valine, tryptophan, or tyrosine, among others.

The term "azido" refers to the group -N₃.

The term "aryl" or "aromatic", in context, refers to a substituted (as otherwise described herein) or unsubstituted monovalent aromatic radical having a single ring (e.g., phenyl or benzyl) or condensed rings (e.g., naphthyl, anthracenyl, phenanthrenyl, etc.) and can be bound to the compound according to the present invention at any available stable position on the ring(s) or as

otherwise indicated in the chemical structure presented. The aryl group can be optionally substituted as described herein.

"Cycloalkyl", "carbocycle", or "carbocyclyl" refers to a saturated (i.e., cycloalkyl) or partially unsaturated (e.g., cycloalkenyl, cycloalkadienyl, etc.) ring having 3 to 7 carbon atoms as a monocycle. Monocyclic carbocycles have 3 to 7 ring atoms, still more typically 5 or 6 ring atoms. Non-limiting examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, and 1-cyclohex-3-enyl.

The term "cyano" refers to the group -CN.

The term "halogen" or "halo" refers to chloro, bromo, fluoro or iodo.

A heteroaryl ring system is a saturated or unsaturated ring with one or more nitrogen, oxygen, or sulfur atoms in the ring (monocyclic) including but not limited to imidazole, furyl, pyrrole, furanyl, thiene, thiazole, pyridine, pyrimidine, purine, pyrazine, triazole, oxazole, or fused ring systems such as indole, quinoline, etc., among others, which may be optionally substituted as described above. Heteroaryl groups include nitrogen-containing heteroaryl groups such as pyrrole, pyridine, pyridone, pyridazine, pyrimidine, pyrazine, pyrazole, imidazole, triazole, triazine, tetrazole, indole, isoindole, indolizine, purine, indazole, quinoline, isoquinoline, quinolizine, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, imidazopyridine, imidazotriazine, pyrazinopyridazine, acridine, phenanthridine, carbazole, carbazoline, perimidine, phenanthroline, phenacene, oxadiazole, benzimidazole, pyrrolopyridine, pyrrolopyrimidine and pyridopyrimidine; sulfur-containing aromatic heterocycles such as thiophene and benzothiophene; oxygen-containing aromatic heterocycles such as furan, pyran, cyclopentapyran, benzofuran and isobenzofuran; and aromatic heterocycles comprising two or more hetero atoms selected from among nitrogen, sulfur and oxygen, such as thiazole, thiadiazole, isothiazole, benzoxazole, benzothiazole, benzothiadiazole, phenothiazine, isoxazole, furazan, phenoxazine, pyrazoloxazole, imidazothiazole, thienofuran, furopyrrrole, pyridoxazine, furopyridine, furopyrimidine, thienopyrimidine and oxazole, among others, all of which may be optionally substituted.

The term "heterocycle" or "heterocyclo" refers to a cyclic group which contains at least one heteroatom, i.e., O, N, or S, and may be aromatic (heteroaryl) or non-aromatic. Exemplary non-aromatic heterocyclic groups for use in the present invention include, for example,

pyrrolidinyl, piperidinyl, piperazinyl, N-methylpiperazinyl, imidazoliny, pyrazolidinyl, imidazolidinyl, morpholinyl, tetrahydropyranyl, azetidiny, oxetanyl, oxathiolanyl, pyridone, 2-pyrrolidone, ethyleneurea, 1,3-dioxolane, 1,3-dioxane, 1,4-dioxane, phthalimide, and succinimide, among others, all of which may be optionally substituted.

5 The term "hydroxyl" refers to the group –OH.

 The term "nitro" refers to the group –NO₂.

 The term "pharmaceutically acceptable salt" or prodrug" is used throughout the specification to describe any pharmaceutically acceptable form (such as an ester, phosphoramidate, thiophosphoramidate, phosphate ester, salt of an ester, or a related group) of a
10 β-D-2'-D-2'-α-fluoro-2'-β-C-substituted-2'-modified-N⁶-substituted purine nucleotide which, upon administration to a patient, provides the desired active compound. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids, which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartrate, succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate.
15 Suitable inorganic salts may also be formed, including sulfate, nitrate, bicarbonate, and carbonate salts. Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium, or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can
20 also be made.

 "Pharmaceutically acceptable prodrug" refers to a compound that is metabolized, for example hydrolyzed or oxidized, in the host to form the compound of the present invention. Typical examples of prodrugs include compounds that have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be
25 oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated, thiophosphoramidated, dethiophosphoramidated, phosphoramidated or dephosphoramidated to produce the active compound. The compounds of this invention possess antiviral activity against HCV, or are metabolized to a compound that exhibits such activity. The β-D-2'-D-2'-α-fluoro-2'-
30 β-C-substituted-2'-modified-N⁶-substituted purine nucleoside can also be administered as a 5'-phosphoether lipid, a bisphosphoramidate, a 3',5'-cyclic phosphoramidate, a 3',5'-cyclic

thiophosphoramidate, a DTE conjugate, a mixed phosphoramidate-SATE derivative or a "SATE" derivative.

The term "phosphonic acid" refers to the group $-P(O)(OH)_2$.

In one embodiment, the term purine or pyrimidine base includes, but is not limited to, adenine, N⁶-alkylpurines, N⁶-acylpurines (wherein acyl is -C(O)alkyl, -C(O)(aryl)C₀-C₄alkyl, or -C(O)(C₀-C₄alkyl)aryl), N⁶-benzylpurine, N⁶-halopurine, N⁶-vinylpurine, N⁶-acetylenic purine, N⁶-acyl purine, N⁶-hydroxyalkyl purine, N⁶-thioalkyl purine, N²-alkylpurines, N²-alkyl-6-thiopurines, thymine, cytosine, 5-fluorocytosine, 5-methylcytosine, 6-azapyrimidine, including 6-azacytosine, 2- and/or 4-mercaptopyrimidine, uracil, 5-halouracil, including 5-fluorouracil, C⁵-alkylpyrimidines, C⁵-benzylpyrimidines, C⁵-halopyrimidines, C⁵-vinylpyrimidine, C⁵-acetylenic pyrimidine, C⁵-acyl pyrimidine, C⁵-hydroxyalkyl purine, C⁵-amidopyrimidine, C⁵-cyanopyrimidine, C⁵-nitropyrimidine, C⁵-aminopyrimidine, N²-alkylpurines, N²-alkyl-6-thiopurines, 5-azacytidinyl, 5-azauracilyl, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, and pyrazolo-pyrimidinyl. Purine bases include, but are not limited to, guanine, adenine, hypoxanthine, 2,6-diaminopurine, and 6-chloropurine. Functional oxygen and nitrogen groups on the base can be protected as necessary or desired. Suitable protecting groups are well known to those skilled in the art, and include benzyl, trimethylsilyl, dimethylhexylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, trityl, alkyl groups, and acyl groups such as acetyl and propionyl; methanesulfonyl, and p-toluenesulfonyl. Alternatively, the purine or pyrimidine base can optionally be substituted such that it forms a viable prodrug, which can be cleaved in vivo. Examples of appropriate substituents include an acyl moiety.

The term "substituted" or "optionally substituted" indicates that the moiety can have at least one additional substituent including, but not limited to, halogen (F, Cl, Br, I), OH, phenyl, benzyl, N₃, CN, acyl, alkyl, including methyl; alkenyl, alkynyl, alkoxy, haloalkyl; including CHF₂, CH₂F and CF₃; etc. In one embodiment, the term "substituted" or "optionally substituted" indicates that the moiety can have at least one additional substituent including, but not limited to, azido, cyano, halogen (fluoro, chloro, bromo, or iodo), alkyl, alkenyl, alkynyl, cycloalkyl, heterocycle, aryl, heteroaryl, haloalkyl, hydroxyl, alkoxy, amino, -NH(C₁-C₆ unsubstituted alkyl), -NH(C₁-C₆ substituted alkyl), -NH-(C₀-C₂alkyl)(C₃-C₈cycloalkyl), -NH-(C₀-C₂alkyl)(C₃-C₈heterocycle), -NH-(C₀-C₂alkyl)(aryl), -N(C₁-C₆ unsubstituted alkyl)₂, -N(C₁-C₆ substituted alkyl)(C₁-C₆ substituted alkyl), -N(C₁-C₆ substituted alkyl)₂, -NH-(C₀-C₂alkyl)(C₃-C₈cycloalkyl),

-NH-(C₀-C₂alkyl)(C₃-C₈heterocycle), -NH-(C₀-C₂alkyl)(aryl), acyl, nitro, sulfonic acid, sulfate, phosphonic acid, phosphate, phosphonate, or thiol.

The term "sulfonate esters", represented by the formula, R¹⁴S(O)₂OR¹⁵, comprise R¹⁴ wherein R¹⁴ is alkyl, haloalkyl, aralkyl or aryl. R¹⁵ is alkyl, aryl or aralkyl.

5 The term "sulfonic acid" refers to the group -SO₂OH.

The term "thiol" refers to the group -SH.

The term "nitrogen-protecting group" as used herein refers to a moiety that is covalently attached to nitrogen and which can be removed, and typically replaced with hydrogen, when appropriate. For example, a nitrogen-protecting group may be a group that is removed *in vivo* after administration to a host, *in vitro* by a cell, or it may be removed during a manufacturing process. Suitable nitrogen-protecting groups useful in the present invention are described by Greene and Wuts in Protective Groups in Organic Synthesis (1991) New York, John Wiley and Sons, Inc.

15 The term "oxygen-protecting group" as used herein refers to a moiety that is covalently attached to oxygen and which can be removed, and typically replaced with hydrogen, when appropriate. For example, an oxygen-protecting group may be a group that is removed *in vivo* after administration to a host, *in vitro* by a cell, or it may be removed during a manufacturing process. Suitable oxygen-protecting groups useful in the present invention are described by Greene and Wuts in Protective Groups in Organic Synthesis (1991) New York, John Wiley and Sons, Inc.

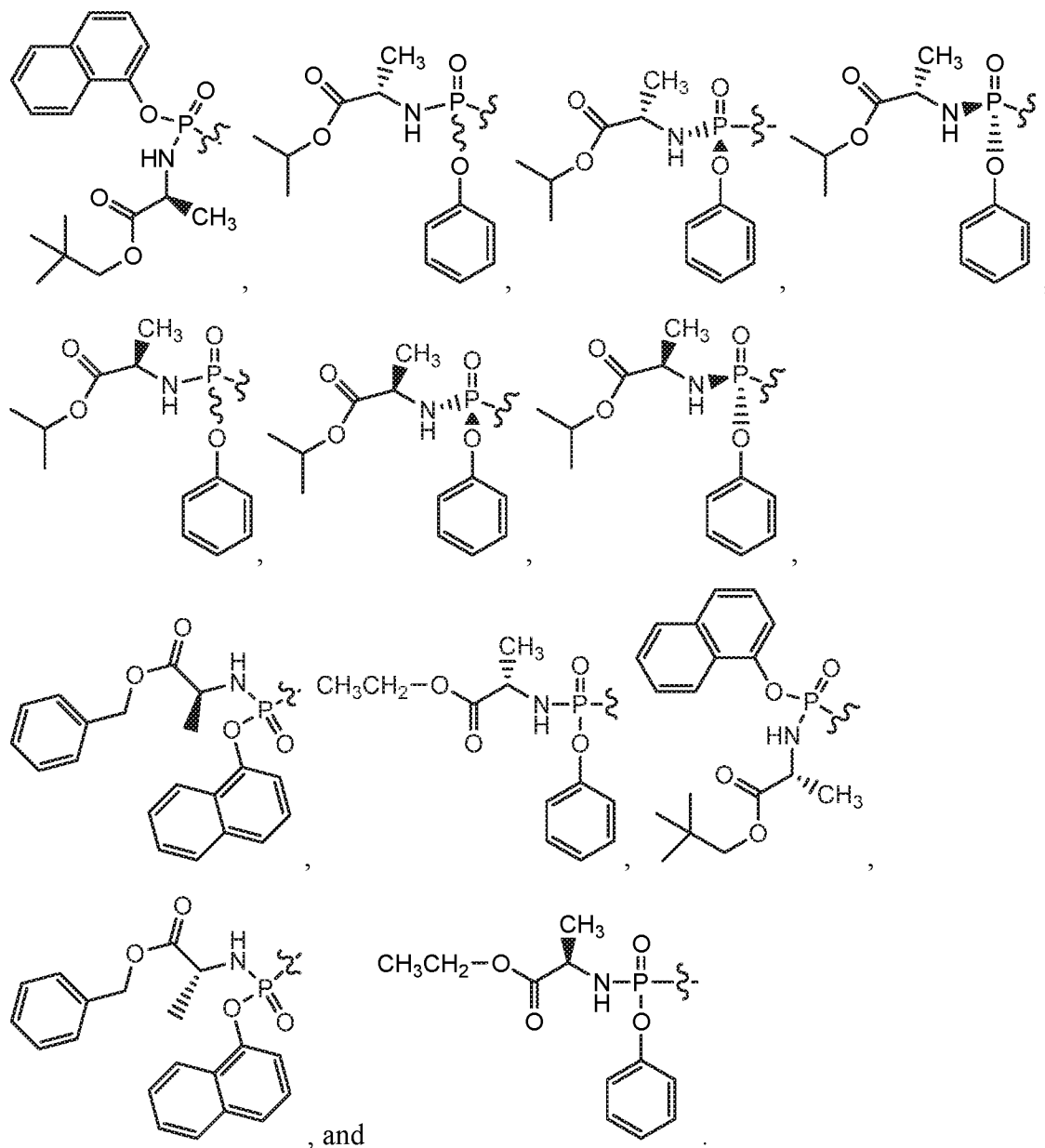
"Phosphate" refers to the group -OP(O)(OH)₂.

"Phosphate ester" refers to mono, di, and tri phosphates unless otherwise indicated.

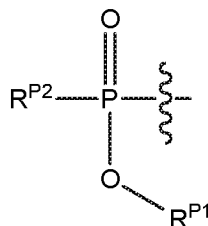
25 The term "phosphoamidate", "phosphoramidate", or "phosphoroamidate" is a moiety that has a phosphorus bound to three oxygen groups and an amine (which may optionally be substituted). Suitable phosphoramidates useful in the present invention are described by Madela, Karolina and McGuigan in 2012, "Progress in the development of anti-hepatitis C virus nucleoside and nucleotide prodrugs", *Future Medicinal Chemistry* 4(5), pages 625-650 10:1021/jm300074y and Dominique, McGuigan and Balzarini in 2004, "Aryloxy Phosphoramidate Triesters as Pro-Tides", *Mini Reviews in Medicinal Chemistry* 4(4), pages 371-381. Additional phosphoramidates useful in the present invention are described in U.S. Patent Nos. 5,233,031, 7,115,590, 7,547,704, 7,879,815, 7,888,330, 7,902,202, 7,951,789, 7,964,580,

8,071,568; 8,148,349, 8,263,575, 8,324,179, 8,334,270, 8,552,021, 8,563,530, 8,580,765, 8,735,372, 8,759,318; EP 2120565; EP 1143995; 6,455,513; and 8,334,270. Other phosphoramidates are described in the nucleoside patents described in the Background of the Invention.

5 Phosphoramidate groups for use in the present invention include those of the structures:



Other phosphoramidates for use in the present invention include those of the structure:



wherein:

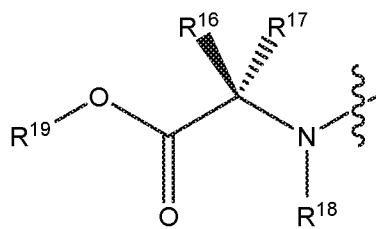
R^{P1} is an optionally substituted linear, branched, or cyclic alkyl group, or an optionally substituted aryl, heteroaryl or heterocyclic group or a linked combination thereof; and

R^{P2} is a $-NR^{N1}R^{N2}$ group or a B' group;

wherein:

R^{N1} and R^{N2} are each independently H, C_1 - 8 alkyl, $(C_3$ - C_7 cycloalkyl) C_0 - C_4 alkyl-, (aryl) C_0 - C_4 alkyl-, $(C_3$ - C_6 heterocyclo) C_0 - C_4 alkyl-, or (heteroaryl) C_0 - C_4 alkyl-, which may be optionally substituted; or

R^{N1} and R^{N2} along with the nitrogen atom to which that are attached, join to form a 3 to 7 membered heterocyclic ring;



B' is a group;

wherein:

R^{16} is hydrogen, $(C_1$ - C_8)alkyl, $(C_2$ - C_8)alkenyl, $(C_2$ - C_8)alkynyl, $(C_3$ - C_8 cycloalkyl) C_0 - C_4 alkyl-, (aryl) C_0 - C_4 alkyl-, $(C_3$ - C_6 heterocyclo) C_0 - C_4 alkyl-, (heteroaryl) C_0 - C_4 alkyl-, or the sidechain of an amino acid, for example a sidechain of an amino acid (as otherwise described herein) often selected from the group consisting of alanine, β -alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, phenylalanine, histidine, isoleucine, lysine, leucine, methionine, proline, serine, threonine, valine, tryptophan, or tyrosine (often R^{16} is hydrogen, methyl, isopropyl, or isobutyl);

R^{17} is hydrogen, $(C_1$ - C_8)alkyl, $(C_2$ - C_8)alkenyl, $(C_2$ - C_8)alkynyl, $(C_3$ - C_8 cycloalkyl) C_0 - C_4 alkyl-, (aryl) C_0 - C_4 alkyl-, $(C_3$ - C_6 heterocyclo) C_0 - C_4 alkyl-, (heteroaryl) C_0 - C_4 alkyl-, or the sidechain of an amino acid, for example a sidechain of an amino acid (as otherwise described

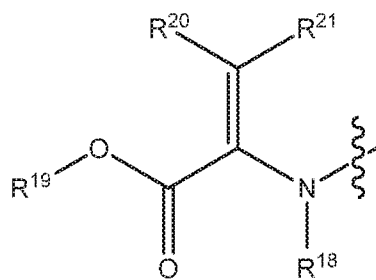
herein) often selected from the group consisting of alanine, β -alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, phenylalanine, histidine, isoleucine, lysine, leucine, methionine, proline, serine, threonine, valine, tryptophan, or tyrosine (often R^{17} is hydrogen, methyl, isopropyl, or isobutyl);

5 R^{18} is hydrogen or C_1 - C_3 alkyl; or

R^{16} and R^{17} can form a (C_3 - C_7)cycloalkyl or (C_3 - C_7)heterocyclic group; or

R^{18} and R^{16} or R^{17} can form (C_3 - C_6)heterocyclic group; and

R^{19} is hydrogen, (C_1 - C_6)alkyl, (C_3 - C_6)alkenyl, (C_3 - C_6)alkynyl, (C_3 - C_8 cycloalkyl) C_0 - C_4 alkyl-, (aryl) C_0 - C_4 alkyl-, (C_3 - C_6 heterocyclo) C_0 - C_4 alkyl-, (heteroaryl) C_0 - C_4 alkyl-; or



10 B' is a group;

wherein:

R^{20} is hydrogen, (C_1 - C_3)alkyl, (C_3 - C_8 cycloalkyl) C_0 - C_4 alkyl-, (aryl) C_0 - C_4 alkyl-, (C_3 - C_6 heterocyclo) C_0 - C_4 alkyl-, or (heteroaryl) C_0 - C_4 alkyl-;

15 R^{21} is hydrogen, (C_1 - C_3)alkyl, (C_3 - C_8 cycloalkyl) C_0 - C_4 alkyl-, (aryl) C_0 - C_4 alkyl-, (C_3 - C_6 heterocyclo) C_0 - C_4 alkyl-, or (heteroaryl) C_0 - C_4 alkyl-; and

R^{18} and R^{19} are as defined above.

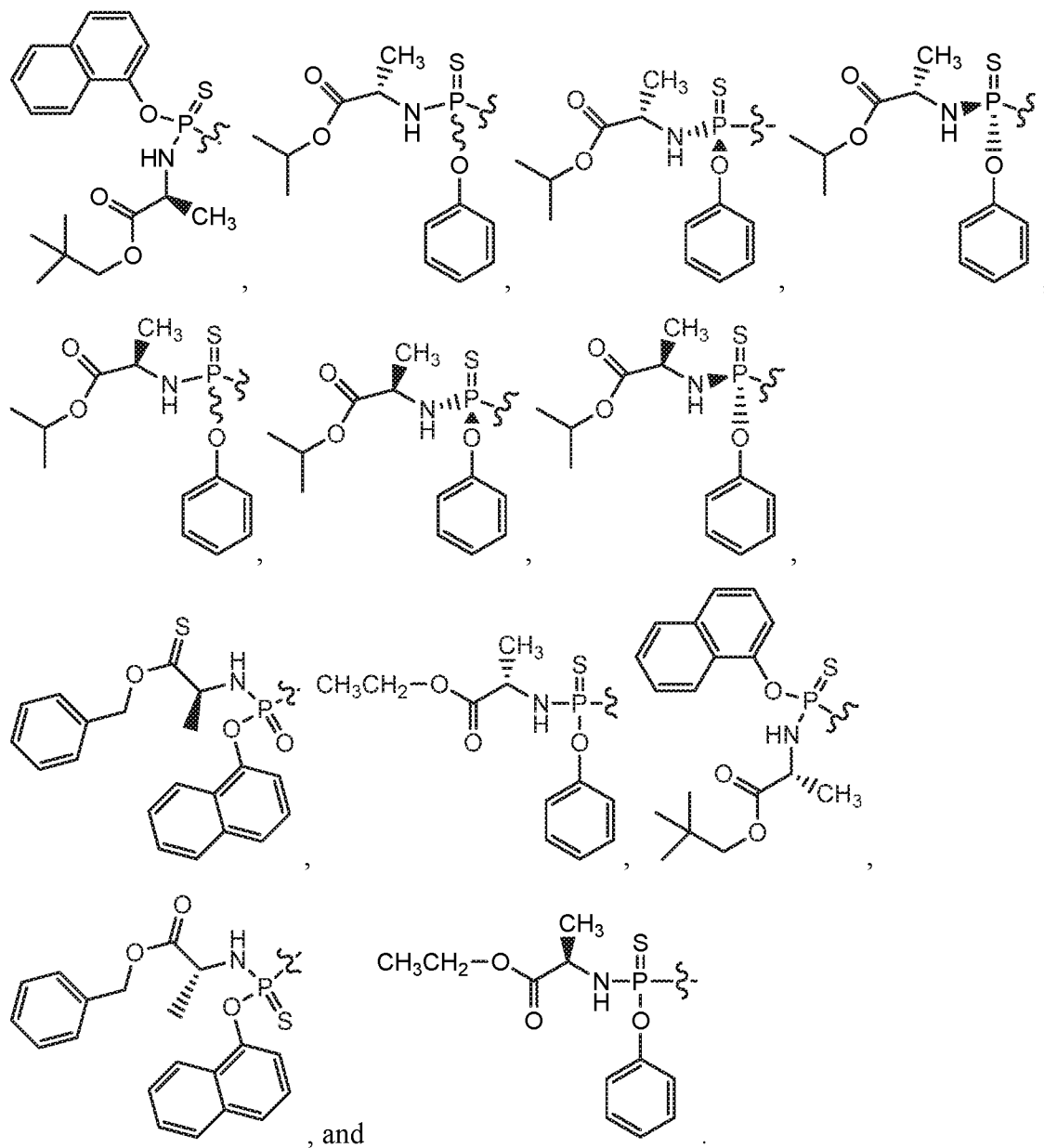
Preferred R^{P1} groups include optionally substituted phenyl, naphthyl, and monocyclic heteroaryl groups, especially those groups (particularly lipophilic groups) which enhance bioavailability of the compounds in the cells of the patient and which exhibit reduced toxicity, enhanced therapeutic index and enhanced pharmacokinetics (the compounds are metabolized and excreted more slowly).

25 The term phosphoramidate is used throughout the specification to describe a group that is found at the 5' or 3' position of the furanose ring of the nucleoside compound and forms a prodrug form of the nucleoside compound. In one embodiment, phosphoramidates can be found at both the 5' and 3' position of the furanose ring of the nucleoside compound and form a prodrug form of the nucleoside compound. In another embodiment, the phosphoramidate found at the 5' position of the furanose ring of the nucleoside can form a cyclic phosphoramidate

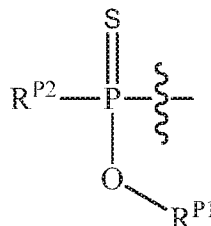
compound by forming a bond with the 3'-hydroxyl substituent at the 3' position of the furanose ring of the nucleoside compound and form a prodrug form of the nucleoside compound.

The term "thiophosphoamidate", "thiophosphoramidate", or "thiophosphoroamidate" is a moiety that has a phosphorus bound to sulfur, two oxygen groups and an amine (which may optionally be substituted). Thiophosphoramidates useful in the present invention are described in US Patent No. 8,772,474 and WO 2012/040124.

Thiophosphoramidate groups for use in the present invention include those of the structures:



Other thiophosphoramidates include those of the structure:



wherein:

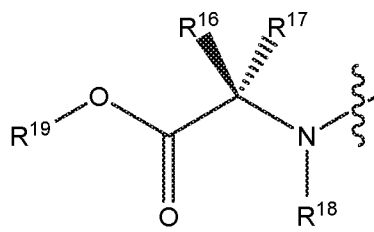
R^{P1} is an optionally substituted linear, branched, or cyclic alkyl group, or an optionally substituted aryl, heteroaryl or heterocyclic group or a linked combination thereof; and

R^{P2} is a $-NR^{N1}R^{N2}$ group or a B' group;

wherein:

R^{N1} and R^{N2} are each independently H, C₁-C₈ alkyl, (C₃-C₇cycloalkyl)C₀-C₄alkyl-, (aryl)C₀-C₄alkyl-, (C₃-C₆heterocyclo)C₀-C₄alkyl-, or (heteroaryl)C₀-C₄alkyl-; or

R^{N1} and R^{N2} along with the nitrogen atom to which that are attached, join to form a 3 to 7 membered heterocyclic ring;



B' is a group;

wherein:

R^{16} is hydrogen, (C₁-C₈)alkyl, (C₂-C₈)alkenyl, (C₂-C₈)alkynyl, (C₃-C₈cycloalkyl)C₀-C₄alkyl-, (aryl)C₀-C₄alkyl-, (C₃-C₆heterocyclo)C₀-C₄alkyl-, (heteroaryl)C₀-C₄alkyl-, or the sidechain of an amino acid, for example a sidechain of an amino acid (as otherwise described herein) often selected from the group consisting of alanine, β -alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, phenylalanine, histidine, isoleucine, lysine, leucine, methionine, proline, serine, threonine, valine, tryptophan, or tyrosine (often R^{16} is hydrogen, methyl, isopropyl, or isobutyl);

R^{17} is hydrogen, (C₁-C₈)alkyl, (C₂-C₈)alkenyl, (C₂-C₈)alkynyl, (C₃-C₈cycloalkyl)C₀-C₄alkyl-, (aryl)C₀-C₄alkyl-, (C₃-C₆heterocyclo)C₀-C₄alkyl-, (heteroaryl)C₀-C₄alkyl-, or the sidechain of an amino acid, for example a sidechain of an amino acid (as otherwise described herein) often selected from the group consisting of alanine, β -alanine, arginine, asparagine,

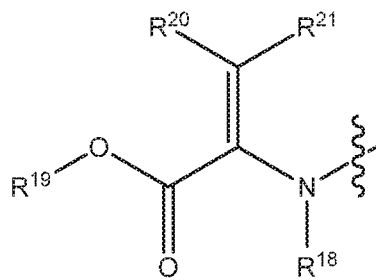
aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, phenylalanine, histidine, isoleucine, lysine, leucine, methionine, proline, serine, threonine, valine, tryptophan, or tyrosine (often R¹⁷ is hydrogen, methyl, isopropyl, or isobutyl);

R¹⁸ is hydrogen or C₁-C₃alkyl; or

5 R¹⁶ and R¹⁷ can form a (C₃-C₇)cycloalkyl or (C₃-C₇)heterocyclic group; or

R¹⁸ and R¹⁶ or R¹⁷ can form (C₃-C₆) heterocyclic group; and

R¹⁹ is hydrogen, (C₁-C₆)alkyl, (C₃-C₆)alkenyl, (C₃-C₆)alkynyl, (C₃-C₈cycloalkyl)C₀-C₄alkyl-, (aryl)C₀-C₄alkyl-, (C₃-C₆heterocyclo)C₀-C₄alkyl-, (heteroaryl)C₀-C₄alkyl-; or



B' is a group; and

10 R¹⁸, R¹⁹, R²⁰ and R²¹ are as defined above.

Preferred R^{P1} groups include optionally substituted phenyl, naphthyl, and monocyclic heteroaryl groups, especially those groups (particularly lipophilic groups) which enhance bioavailability of the compounds into the cells of the patient and which exhibit reduced toxicity, enhanced therapeutic index and enhanced pharmacokinetics (the compounds are metabolized and excreted more slowly).

The thiophosphoramidate can be at the 5' or 3' position of the furanose ring of the nucleoside compound to form a prodrug form of the nucleoside compound. In one embodiment, thiophosphoramidates can be found at both the 5' and 3' position of the furanose ring of the nucleoside compound and form a prodrug form of the nucleoside compound. In another embodiment, the thiophosphoramidate found at the 5' position of the furanose ring of the nucleoside can form a cyclic thiophosphoramidate compound by forming a bond with the 3'-hydroxyl substituent at the 3' position of the furanose ring of the nucleoside compound and form a prodrug form of the nucleoside compound.

The term "D-configuration" as used in the context of the present invention refers to the principle configuration which mimics the natural configuration of sugar moieties as opposed to the unnatural occurring nucleosides or "L" configuration. The term "β" or "β anomer" is used

with reference to nucleoside analogs in which the nucleoside base is configured (disposed) above the plane of the furanose moiety in the nucleoside analog.

The terms "coadminister" and "coadministration" or combination therapy are used to describe the administration of at least one of the 2'-deoxy-2'- α -fluoro-2'- β -C-nucleoside compounds according to the present invention in combination with at least one other active agent, for example where appropriate at least one additional anti-HCV agent, including other 2'-deoxy-2'- α -fluoro-2'- β -C-nucleoside agents which are disclosed herein. The timing of the coadministration is best determined by the medical specialist treating the patient. It is sometimes preferred that the agents be administered at the same time. Alternatively, the drugs selected for combination therapy may be administered at different times to the patient. Of course, when more than one viral or other infection or other condition is present, the present compounds may be combined with other agents to treat that other infection or condition as required.

The term "host", as used herein, refers to a unicellular or multicellular organism in which a HCV virus can replicate, including cell lines and animals, and typically a human. The term host specifically refers to infected cells, cells transfected with all or part of a HCV genome, and animals, in particular, primates (including chimpanzees) and humans. In most animal applications of the present invention, the host is a human patient. Veterinary applications, in certain indications, however, are clearly anticipated by the present invention (such as chimpanzees). The host can be for example, bovine, equine, avian, canine, feline, etc.

Isotopic Substitution

The present invention includes compounds and the use of compounds with desired isotopic substitutions of atoms, at amounts above the natural abundance of the isotope, i.e., enriched. Isotopes are atoms having the same atomic number but different mass numbers, i.e., the same number of protons but a different number of neutrons. By way of general example and without limitation, isotopes of hydrogen, for example, deuterium (^2H) and tritium (^3H) may be used anywhere in described structures. Alternatively or in addition, isotopes of carbon, e.g., ^{13}C and ^{14}C , may be used. A preferred isotopic substitution is deuterium for hydrogen at one or more locations on the molecule to improve the performance of the drug. The deuterium can be bound in a location of bond breakage during metabolism (an α -deuterium kinetic isotope effect) or next to or near the site of bond breakage (a β -deuterium kinetic isotope effect). Achillion

Pharmaceuticals, Inc. (WO/2014/169278 and WO/2014/169280) describes deuteration of nucleotides to improve their pharmacokinetics or pharmacodynamics, including at the 5-position of the molecule.

Substitution with isotopes such as deuterium can afford certain therapeutic advantages resulting from greater metabolic stability, such as, for example, increased in vivo half-life or reduced dosage requirements. Substitution of deuterium for hydrogen at a site of metabolic break down can reduce the rate of or eliminate the metabolism at that bond. At any position of the compound that a hydrogen atom may be present, the hydrogen atom can be any isotope of hydrogen, including protium (^1H), deuterium (^2H) and tritium (^3H). Thus, reference herein to a compound encompasses all potential isotopic forms unless the context clearly dictates otherwise.

The term "isotopically-labeled" analog refers to an analog that is a "deuterated analog", a " ^{13}C -labeled analog," or a "deuterated/ ^{13}C -labeled analog." The term "deuterated analog" means a compound described herein, whereby a H-isotope, i.e., hydrogen/protium (^1H), is substituted by a H-isotope, i.e., deuterium (^2H). Deuterium substitution can be partial or complete. Partial deuterium substitution means that at least one hydrogen is substituted by at least one deuterium. In certain embodiments, the isotope is 90, 95 or 99% or more enriched in an isotope at any location of interest. In some embodiments it is deuterium that is 90, 95 or 99% enriched at a desired location. Unless indicated to the contrary, the deuteration is at least 80% at the selected location. Deuteration of the nucleoside can occur at any replaceable hydrogen that provides the desired results.

III. Methods of Treatment or Prophylaxis

Treatment, as used herein, refers to the administration of an active compound to a host that is infected with a HCV virus.

The term "prophylactic" or preventative, when used, refers to the administration of an active compound to prevent or reduce the likelihood of an occurrence of the viral disorder. The present invention includes both treatment and prophylactic or preventative therapies. In one embodiment, the active compound is administered to a host who has been exposed to and thus at risk of infection by a hepatitis C virus infection.

The invention is directed to a method of treatment or prophylaxis of a hepatitis C virus, including drug resistant and multidrug resistant forms of HCV and related disease states, conditions, or

complications of an HCV infection, including cirrhosis and related hepatotoxicities, as well as other conditions that are secondary to a HCV infection, such as weakness, loss of appetite, weight loss, breast enlargement (especially in men), rash (especially on the palms), difficulty with clotting of blood, spider-like blood vessels on the skin, confusion, coma (encephalopathy),
5 buildup of fluid in the abdominal cavity (ascites), esophageal varices, portal hypertension, kidney failure, enlarged spleen, decrease in blood cells, anemia, thrombocytopenia, jaundice, and hepatocellular cancer, among others. The method comprises administering to a host in need thereof an effective amount of at least one β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleotide as described herein, optionally in combination with at least one
10 additional bioactive agent, for example, an additional anti-HCV agent, further in combination with a pharmaceutically acceptable carrier additive and/or excipient.

In yet another aspect, the present invention is a method for prevention or prophylaxis of a an HCV infection or a disease state or related or follow-on disease state, condition or complication of an HCV infection, including cirrhosis and related hepatotoxicities, weakness,
15 loss of appetite, weight loss, breast enlargement (especially in men), rash (especially on the palms), difficulty with clotting of blood, spider-like blood vessels on the skin, confusion, coma (encephalopathy), buildup of fluid in the abdominal cavity (ascites), esophageal varices, portal hypertension, kidney failure, enlarged spleen, decrease in blood cells, anemia, thrombocytopenia, jaundice, and hepatocellular (liver) cancer, among others, said method comprising administering
20 to a patient at risk with an effective amount of at least one compound according to the present invention as described above in combination with a pharmaceutically acceptable carrier, additive, or excipient, optionally in combination with another anti-HCV agent. In another embodiment, the active compounds of the invention can be administered to a patient after a hepatitis-related liver transplantation to protect the new organ.

25 The 5'-stabilized β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleotide can be administered if desired as any salt or prodrug that upon administration to the recipient is capable of providing directly or indirectly the parent compound, or that exhibits activity itself. Nonlimiting examples are the pharmaceutically acceptable salts and a compound, which has been modified at a function group, such as a hydroxyl or amine function,
30 to modify the biological activity, pharmacokinetics, half-life, controlled delivery, lipophilicity, absorption kinetics, ease of phosphorylation to the active 5'-triphosphate or efficiency of

delivery using a desired route of administration; of the compound. Methods to modify the properties of an active compound to achieve target properties are known to those of skill in the art or can easily be assessed by standard methods, for example, acylation, phosphorylation, thiophosphoramidation, phosphoramidation, phosphonation, alkylation, or pegylation.

5

IV. Pharmaceutical Compositions

In an aspect of the invention, pharmaceutical compositions according to the present invention comprise an anti-HCV virus effective amount of at least one of the 5'-stabilized β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleotide compounds described herein, optionally in combination with a pharmaceutically acceptable carrier, additive, or excipient, further optionally in combination or alternation with at least one other active compound.

In an aspect of the invention, pharmaceutical compositions according to the present invention comprise an anti-HCV effective amount of at least one of the active β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleotide compounds described herein, optionally in combination with a pharmaceutically acceptable carrier, additive, or excipient, further optionally in combination with at least one other antiviral, such as an anti-HCV agent.

The invention includes pharmaceutical compositions that include an effective amount to treat a hepatitis C virus infection, of one of the β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleotide compounds of the present invention or its salt or prodrug, in a pharmaceutically acceptable carrier or excipient. In an alternative embodiment, the invention includes pharmaceutical compositions that include an effective amount to prevent a hepatitis C virus infection, of one of the β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleotide compounds of the present invention or its salt or prodrug, in a pharmaceutically acceptable carrier or excipient.

One of ordinary skill in the art will recognize that a therapeutically effective amount will vary with the infection or condition to be treated, its severity, the treatment regimen to be employed, the pharmacokinetics of the agent used, as well as the patient or subject (animal or human) to be treated, and such therapeutic amount can be determined by the attending physician or specialist.

The 5'-stabilized β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2- modified -N⁶-substituted purine nucleotide compounds according to the present invention can be formulated in an admixture with a pharmaceutically acceptable carrier. In general, it is preferable to administer the pharmaceutical composition in orally-administrable form, but certain formulations may be administered via a parenteral, intravenous, intramuscular, topical, transdermal, buccal, subcutaneous, suppository, or other route, including intranasal spray. Intravenous and intramuscular formulations are often administered in sterile saline. One of ordinary skill in the art may modify the formulations to render them more soluble in water or other vehicle, for example, this can be easily accomplished by minor modifications (salt formulation, esterification, etc.) which are well within the ordinary skill in the art. It is also well within the routine skill to modify the route of administration and dosage regimen of a particular compound in order to manage the pharmacokinetics of the present compounds for maximum beneficial effect in patients.

In certain pharmaceutical dosage forms, the prodrug form of the compounds, especially including acylated (acetylated or other), and ether (alkyl and related) derivatives, phosphate esters, thiophosphoramidates, phosphoramidates, and various salt forms of the present compounds, are preferred. One of ordinary skill in the art will recognize how to readily modify the present compounds to prodrug forms to facilitate delivery of active compounds to a targeted site within the host organism or patient. The routine skill also will take advantage of favorable pharmacokinetic parameters of the prodrug forms, where applicable, in delivering the present compounds to a targeted site within the host organism or patient to maximize the intended effect of the compound.

The amount of compound included within therapeutically active formulations according to the present invention is an effective amount for treating the HCV infection, reducing the likelihood of a HCV infection or the inhibition, reduction, and/or abolition of HCV or its secondary effects, including disease states, conditions, and/or complications which occur secondary to HCV. In general, a therapeutically effective amount of the present compound in pharmaceutical dosage form usually ranges from about 0.001 mg/kg to about 100 mg/kg per day or more, more often, slightly less than about 0.1 mg/kg to more than about 25 mg/kg per day of the patient or considerably more, depending upon the compound used, the condition or infection treated and the route of administration. The active nucleoside compound according to the

present invention is often administered in amounts ranging from about 0.1 mg/kg to about 15 mg/kg per day of the patient, depending upon the pharmacokinetics of the agent in the patient. This dosage range generally produces effective blood level concentrations of active compound which may range from about 0.001 to about 100, about 0.05 to about 100 micrograms/cc of blood in the patient.

Often, to treat, prevent or delay the onset of these infections and/or to reduce the likelihood of an HCV virus infection, or a secondary disease state, condition or complication of HCV, the compositions will be administered in oral dosage form in amounts ranging from about 250 micrograms up to about 500 mg or more at least once a day, for example, at least 25, 50, 100, 150, 250 or 500 milligrams, up to four times a day. The present compounds are often administered orally, but may be administered parenterally, topically, or in suppository form, as well as intranasally, as a nasal spray or as otherwise described herein.

In the case of the co-administration of the present compounds in combination with another anti-HCV compound as otherwise described herein, the amount of the compound according to the present invention to be administered ranges from about 0.01 mg/kg of the patient to about 500 mg/kg. or more of the patient or considerably more, depending upon the second agent to be co-administered and its potency against the virus, the condition of the patient and severity of the disease or infection to be treated and the route of administration. The other anti-HCV agent may for example be administered in amounts ranging from about 0.01 mg/kg to about 500 mg/kg. In certain preferred embodiments, these compounds may be often administered in an amount ranging from about 0.5 mg/kg to about 50 mg/kg or more (usually up to about 100 mg/kg), generally depending upon the pharmacokinetics of the two agents in the patient. These dosage ranges generally produce effective blood level concentrations of active compound in the patient.

For purposes of the present invention, a prophylactically or preventive effective amount of the compositions according to the present invention falls within the same concentration range as set forth above for therapeutically effective amount and is usually the same as a therapeutically effective amount.

Administration of the active compound may range from continuous (intravenous drip) to several oral or intranasal administrations per day (for example, Q.I.D.) or transdermal administration and may include oral, topical, parenteral, intramuscular, intravenous,

sub-cutaneous, transdermal (which may include a penetration enhancement agent), buccal, and suppository administration, among other routes of administration. Enteric coated oral tablets may also be used to enhance bioavailability of the compounds for an oral route of administration. The most effective dosage form will depend upon the bioavailability/pharmacokinetics of the particular agent chosen as well as the severity of disease in the patient. Oral dosage forms are particularly preferred, because of ease of administration and prospective favorable patient compliance.

To prepare the pharmaceutical compositions according to the present invention, a therapeutically effective amount of one or more of the compounds according to the present invention is often intimately admixed with a pharmaceutically acceptable carrier according to conventional pharmaceutical compounding techniques to produce a dose. A carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral. In preparing pharmaceutical compositions in oral dosage form, any of the usual pharmaceutical media may be used. Thus, for liquid oral preparations such as suspensions, elixirs, and solutions, suitable carriers and additives including water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like may be used. For solid oral preparations such as powders, tablets, capsules, and for solid preparations such as suppositories, suitable carriers and additives including starches, sugar carriers, such as dextrose, manifold, lactose, and related carriers, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like may be used. If desired, the tablets or capsules may be enteric-coated or sustained release by standard techniques. The use of these dosage forms may significantly enhance the bioavailability of the compounds in the patient.

For parenteral formulations, the carrier will usually comprise sterile water or aqueous sodium chloride solution, though other ingredients, including those which aid dispersion, also may be included. Of course, where sterile water is to be used and maintained as sterile, the compositions and carriers must also be sterilized. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents, and the like may be employed.

Liposomal suspensions (including liposomes targeted to viral antigens) may also be prepared by conventional methods to produce pharmaceutically acceptable carriers. This may be appropriate for the delivery of free nucleosides, acyl/alkyl nucleosides or phosphate ester pro-drug forms of the nucleoside compounds according to the present invention.

In typical embodiments according to the present invention, the compounds and compositions are used to treat, prevent or delay a HCV infection or a secondary disease state, condition or complication of HCV.

5 V. Combination and Alternation Therapy

It is well recognized that drug-resistant variants of viruses can emerge after prolonged treatment with an antiviral agent. Drug resistance most typically occurs by mutation of a gene that encodes for an enzyme used in viral replication. The efficacy of a drug against an HCV infection, can be prolonged, augmented, or restored by administering the compound in
10 combination or alternation with another, and perhaps even two or three other, antiviral compounds that induce a different mutation or act through a different pathway, from that of the principle drug. Alternatively, the pharmacokinetics, bio distribution, half-life, or other parameter of the drug can be altered by such combination therapy (which may include alternation therapy if considered concerted). Since the disclosed β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2- modified-
15 N⁶-substituted purine nucleotides are NS5B polymerase inhibitors, it may be useful to administer the compound to a host in combination with, for example a:

- (1) Protease inhibitor, such as an NS3/4A protease inhibitor;
- (2) NS5A inhibitor;
- (3) Another NS5B polymerase inhibitor;
- 20 (4) NS5B non-substrate inhibitor;
- (5) Interferon alfa-2a, which may be pegylated or otherwise modified, and/or ribavirin;
- (6) Non-substrate-based inhibitor;
- (7) Helicase inhibitor;
- 25 (8) Antisense oligodeoxynucleotide (S-ODN);
- (9) Aptamer;
- (10) Nuclease-resistant ribozyme;
- (11) iRNA, including microRNA and SiRNA;
- (12) Antibody, partial antibody or domain antibody to the virus, or
- 30 (13) Viral antigen or partial antigen that induces a host antibody response.

Non limiting examples of anti-HCV agents that can be administered in combination with the β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2- modified-N⁶-substituted purine nucleotides of the invention are:

- (i) protease inhibitors such as telaprevir (Incivek[®]), boceprevir (Victrelis[™]), simeprevir (Olysio[™]), paritaprevir (ABT-450), ACH-2684; AZD-7295; BMS-791325; danoprevir; Filibuvir; GS-9256; GS-9451; MK-5172; Setrobuvir; Sovaprevir; Tegobuvir; VX-135; VX-222 and ALS-220;
- (ii) NS5A inhibitor such as ACH-2928, ACH-3102, IDX-719, daclatasvir, ledipasvir and Ombitasvir (ABT-267);
- (iii) NS5B inhibitors such as ACH-3422; AZD-7295; Clemizole; ITX-5061; PPI-461; PPI-688, Sovaldi[®], MK-3682, and mericitabine;
- (iv) NS5B inhibitors such as ABT-333, MBX-700; and,
- (v) Antibody such as GS-6624.

If the β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2- modified-N⁶-substituted purine nucleotide is administered to treat advanced hepatitis C virus leading to liver cancer or cirrhosis, in one embodiment, the compound can be administered in combination or alternation with another drug that is typically used to treat hepatocellular carcinoma (HCC), for example, as described by Andrew Zhu in "New Agents on the Horizon in Hepatocellular Carcinoma" Therapeutic Advances in Medical Oncology, V 5(1), January 2013, 41-50. Examples of suitable compounds for combination therapy where the host has or is at risk of HCC include anti-angiogenic agents, sunitinib, brivanib, linifanib, ramucirumab, bevacizumab, cediranib, pazopanib, TSU-68, lenvatinib, antibodies against EGFR, mTor inhibitors, MEK inhibitors, and histone decetylase inhibitors.

Drugs that are currently approved for influenza are Amantadine, Rimantadine and Oseltamivir. Any of these drugs can be used in combination or alternation with an active compound provided herein to treat a viral infection susceptible to such. Ribavirin is used to treat measles, Influenza A, influenza B, parainfluenza, severe RSV bronchiolitis and SARS as well as other viral infections, and therefore is particularly useful in combination with the present compound for treatment of the host infected with a single stranded RNA virus. Palivizumab is approved for use in infants with high risk for RSV infection.

Currently, there are no approved drugs for West Nile virus. Physicians are recommended to provide intensive support therapy, which may involve hospitalization, intravenous fluids, use of a ventilator to assist breathing, medications to control seizures, brain swelling, nausea and vomiting, and the use of antibiotics to prevent bacterial infections for making the disease even worse. This highlights the importance of the present compounds for viral medical therapy.

VI. Process of Preparation of β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-Substituted Purine Nucleotides of the Invention

General methods for providing the compounds of the present invention are known in the art or described herein. The synthesis of 2'-chloro nucleotides is described in US 20150366888, WO 2014058801; WO 2015/066370 and WO 2015200219.

The following abbreviations are used in the synthetic schemes.

CBr₄: Carbon tetrabromide

DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene

DCM: Dichloromethane

THF: Tetrahydrofuran (THF), anhydrous

EtOAc: Ethyl acetate

EtOH: Ethanol

Li(OtBu)₃AlH: Lithium tri-tert-butoxyaluminum hydride

Na₂SO₄: Sodium sulphate (anhydrous)

MeCN: Acetonitrile

MeNH₂:Methylamine

MeOH: Methanol

Na₂SO₄: Sodium sulfate

NaHCO₃: Sodium bicarbonate

NH₄Cl: Ammonium chloride

NH₄OH: Ammonium hydroxide

PE: Petroleum ether

Ph₃P: Triphenylphosphine

Silica gel (230 to 400 mesh, Sorbent)

t-BuMgCl: *t*-Butyl magnesium chloride

t-BuOK: Sodium tert-butoxide

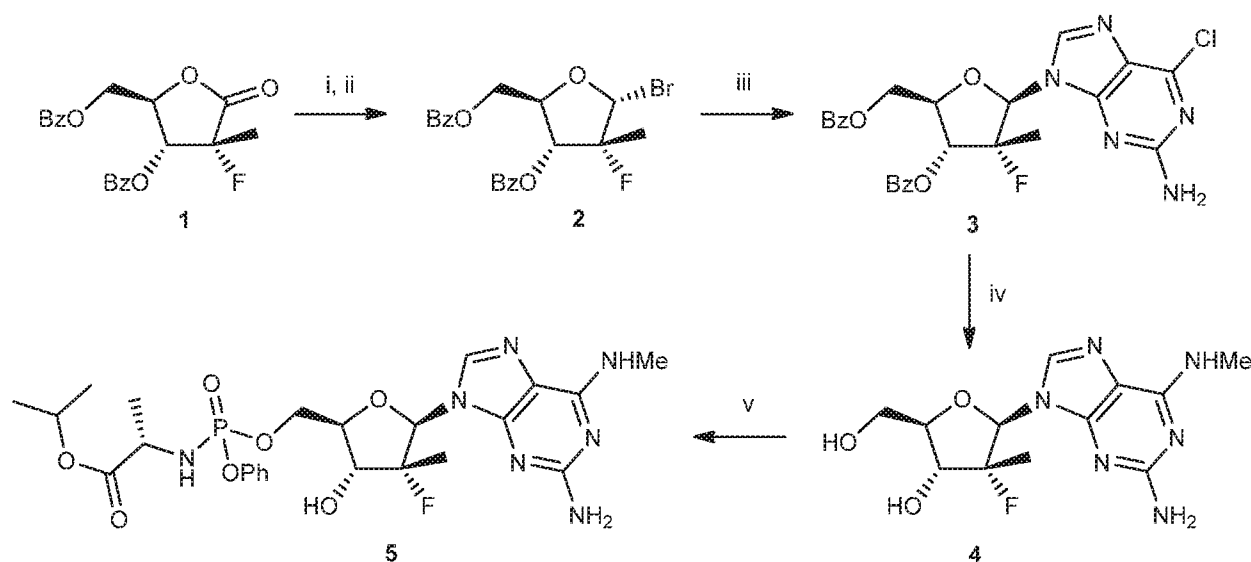
t-BuOH: Tert-butanol

EXAMPLES

5 General Methods

^1H , ^{19}F and ^{31}P NMR spectra were recorded on a 300 MHz Fourier transform Brücker spectrometer. Spectra were obtained from samples prepared in 5 mm diameter tubes in CDCl_3 , CD_3OD or $\text{DMSO}-d_6$. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), m (multiplet) and, br (broad). Coupling constants (J) are reported in Hz.

10 MS spectra were obtained using electrospray ionization (ESI) on an Agilent Technologies 6120 quadrupole MS apparatus. The reactions were generally carried out under a dry nitrogen atmosphere using Sigma-Aldrich anhydrous solvents. All common chemicals were purchased from commercial sources.



i) $\text{Li}(\text{OtBu})_3\text{AlH}$, THF, $-30\text{ }^\circ\text{C} \rightarrow -15\text{ }^\circ\text{C}$; ii) PPh_3 , CBr_4 , DCM, $-20\text{ }^\circ\text{C} \rightarrow 0\text{ }^\circ\text{C}$; iii) 2-amino-6-chloropurine, $t\text{BuOK}$, $t\text{BuOH}/\text{MeCN}$ 9:1, $65\text{ }^\circ\text{C}$; iv) MeNH_2 (33%), MeOH, $85\text{ }^\circ\text{C}$; v) Isopropyl ((R,S)-(pentafluorophenoxy)-phenoxy-phosphoryl)- L -alaninate, $t\text{BuMgCl}$, THF, $0\text{ }^\circ\text{C} \rightarrow \text{r.t.}$

15

Example 1. Preparation of isopropyl ((((R,S)-(2 R ,3 R ,4 R ,5 R)-5-(2-amino-6-(methylamino)-9 H -purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)- L -alaninate

Step 1. Preparation of ((2*R*,3*R*,4*R*,5*R*)-3-(benzoyloxy)-5-bromo-4-fluoro-4-methyltetrahydrofuran-2-yl)methyl benzoate (**2**).

To a solution of (2*R*)-3,5-di-*O*-benzoyl-2-fluoro-2-*C*-methyl-D-ribo- γ -lactone (24.8 g, 66.6 mmol) in dry THF (333 mL), under a nitrogen atmosphere and cooled to -30 °C, was added lithium tri-*tert*-butoxyaluminum hydride (1.0 M in THF, 22.6 mL, 22.6 mmol) dropwise. After completion of the addition the reaction mixture was slowly warmed up to -15 °C over 90 min then EtOAc was added (300 mL) and the mixture was quenched with a saturated aq. NH₄Cl solution (200 mL). The resulting solution was filtered on Celite® and the filtrate was extracted twice with EtOAc. The combined organics were dried (Na₂SO₄), filtered and concentrated. The residue was taken up in dry DCM (225 mL) under a nitrogen atmosphere, cooled to -20 °C, then PPh₃ (19.1 g, 72.8 mmol) was added. After 10 min of stirring at -20 °C, CBr₄ (26.0 g, 78.4 mmol) was added and the reaction mixture was allowed to slowly warm up to 0 °C over 2 h. The resulting mixture was poured onto a silica gel column and eluted with PE/EtOAc (gradient 100:0 to 80:20). The fractions containing the α -bromofuranoside were collected and concentrated to afford the product **2** (18.1 g, 41.3 mmol, 62% over two steps) as a thick colorless oil.

¹H NMR (300 MHz, CDCl₃) δ 8.15-8.11 (m, 2H), 8.04-8.01 (m, 2H), 7.64-7.55 (m, 2H), 7.51-7.41 (m, 4H), 6.34 (d, *J* = 1.6 Hz, 1H), 5.29 (dd, *J* = 5.5, 3.1 Hz, 1H), 4.89-4.85 (m, 1H), 4.78 (dd, *J* = 12.5, 3.2 Hz, 1H), 4.63 (dd, *J* = 12.5, 4.5 Hz, 1H), 1.72 (d, *J* = 21.6 Hz, 3H). ¹⁹F NMR (282 MHz, CDCl₃) δ -150.0.

Step 2. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-chloro-9*H*-purin-9-yl)-2-(benzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl benzoate (**3**).

2-Amino-6-chloropurine (2.63 g, 15.5 mmol) was suspended in *t*-BuOH (54 mL) under a nitrogen atmosphere. The reaction mixture was heated to 30 °C then potassium *tert*-butoxide (1.69 g, 15.1 mmol) was added. After 45 min a solution of bromofuranoside **2** (2.24 g, 5.12 mmol) dissolved in anhydrous MeCN (6 mL) was added, the reaction mixture was heated to 65 °C for 16 h then cooled down to room temperature. A saturated aq. NH₄Cl solution (70 mL) was added and the resulting solution was extracted with EtOAc (3 x 60mL). The combined organics were dried (Na₂SO₄), filtered and concentrated. The residue was purified twice by column chromatography (gradient PE/EtOAc 80:20 to 0:100 then 60:40 to 20:80) to afford the product **3** (1.56 g, 2.96 mmol, 57%) as a white solid.

¹H NMR (300 MHz, CDCl₃) δ 8.05-8.02 (m, 2H), 7.95-7.92 (m, 2H), 7.88 (s, 1H), 7.63-7.57 (m, 1H), 7.53-7.41 (m, 3H), 7.35-7.30 (m, 2H), 6.43 (dd, *J* = 22.6, 9.1 Hz, 1H), 6.12 (d, *J* = 18.3 Hz, 1H), 5.34 (br s, 2H), 5.00 (dd, *J* = 11.9, 4.5 Hz, 1H), 4.79-4.73 (m, 1H), 4.60 (dd, *J* = 11.9, 5.3 Hz, 1H), 1.34 (d, *J* = 22.6 Hz, 3H). ¹⁹F NMR (282 MHz, CDCl₃) δ -157.0. MS (ESI) *m/z* calcd. for C₂₅H₂₂FN₅O₅ [M+H]⁺ 526.9; found 527.0.

Step 3. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (**4**).

To a solution of compound **3** (575 mg, 1.09 mmol) in MeOH (9 mL) was added methylamine (33% in absolute EtOH, 1.7 mL, 1.81 mmol). The reaction mixture was heated to 85 °C in a sealed tube for 16 h, cooled down to room temperature and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 85:15) then reverse phase column chromatography (gradient H₂O/MeOH 100:0 to 0:100) to afford the product **4** (286 mg, 0.91 mmol, 84%) as a white solid.

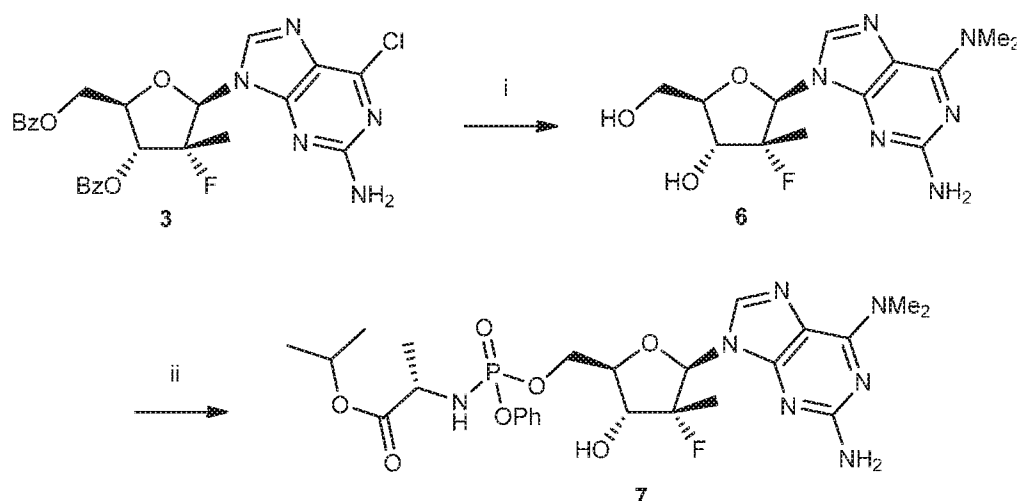
¹H NMR (300 MHz, CD₃OD) δ 8.06 (s, 1H), 6.11 (d, *J* = 18.1 Hz, 1H), 4.41 (dd, *J* = 24.4, 9.1 Hz, 1H), 4.07-4.01 (m, 2H), 3.86 (dd, *J* = 12.9, 3.3 Hz, 1H), 3.04 (br s, 3H), 1.16 (d, *J* = 22.3 Hz, 3H). ¹⁹F NMR (282 MHz, CD₃OD) δ -163.7. MS (ESI) *m/z* calcd. for C₁₂H₁₉FN₆O₃ [M+H]⁺ 313.1; found 313.2.

Step 4. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (**5**).

To a solution of compound **4** (114 mg, 365 μmol) in dry THF (4 mL), under a nitrogen atmosphere and cooled to 0 °C was added *t*-butyl magnesium chloride (1.0 M in THF, 0.66 mL, 660 μmol) dropwise over 10 min. The reaction mixture was stirred 15 min at 0 °C then another 15 min at room temperature. The reaction mixture was cooled down to 0 °C then a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, Ross, B.S., Reddy, P.G., Zhang, H.R., Rachakonda, S., and Sofia, M.J., J. Org. Chem., (2011), (253 mg, 558 μmol) dissolved in dry THF (1 mL) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30 min followed by 18 h at room temperature then quenched with a saturated aq. NH₄Cl solution (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organics were

dried, filtered (Na₂SO₄) and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) then reverse phase column chromatography (gradient H₂O/MeOH 100:0 to 0:100) to afford product **5** (a mixture of diastereomers, 101 mg, 174 μ mol, 48%) as a white solid.

- 5 ¹H NMR (300 MHz, CD₃OD) δ 7.83 (s, 0.55H), 7.82 (s, 0.45H), 7.38-7.16 (m, 5H), 6.15 (d, J = 18.5 Hz, 0.45 H), (d, J = 18.8 Hz, 0.55 H), 4.99-4.88 (overlapped with H₂O, m, 1H), 4.65-4.36 (m, 3H), 4.25-4.17 (m, 1H), 3.97-3.85 (m, 1H), 3.05 (br s, 3H), 1.32-1.28 (m, 3H), 1.25-1.15 (m, 9H). ¹⁹F NMR (282 MHz, CD₃OD) δ -162.8 (s), -163.3 (s). ³¹P NMR (121 MHz, CD₃OD) δ 4.10 (s), 3.99 (s). MS (ESI) m/z calcd. for C₂₄H₃₄FN₇O₇P [M+H]⁺ 582.2; found 582.2.



- 10 i) Me₂NH HCl, DBU, MeOH, 85 °C; v) Isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, *t*BuMgCl, THF, 0 °C.

Example 2. Preparation of isopropyl (((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-Amino-6-(dimethylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (7).

- 15 Step 1. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(dimethylamino)-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (**6**).

- To a solution of compound **3**, from Example 1, (500 mg, 0.95 mmol) in MeOH (6 mL) was added dimethylamine hydrochloride (783 mg, 9.6 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.43 mL, 9.6 mmol). The reaction mixture was heated at 85 °C in a sealed tube for 6 h,
20 cooled down to room temperature and concentrated. The residue was purified by column

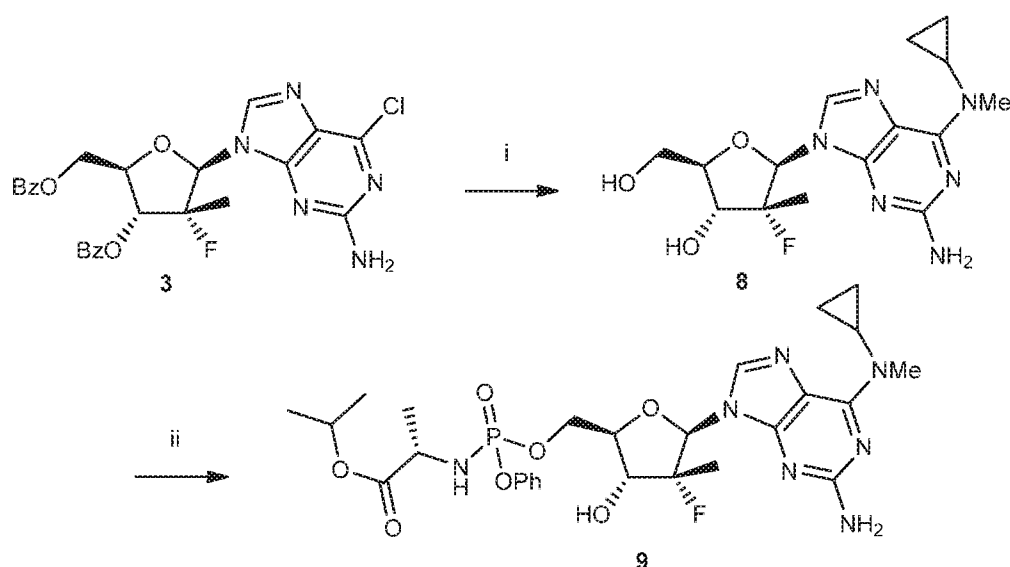
chromatography (gradient DCM/MeOH 100:0 to 85:15) then by reverse phase column chromatography (gradient H₂O/MeOH 100:0 to 0:100) to afford product **6** (200 mg, 0.61 mmol, 64%) as a white solid.

¹H NMR (300 MHz, CD₃OD) δ 8.07 (s, 1H), 6.14 (d, J = 18.1 Hz, 1H), 4.41 (dd, J = 24.4, 9.2 Hz, 1H), 4.08-4.02 (m, 2H), 3.87 (dd, J = 12.8, 2.9 Hz, 1H), 3.42 (br s, 6H), 1.16 (d, J = 22.0 Hz, 3H). ¹⁹F NMR (282 MHz, CD₃OD) δ -163.8. MS (ESI) m/z calcd. for C₁₃H₂₀FN₆O₃ [M+H]⁺ 327.2; found 327.2.

Step 2. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(dimethylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (**7**).

To a solution of compound **6** (80 mg, 245 μ mol) in dry THF (4 mL), under a nitrogen atmosphere and cooled to 0 °C was added *tert*-butyl magnesium chloride (1.0 M in THF, 0.64 mL, 640 μ mol) drop-wise over 10 min. The reaction mixture was stirred 15 min at 0 °C then another 15 min at room temperature. The reaction mixture was cooled down to 0 °C then a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate (167 mg, 367 μ mol) dissolved in dry THF (4 mL) was added drop-wise over 10 min. The reaction mixture was stirred at 0 °C for 30 min and 18 h at room temperature. The reaction was quenched with a saturated aq. NH₄Cl solution (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organics were dried, filtered (Na₂SO₄) and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) and then by reverse phase column chromatography (gradient H₂O/MeOH 100:0 to 0:100) to afford the product **7** (mixture of diastereomers, 35 mg, 58 μ mol, 24%) as a white solid.

¹H NMR (300 MHz, CD₃OD) δ 7.83 (s, 0.5H), 7.82 (s, 0.5H), 7.34-7.16 (m, 5H), 6.15 (d, J = 18.7 Hz, 0.5 H), 6.13 (d, J = 18.8 Hz, 0.5 H), 4.99-4.85 (overlapped with H₂O, m, 1H), 4.65-4.26 (m, 3H), 4.27-4.12 (m, 1H), 3.99-3.81 (m, 1H), 3.42, 3.41 (2br s, 6H), 1.36-1.25 (m, 3H), 1.24-1.11 (m, 9H). ¹⁹F NMR (282 MHz, CD₃OD) δ -162.7 (s), -163.2 (s). ³¹P NMR (121 MHz, CD₃OD) δ 4.08 (s), 4.00 (s). MS (ESI) m/z calcd. for C₂₅H₃₆FN₇O₇P [M+H]⁺ 596.5; found 596.2.



i) a) *N*-Methylcyclopropylamine hydrochloride, Et₃N, MeOH, 100 °C; b) NH₄OH, MeOH, 100 °C; ii) Isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, *t*BuMgCl, THF, 0 °C.

Example 3. Preparation of Isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methylcyclopropylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (9).

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Step 1. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-Amino-6-(*N*-methyl-cyclopropylamino)-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (**8**).

To a solution of compound **3** (600 mg, 1.14 mmol) in MeOH (10 mL) was added *N*-methylcyclopropylamine hydrochloride (366 mg, 3.40 mmol) and triethylamine (470 μL, 3.40 mmol). The reaction mixture was heated at 100° C in a sealed tube for 15 h and cooled down to room temperature. An aqueous solution containing 30% NH₄OH (4 mL) was added and the reaction mixture was heated at 100° C in a sealed tube for 2 h, cooled down and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) to afford product **8** (351 mg, 0.99 mmol, 87%) as a white solid.

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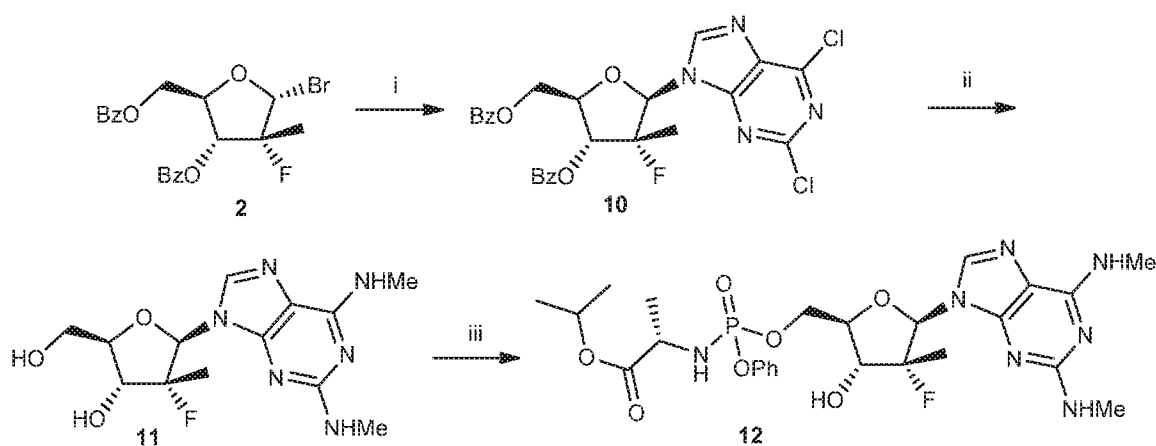
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¹H NMR (300 MHz, CD₃OD) δ 8.13 (s, 1H), 6.15 (d, *J* = 18.0 Hz, 1H), 4.40 (dd, *J* = 24.3, 9.0 Hz, 1H), 4.06-4.02 (m, 2H), 3.89-3.83 (m, 1H), 3.32 (m, 3H), 3.18-3.11 (m, 1H), 1.16 (d, *J* = 22.2 Hz, 3H), 0.96-0.89 (m, 2H), 0.74-0.69 (m, 2H). ¹⁹F NMR (282 MHz, CD₃OD) δ -163.8. MS (ESI) *m/z* calcd. for C₁₅H₂₂FN₆O₃ [M+H]⁺ 353.2; found 353.2.

Step 2. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methyl-cyclopropylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (**9**).

To a solution of compound **8** (200 mg, 0.57 mmol) in dry THF (15 mL) at 0° C was added *tert*-butyl magnesium chloride (1.0 M in THF, 680 µL, 0.68 mmol) dropwise over 10 min. The reaction mixture was stirred 15 min at 0° C then another 15 min at room temperature. The reaction mixture was cooled down to 0° C and a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate (283 mg, 0.62 mmol) dissolved in dry THF (4 mL) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30 min and 18 h at room temperature. The reaction was quenched with a saturated aq. NH₄Cl solution (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) and then by reverse phase column chromatography (gradient H₂O/MeOH 100:0 to 0:100) to afford product **9** (mixture of 2 diastereoisomers, 160 mg, 0.26 mmol, 45%) as a white solid.

¹H NMR (300 MHz, CD₃OD) δ 7.85 (m, 1H), 7.38-7.16 (m, 5H), 6.18 (d, *J* = 18.6 Hz) and 6.16 (d, *J* = 18.9 Hz, 1H), 4.95-4.90 (overlapped with H₂O, m, 1H), 4.58-4.47 (m, 3H), 4.22-4.19 (m, 1H), 3.95-3.87 (m, 1H), 3.36-3.34 (overlapped with MeOH, m, 3H), 3.19-3.12 (m, 1H), 1.32-1.22 (m, 12H), 0.96-0.89 (m, 2H), 0.74-0.69 (m, 2H). ³¹P NMR (121 MHz, CD₃OD) δ 4.11 (s), 4.02 (s). MS (ESI) *m/z* calcd. for C₂₇H₃₈FN₇O₇P [M+H]⁺ 622.2; found 622.2.



i) 2,6-dichloropurine, *t*BuOK, *t*BuOH/MeCN, 65 °C; ii) MeNH₂, MeOH, 130°C; iii) Isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, *t*BuMgCl, THF, 0 °C to RT

Example 4. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2,6-bis-methylamino-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (12).

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Step 1. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2,6-dichloro-9*H*-purin-9-yl)-2-(benzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl benzoate (**10**).

The compound 2,6-dichloropurine (1.30 g, 6.86 mmol) was suspended in *t*-BuOH (25 mL) under a nitrogen atmosphere. Potassium *tert*-butoxide (778 mg, 6.92 mmol) was added portion-wise then the reaction mixture was stirred at room temperature. After 1 h, a solution of bromofuranoside **2** (1.0 g, 2.29 mmol) dissolved in anhydrous MeCN (20 mL) was added and the reaction mixture was heated at 65 °C overnight and then cooled down to room temperature. A saturated aq. NH₄Cl solution was added and the resulting solution was extracted with EtOAc (3 times). The combined organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient PE/EtOAc 100:0 to 0:100) to afford product **10** (148 mg, 0.27 mmol, 12%) as a sticky solid.

¹H NMR (300 MHz, CDCl₃) δ 8.31 (s, 1H), 8.12-8.09 (m, 2H), 8.02-7.99 (m, 2H), 7.64-7.39 (m, 6H), 6.38 (d, *J* = 17.2 Hz, 1H), 6.02 (dd, *J* = 21.2, 8.9 Hz, 1H), 4.90-4.68 (m, 3H), 1.33 (d, *J* = 22.4 Hz, 3H). ¹⁹F NMR (282 MHz, CDCl₃) δ -158.0. MS (ESI) *m/z* calcd. for C₂₅H₂₀Cl₂FN₄O₅ [M+H]⁺ 546.4; found 546.3.

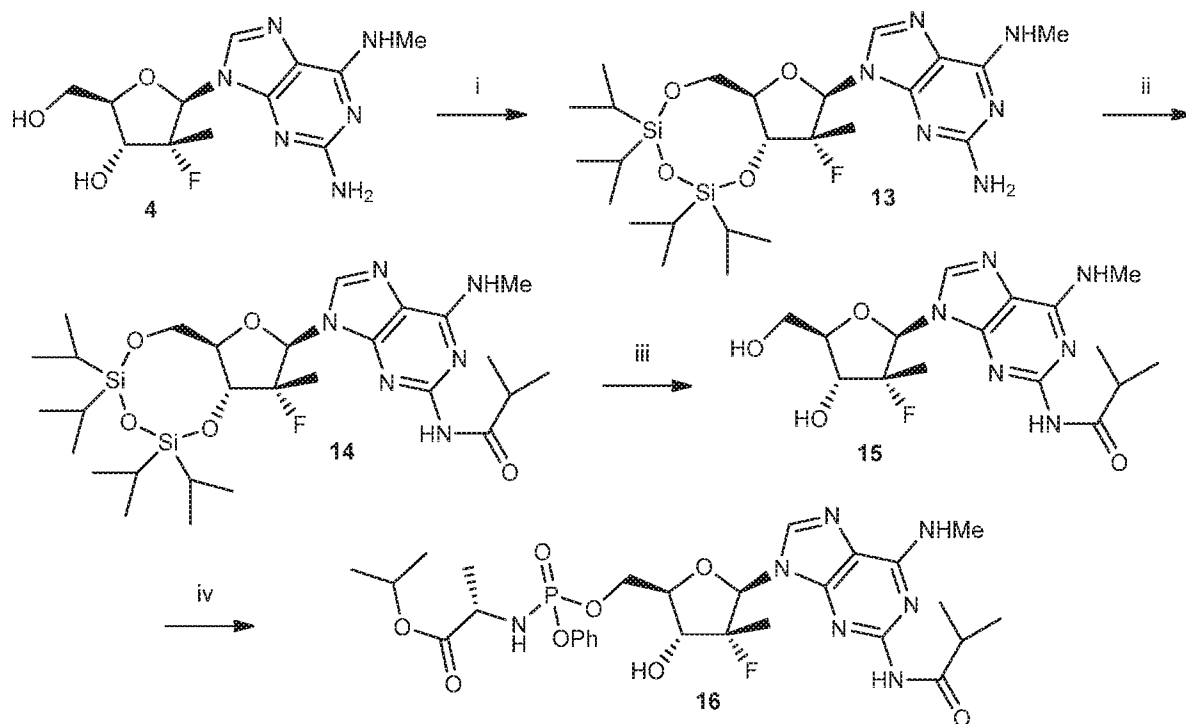
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Step 2. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2,6-*bis*-methylamino-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (**11**).

A solution of compound **10** (148 mg, 0.27 mmol) in methylamine (33% in EtOH, 30 mL) was heated at 130 °C in a sealed tube for 4 days, cooled down to room temperature and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 50:50) followed by reverse phase column chromatography (gradient H₂O/MeOH 100:0 to 0:100) to afford product **11** (33 mg, 0.10 mmol, 37%) as a white solid. ¹H NMR (300 MHz, CD₃OD) δ 8.00 (s, 1H), 6.12 (d, *J* = 18.5 Hz, 1H), 4.51 (dd, *J* = 24.4, 9.5 Hz, 1H), 4.06-3.85 (m, 3H), 3.04 (s, 3H), 2.93 (s, 3H), 1.20 (d, *J* = 22.4 Hz, 3H). ¹⁹F NMR (282 MHz, CD₃OD) δ -163.2. MS (ESI) *m/z* calcd. for C₁₃H₂₀FN₆O₃ [M+H]⁺ 327.2; found 327.2.

Step 3. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2,6-*bis*-methylamino-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (**12**).

To a solution of compound **11** (55 mg, 0.17 mmol) in dry THF (2 mL) at 0 °C was added *tert*-butyl magnesium chloride (1 M in THF, 304 μL, 0.30 mmol) dropwise over 10 min. The reaction mixture was stirred 15 min at 0 °C and then 15 min at room temperature. The solution was cooled down to 0 °C and a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate (115 mg, 0.25 mmol) dissolved in dry THF (1 mL) was dropwise added over 10 min. The mixture was warmed slowly to room temperature and stirred for 4 days. The reaction was quenched with a saturated aq. NH₄Cl solution and extracted with EtOAc (3 times). The combined organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 50:50) to yield product **12** (mixture of diastereomers, 13 mg, 0.02 mmol, 13%) as a white solid. ¹H NMR (300 MHz, CD₃OD) δ 7.78 (s, 1H), 7.35-7.12 (m, 5H), 6.13 (d, *J* = 19.1 Hz, 0.53H), 6.10 (d, *J* = 19.2 Hz, 0.47H), 4.99-4.78 (overlapped with H₂O, m, 1H), 4.72-4.46 (m, 3H), 4.24-4.15 (m, 1H), 3.79-3.92 (m, 1H), 3.02 (br s, 3H), 2.92 (s+s, 3H), 1.29-1.11 (m, 12H). ¹⁹F NMR (282 MHz, CD₃OD) δ -162.0 (s), -162.3 (s). ³¹P NMR (121 MHz, CD₃OD) δ 3.97 (s), 3.89 (s). MS (ESI) *m/z* calcd. for C₂₅H₃₆FN₇O₇P [M+H]⁺ 596.6; found 596.2.



i) TIPDSCl₂, imidazole, DMF; ii) isobutyryl chloride, pyridine; iii) TBAF, THF; iv) Isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, *t*BuMgCl, THF, 0 °C.

Example 5. Preparation of isopropyl (((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-isobutyramido-6-methylamino-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (16).

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Step 1. Preparation of compound 13.

To a solution of compound **4** (286 mg, 0.92 mmol) and imidazole (370 mg, 5.43 mmol) in dry DMF (6 mL) at 0 °C was added 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (300 µL, 0.94 mmol). The reaction mixture was stirred for 2 h at RT, diluted with EtOAc (50 mL) and the suspension was washed with saturated aq. NH₄Cl solution and brine (40 mL each). The organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient PE/EtOAc 7:3 to 3:7) to afford product **13** (283 mg, 0.51 mmol, 56%) as a white solid. MS (ESI) *m/z* calcd. for C₂₄H₄₄FN₆O₄Si₂ [M+H]⁺ 555.8; found 555.2.

Step 2. Preparation of compound 14.

To a solution of compound **13** (200 mg, 0.36 mmol) in dry pyridine (3 mL) at 0 °C was added isobutyryl chloride (38 µL, 0.36 mmol). The reaction mixture was stirred for 2 h at RT.

The reaction was quenched by the addition of water (500 μ L). The mixture was concentrated and co-evaporated with toluene (3 x 10 mL). The residue was purified by column chromatography (gradient PE/EtOAc 1:0 to 1:1) to afford product **14** (99 mg, 0.16 mmol, 44%) as a white solid. MS (ESI) m/z calcd. for $C_{28}H_{50}FN_6O_5Si_2$ $[M+H]^+$ 625.9; found 625.3.

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Step 3. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-isobutyramido-6-methylamino-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (**15**).

To a solution of compound **14** (90 mg, 0.14 mmol) in dry THF (2 mL) was added tetrabutylammonium fluoride (1 M in THF, 38 μ L, 0.38 mmol). The mixture was stirred for 2 h at RT and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 10:0 to 9:1) followed by reverse phase column chromatography (gradient H₂O/MeOH 100:0 to 0:100) to give product **15** (42 mg, 0.11 mmol, 77%) as a white solid. ¹H NMR (300 MHz, CD₃OD) δ 8.31 (s, 1H), 6.29 (d, J = 17.9 Hz, 1H), 4.70-4.60 (m, 1H), 4.07-3.98 (m, 2H), 3.89 (dd, J = 12.5, 3.4 Hz, 1H), 3.10 (br s, 3H), 2.87 (br s, 1H), 1.23-1.16 (m, 9H). ¹⁹F NMR (282 MHz, CD₃OD) δ -163.8. MS (ESI) m/z calcd. for $C_{16}H_{24}FN_6O_4$ $[M+H]^+$ 383.4; found 383.2.

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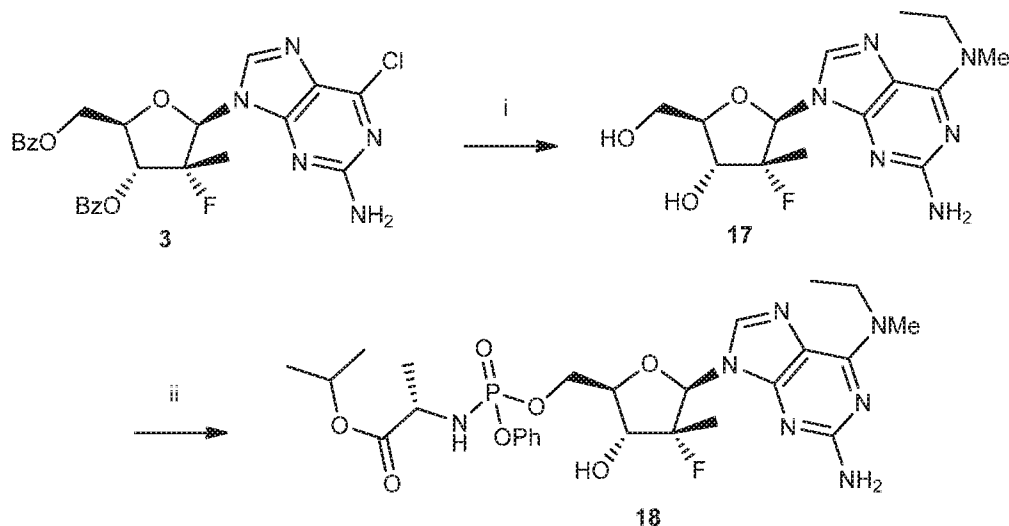
Step 4. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-isobutyramido-6-methylamino-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (**16**).

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To a solution of compound **15** (27 mg, 0.07 mmol) in dry THF (1 mL) at 0 °C was added *t*-butyl magnesium chloride (1.0 M in THF, 130 μ L, 0.13 mmol) dropwise over 10 min. The reaction mixture was stirred 15 min at 0 °C then another 15 min at room temperature. The reaction mixture was cooled down to 0 °C and a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate (50 mg, 0.11 mmol) dissolved in dry THF (1 mL) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30 min followed by 18 h at room temperature then quenched with a saturated aq. NH₄Cl solution (2 mL) and extracted with EtOAc (3 x 5 mL). The combined organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 95:5) then reverse phase column chromatography (gradient H₂O/MeOH 100:0 to 0:100) to afford product **16** (mixture of 2 diastereoisomers, 25 mg, 0.04 mmol, 54%) as a white solid.

¹H NMR (300 MHz, CD₃OD) δ 8.05 (s, 1H), 7.33-7.13 (m, 5H), 6.27 (d, J = 18.6 Hz) and 6.21 (d, J = 19.1 Hz, 1H), 5.10-4.95 (m, 1H), 4.93-4.78 (overlapped with H₂O, m, 1H), 4.60-4.42 (m, 2H), 4.26-4.18 (m, 1H), 3.90-3.80 (m, 1H), 3.09 (br s, 3H), 2.84-2.80 (m, 1H), 1.33-1.15 (m, 18H). ³¹P NMR (121 MHz, CD₃OD) δ 3.69 (s). ³¹P NMR (121 MHz, CD₃OD) δ 4.11 (s), 3.99 (s). MS (ESI) m/z calcd. for C₂₈H₄₀FN₇O₈P [M+H]⁺ 652.6; found 652.3.



i) *N*-Methylethylamine, MeOH, 100 °C; ii) Isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, *t*BuMgCl, THF, 0 °C.

Example 6. Preparation of isopropyl (((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methyl-ethylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (18).

Step 1. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methyl-ethylamino)-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (17).

To a solution of compound 3 (150 mg, 0.29 mmol) in MeOH (4 mL) was added *N*-methylethylamine (245 μ L, 2.90 mmol). The reaction mixture was heated at 100 °C in a sealed tube for 15 h, cooled down to room temperature and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) to afford product 31 (89 mg, 0.26 mmol, 89%) as a white solid.

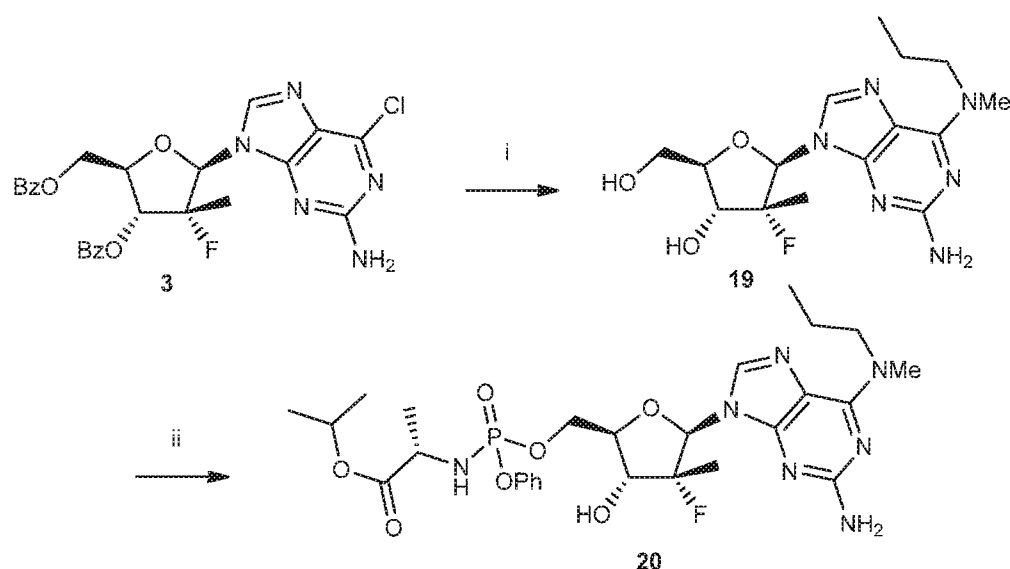
¹H NMR (300 MHz, CD₃OD) δ 8.06 (s, 1H), 6.13 (d, J = 18.0 Hz, 1H), 4.40 (dd, J = 24.9, 8.7 Hz, 1H), 4.11-4.01 (m, 4H), 3.98-3.83 (m, 1H), 3.34 (br. s, 3H), 1.24-1.11 (m, 6H). ¹⁹F NMR

(282 MHz, CD₃OD) δ -163.7. MS (ESI) m/z calcd. for C₁₄H₂₂FN₆O₃ [M+H]⁺ 341.2; found 341.2.

Step 2. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methyl-ethylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (**18**).

To a solution of compound **17** (30 mg, 0.09 mmol) in dry THF (2 mL) at 0 °C was added *tert*-butyl magnesium chloride (1.0 M in THF, 110 μ L, 0.11 mmol) dropwise over 10 min. The reaction mixture was stirred 15 min at 0 °C then another 15 min at room temperature. The reaction mixture was cooled down to 0 °C and a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate (48 mg, 0.11 mmol) dissolved in dry THF (1 mL) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30 min and 18 h at room temperature. The reaction was quenched with a saturated aq. NH₄Cl solution (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) to afford the product **18** (mixture of 2 diastereoisomers, 22 mg, 0.04 mmol, 40%) as a white solid.

¹H NMR (300 MHz, CD₃OD) δ 7.69 (m, 1H), 7.26-7.04 (m, 5H), 6.05 (d, J = 18.6 Hz) and 6.03 (d, J = 18.9 Hz, 1H), 4.86-4.79 (overlapped with H₂O, m, 1H), 4.50-4.32 (m, 3H), 4.12-4.06 (m, 1H), 3.96-3.79 (m, 3H), 3.25 (br. s, 3H), 1.24-1.02 (m, 15H). ³¹P NMR (121 MHz, CD₃OD) δ 4.07 (s), 4.00 (s). MS (ESI) m/z calcd. for C₂₆H₃₈FN₇O₇P [M+H]⁺ 609.3; found 609.2.



i) *N*-Methylpropylamine, MeOH, 100 °C; ii) Isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, *t*BuMgCl, THF, 0 °C.

Example 7. Preparation of isopropyl ((((*R,S*)-(2*R,3R,4R,5R*)-5-(2-amino-6-(*N*-methylpropylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (20).

5

Step 1. Preparation of (2*R,3R,4R,5R*)-5-(2-amino-6-(*N*-methyl-propylamino)-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (**19**).

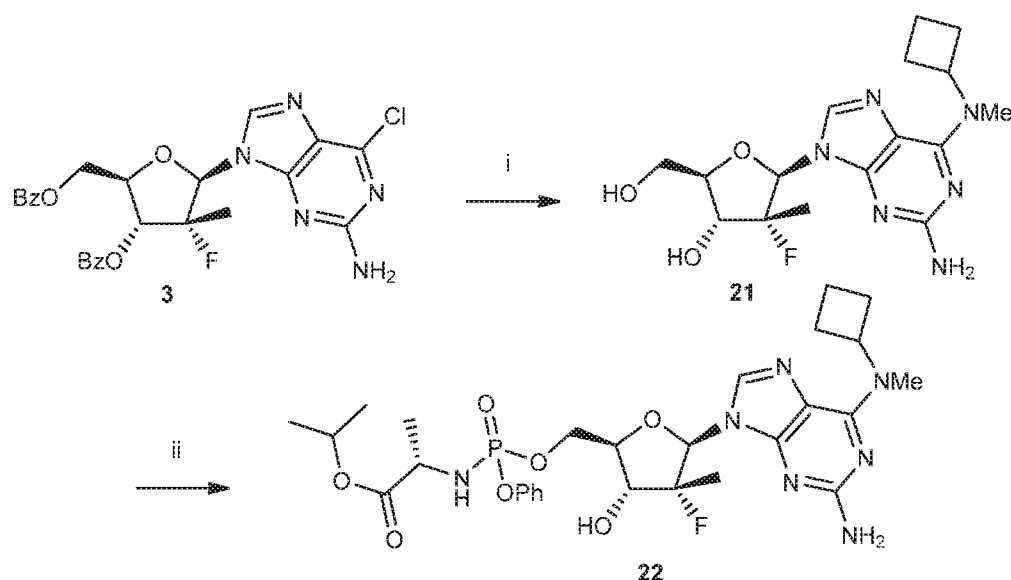
To a solution of compound **3** (150 mg, 0.29 mmol) in MeOH (4 mL) was added *N*-methylpropylamine (295 μ L, 2.90 mmol). The reaction mixture was heated at 100 °C in a sealed tube for 15 h, cooled down to room temperature and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) then reverse phase column chromatography (gradient H₂O/MeOH 100:0 to 0:100) to afford product **19** (80 mg, 0.23 mmol, 78%) as a white solid.

¹H NMR (300 MHz, CD₃OD) δ 8.04 (s, 1H), 6.13 (d, *J* = 18.3, 1H), 4.40 (dd, *J* = 24.2, 9.2 Hz, 1H), m, 4.06-3.84 (m, 5H), 1.68 (sept, *J* = 7.5 Hz, 2H), 1.15 (d, *J* = 22.2 Hz, 3H), 0.93 (t, *J* = 7.5 Hz, 3H). ¹⁹F NMR (282 MHz, CD₃OD) δ -163.8. MS (ESI) *m/z* calcd. for C₁₅H₂₄FN₆O₃ [M+H]⁺ 355.2; found 355.2.

Step 2. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methyl-propylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (**20**).

To a solution of compound **19** (30 mg, 0.09 mmol) in dry THF (2 mL) at 0 °C was added
5 *tert*-butyl magnesium chloride (1.0 M in THF, 110 µL, 0.11 mmol) dropwise over 10 min. The reaction mixture was stirred 15 min at 0 °C then another 15 min at room temperature. The reaction mixture was cooled down to 0 °C and a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate (46 mg, 0.11 mmol) dissolved in dry THF (1 mL) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30
10 min and 18 h at room temperature. The reaction was quenched with a saturated aq. NH₄Cl solution (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) to afford product **20** (mixture of 2 diastereoisomers, 22 mg, 0.03 mmol, 33%) as a white solid.

15 ¹H NMR (300 MHz, CD₃OD) δ 7.78, 7.77 (s+s, 1H), 7.37-7.13 (m, 5H), 6.15 (d, *J* = 18.6 Hz) and 6.13 (d, *J* = 18.9 Hz, 1H), 4.97-4.89 (overlapped with H₂O, m, 1H), 4.63-4.30 (m, 3H), 4.22-4.14 (m, 1H), 4.02-3.84 (m, 2H), 1.74-1.63 (3H, m), 1.32-1.27 (m, 3H), 1.23-1.13 (m, 9H), 0.94 (t, *J* = 7.4 Hz) and 0.93 (t, *J* = 7.4 Hz, 3H). ³¹P NMR (121 MHz, CD₃OD) δ 4.05 (s), 4.00 (s). MS (ESI) *m/z* calcd. for C₂₇H₄₀FN₇O₇P [M+H]⁺ 623.3; found 623.2.



i) a) *N*-Methylcyclobutylamine hydrochloride, Et₃N, MeOH, 100 °C; b) NH₄OH, MeOH, 100° C; ii) Isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, *t*BuMgCl, THF, 0 °C.

Example 8. Preparation of Isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methylcyclobutylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (22).

5

Step 1. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methylcyclobutylamino)-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (**21**).

To a solution of compound **3** (150 mg, 0.29 mmol) in MeOH (4 mL) was added *N*-methylcyclobutylamine hydrochloride (105 mg, 0.90 mmol) and triethylamine (190 μL, 1.00 mmol). The reaction mixture was heated at 100 °C in a sealed tube for 15 h and cooled down to room temperature. An aqueous solution containing 30% NH₄OH (1 mL) was added and the reaction mixture was heated at 100 °C in a sealed tube for 2 h, cooled down and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) to afford product **21** (90 mg, 0.25 mmol, 86%) as a pale yellow solid.

¹H NMR (300 MHz, CD₃OD) δ 8.09 (s, 1H), 6.14 (d, *J* = 18.0 Hz, 1H), 5.80-5.70 (m, 1H), 4.44-4.33 (m, 1H), 4.06-4.02 (m, 2H), 3.88-3.84 (m, 1H), 3.34 (s, 3H), 2.38-2.19 (m, 4H), 1.79-1.71 (m, 2H), 1.17 (d, *J* = 22.2 Hz, 3H). ¹⁹F NMR (282 MHz, CD₃OD) δ -163.8. MS (ESI) *m/z* calcd. for C₁₆H₂₄FN₆O₃ [M+H]⁺ 367.2; found 367.2.

Step 2. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methylcyclobutylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (**22**).

To a solution of compound **21** (50 mg, 0.14 mmol) in dry THF (2 mL) at 0 °C was added
 5 *tert*-butyl magnesium chloride (1.0 M in THF, 210 µL, 0.21 mmol) dropwise over 10 min. The reaction mixture was stirred 15 min at 0 °C then another 15 min at room temperature. The reaction mixture was cooled down to 0 °C and a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate (74 mg, 0.16 mmol) dissolved in dry THF (2 mL) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30
 10 min and 18 h at room temperature. The reaction was quenched with a saturated aq. NH₄Cl solution (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) and then by reverse phase column chromatography (gradient H₂O/MeOH 100:0 to 0:100) to afford product **22** (mixture of 2 diastereoisomers, 24 mg, 0.04
 15 mmol, 28%) as a white solid.

¹H NMR (300 MHz, CD₃OD) δ 7.79 (s, 0.2H), 7.77 (s, 0.8H), 7.38-7.12 (m, 5H), 6.18 (d, *J* = 17.6 Hz) and 6.16 (d, *J* = 17.5 Hz, 1H), 4.95-4.81 (m, 2H), 4.62-4.43 (m, 3H), 4.25-4.18 (m, 1H), 3.96-3.83 (m, 1H), 3.38 (s) and 3.36 (s, 3H), 2.38-2.21 (m, 4H), 1.75-1.63 (m, 2H), 1.32-1.16 (m, 12H). ³¹P NMR (121 MHz, CD₃OD) δ 4.04 (s), 3.97 (s). MS (ESI) *m/z* calcd. for
 20 C₂₈H₄₀FN₇O₇P [M+H]⁺ 636.3; found 636.2.

Modification of the 2-amino moiety in the active compounds

One of ordinary skill in the art can add a substituent to the 2-amino purine moiety by methods well known to those skilled in the art. One non-limiting process is provided here, and
 25 others can be easily adapted. ((2*R*,3*R*,4*R*,5*R*)-3-(benzoyloxy)-5-bromo-4-fluoro-4-methyltetrahydrofuran-2-yl)methyl benzoate, is treated with commercially available 2,6-dichloropurine, a base and a mixture of organic solvents at an elevated temperature to generate (2*R*,3*R*,4*R*,5*R*)-5-(2,6-dichloro-9*H*-purin-9-yl)-2-(benzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl benzoate. In one embodiment, the base is potassium *tert*-butoxide.
 30 In one embodiment, the mixture of organic solvents comprises *tert*-butanol and acetonitrile. The compound, (2*R*,3*R*,4*R*,5*R*)-5-(2,6-dichloro-9*H*-purin-9-yl)-2-(benzoyloxymethyl)-4-fluoro-4-

methyltetrahydrofuran-3-yl benzoate is treated with an amine, a base and an organic solvent at ambient temperature to generate 2-chloro-N⁶-substituted purines. In one embodiment, the amine is methylamine. In one embodiment, the base is triethylamine. In one embodiment, the organic solvent is ethanol. One skilled in the art will also recognize that upon treatment with an amine and base, the benzoate groups on the nucleoside will simultaneously be removed to generate the deprotected furanose moiety. 2-Chloro-N⁶-substituted purines can then be treated with an amine, and an organic solvent in a sealed tube at an elevated temperature of about 100 °C to generate N²,N⁶-disubstituted purine nucleosides of the present invention. In one embodiment, the amine is methylamine. In one embodiment, the organic solvent is ethanol. N²,N⁶-Disubstituted purine nucleosides of the present invention can be treated with a base, isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate and an organic solvent at a reduced temperature to generate compounds of Formula I-V. In one embodiment, the base is tert-butyl magnesium chloride. In one embodiment, the organic solvent is tetrahydrofuran.

Preparation of Stereospecific Phosphorus Enantiomers

Certain of the active compounds described herein have a chiral phosphorus moiety. Any of the active compounds described herein can be provided as an isolated phosphorus enantiomeric form, for example, at least 80, 90, 95 or 98% of the R or S enantiomer, using methods known to those of skill in the art. For example, there are a number of publications that describe how to obtain such compounds, including but not limited to column chromatography, for example as described in Example 17 below and U.S. Patent No. 8,859,756; 8,642,756 and 8,333,309 to Ross, et al.

Example 9. Separation of the stereoisomers of compound 5.

The stereoisomers of Compound 5 were separated on a Phenominex Luna column using the following conditions:

Column: Phenominex Luna 5 micron C18 (2) 250 x 10 mm part# OOG-4252-BO

Sample concentration: Approximately 50 mg/ml in acetonitrile

Injection volume: 50 µl

Mobile phase A: HPLC grade water

Mobile phase B: HPLC grade acetonitrile.

Flow: 5 ml/min

UV: 283 nm

Gradient:

Time	%B
0	2
40	50
41	50
41.1	2
45	2

Run time: 45 minutes

5 Column Temperature: 40 °C

A sample chromatogram of a semi-prep run is illustrated in Figure 1.

The combined fractions were evaluated using an analytical column with the following conditions:

Column: Phenomenex Luna 5 micron C18 (2) 250 x 2mm part# OOG-4252-BO

10 Injection volume: 10 µl

Mobile phase A: HPLC grade water

Mobile phase B: HPLC grade acetonitrile.

Flow: 0.2 ml/min

UV: 283 nm

15 Gradient:

Time	%B
0	2
30	50
40	50
40.1	2
45	2

Run time: 45 minutes

Column Temperature: 40 °C

The combined fractions for each stereoisomer were evaporated to dryness using a rotovap with a bath temperature of 30 °C. The resulting solids were dissolved in 1 ml of acetonitrile,

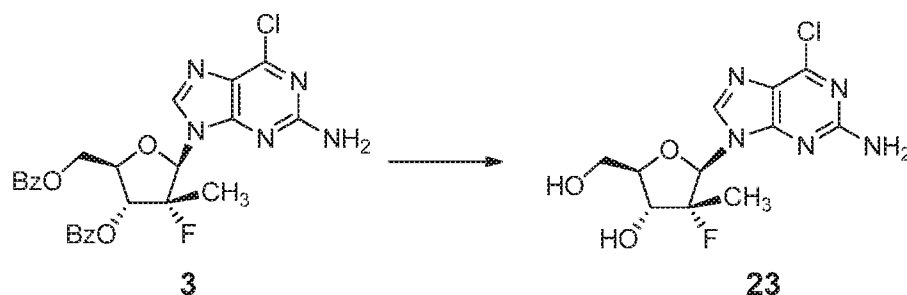
transferred into 1.7 ml microcentrifuge tubes and the solvent evaporated on the vacuum centrifuge at a temperature of 30 °C.

The data on the final samples are as follows:

1. First eluding peak: Compound 5 #1 (**5-1**) (21.7 mgs – 97.8% ee).
2. Second eluding Peak: Compound 5 #2 (**5-2**) (13.2 mgs – 95.9% ee).

The final weights of the 1st and 2nd peak correspond well to their percentages in the original mixture. (62.2% and 37.8% respectively).

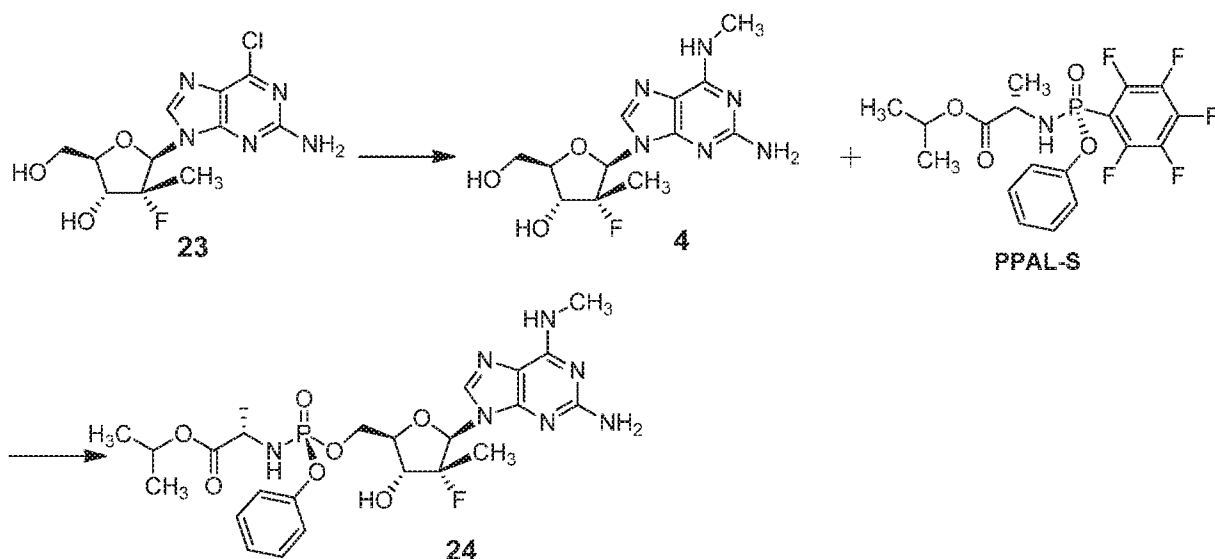
Stereospecific Syntheses of Compounds of Formula I-VII



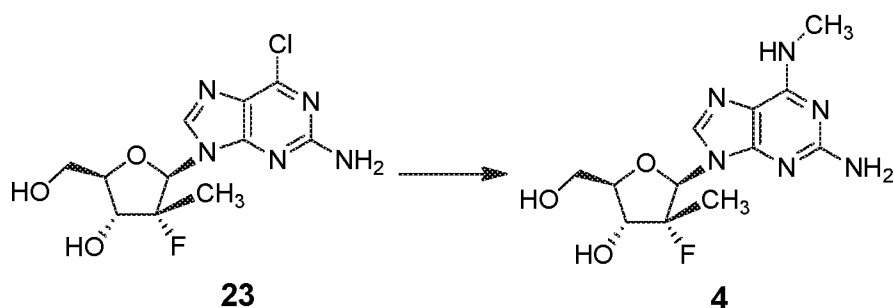
Example 10. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-chloro-9*H*-purin-9-yl)-2-(hydroxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-ol (**23**).

Step 1. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-chloro-9*H*-purin-9-yl)-2-(hydroxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-ol (**23**).

The compound (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-chloro-9*H*-purin-9-yl)-2-(benzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl benzoate, **3**, (80 g, 140 mmol) was added to a solution of trimethylamine in methanol (7 M, 800 mL) and stirred at RT overnight. The mixture was concentrated and then purified by column chromatography (DCM:MeOH = 100:1) to afford (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-chloro-9*H*-purin-9-yl)-2-(hydroxymethyl)-4-fluoro-4-methyl-tetrahydrofuran-3-ol (**23**) (40 g, 90%).



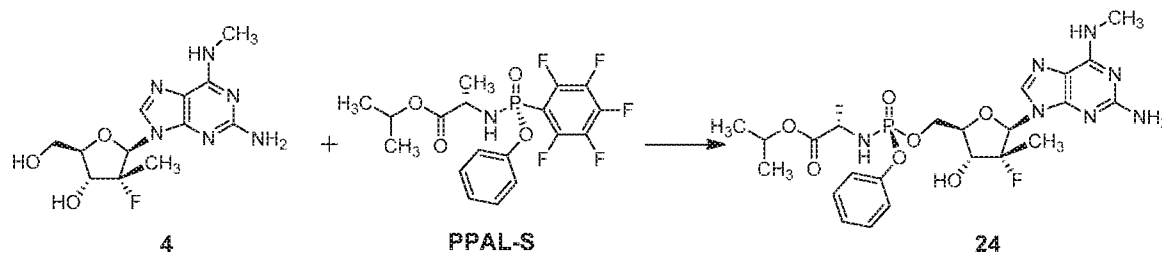
Example 11. Preparation of (((*S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate.



Step 1. Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol (**4**).

To a solution of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-chloro-9*H*-purin-9-yl)-2-(hydroxymethyl)-4-fluoro-4-methyl-tetrahydrofuran-3-ol (2.0 g, 1.0 eq) in dioxane (15 mL) was added MeNH₂ aqueous solution (5.0 eq). After stirring overnight at RT, TLC showed that the starting material was consumed. The mixture was concentrated and purified by column chromatography (DCM:MeOH = 40:1- 30:1) to afford (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol as a white powder (1.6 g, 81.6%).

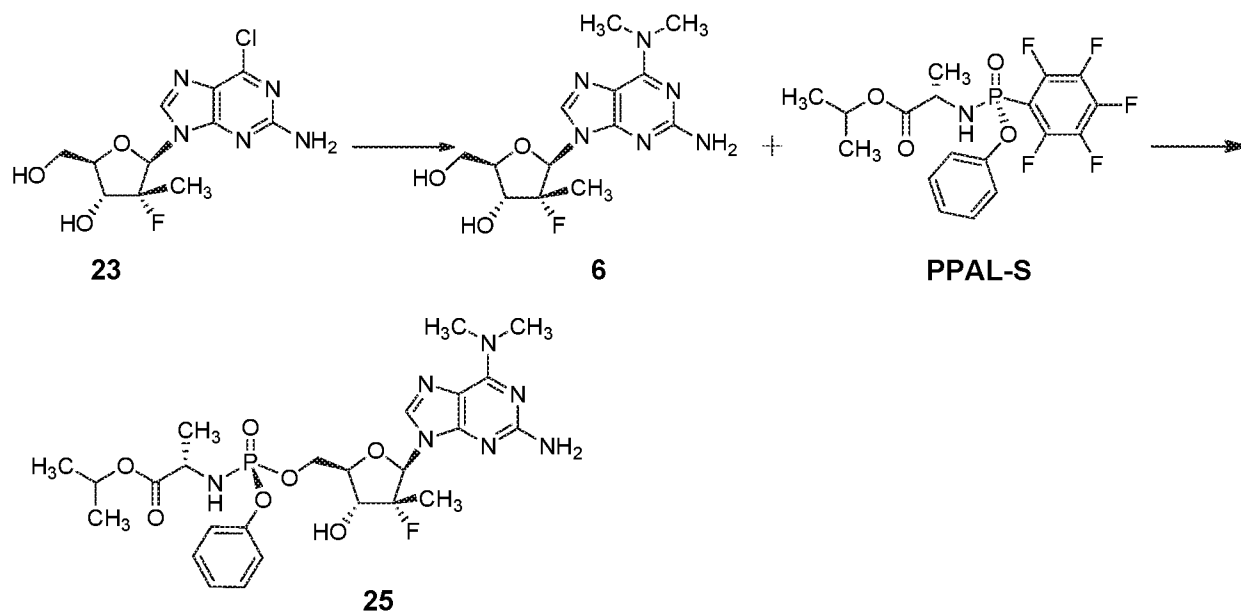
[M+H]⁺ = 313.5



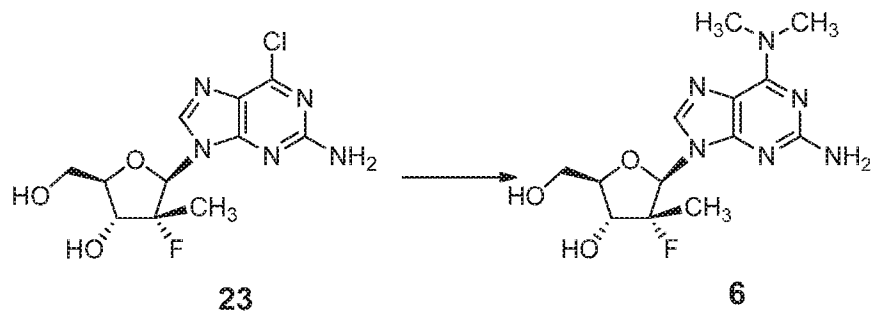
Step 2. Preparation of (((*S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate.

The compound (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol (1.47 g, 1.0 eq) and **PPAL-S** (2.35 g, 1.1 eq) were dissolved in anhydrous THF (29 mL). After cooling the mixture to -10 °C, *t*-BuMgCl (5.8 mL, 1.7 M, 2.1 eq) was slowly added under a blanket of N₂. After stirring at RT for 45 min, the mixture was quenched with aq. saturated NH₄Cl, and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with water, brine (30 mL), dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by column chromatography (DCM:MeOH = 50:1- 20:1) to afford (((*S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate as a white powder (1.1 g, 40.3%).

¹H NMR (400 MHz, CD₃OD) δ 7.81 (s, 1H), 7.33-7.16 (m, 5H), 6.10 (d, *J* = 18.4 Hz, 1H), 4.90-4.84(m, 5H), 4.55-4.46 (m, 3H), 4.20-4.16 (m, 1H), 3.91-3.87 (m, 1H), 3.30 (m, 1H), 3.03 (s, 3H), 1.30-1.20(m, 12H). [M+H]⁺ = 582.8.



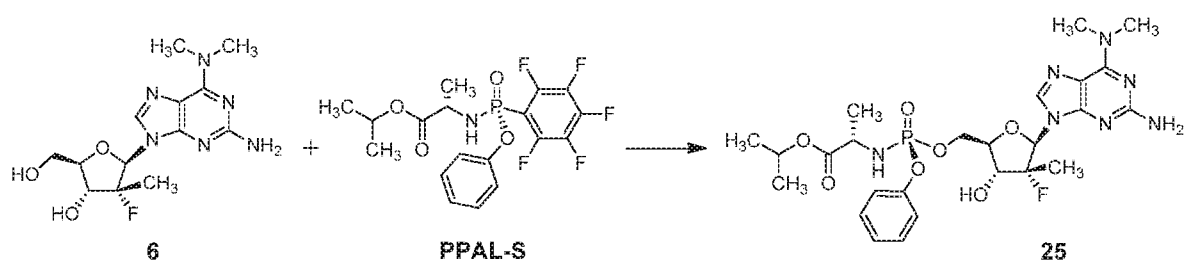
Example 12. Preparation of isopropyl (((S)-2-((2R,3R,4R,5R)-5-(2-amino-6-(dimethylamino)-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy)-phosphoryl)-L-alaninate (25).



Step 1. Preparation of (2R,3R,4R,5R)-5-(2-amino-6-(dimethylamino)-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol.

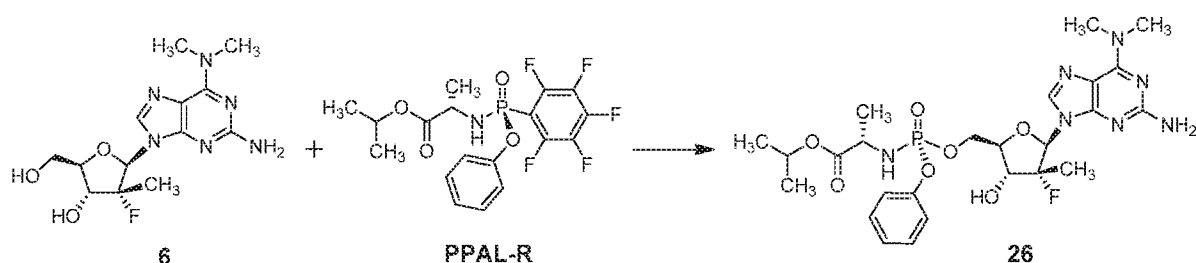
To a solution of (2R,3R,4R,5R)-5-(2-amino-6-chloro-9H-purin-9-yl)-2-(hydroxymethyl)-4-fluoro-4-methyl-tetrahydrofuran-3-ol (2.8 g, 8 mmol) in dioxane (20 mL) was added dimethylamine aqueous solution (5 mL). After stirring at RT for 3 h, TLC showed that the starting material was consumed. The mixture was concentrated and purified by column chromatography (DCM:MeOH = 60:1) to afford (2R,3R,4R,5R)-5-(2-amino-6-(dimethylamino)-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol (2.2 g).

¹H NMR (400 MHz, CD₃OD) δ 8.08 (s, 1H), 6.13 (d, *J* = 18.0 Hz, 1H), 4.43 (dd, *J* = 9.2, 9.2 Hz, 1H), 4.06 (d, *J* = 10.8 Hz, 2H), 3.90 (m, 1H), 3.37 (s, 3H), 3.06 (s, 3H), 1.18 (d, *J* = 22 Hz, 3H).



Step 2. Preparation of isopropyl (((*S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(dimethylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (**25**).

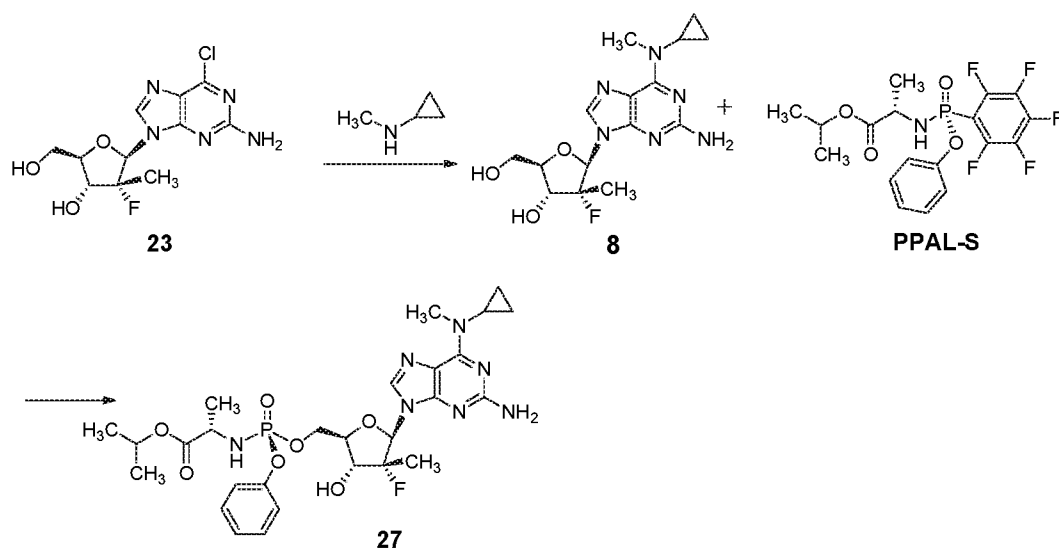
- 5 The compound (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(dimethylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol (8 g, 1.0 eq) and **PPAL-S** (11.1 g, 1 eq) were dissolved in anhydrous THF (100 mL). The mixture was cooled to -5-0 °C and *t*-BuMgCl (30.5 mL, 1.7 M, 2.1 eq) was slowly added under a N₂ atmosphere. After stirring at RT for 2 h, the mixture was quenched with aq. saturated NH₄Cl solution and extracted with EtOAc (70 mL × 3). The
- 10 combined organic layers were washed with water, brine (30 mL), dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by column chromatography (DCM:MeOH = 50:1) to afford isopropyl (((*S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(dimethylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate as a white powder (9.5 g, 65%).
- 15 ¹H NMR (400 MHz, CD₃OD) δ 7.81(s, 1H), 7.35-7.19 (m, 5H), 6.15 (d, *J* = 18.8 Hz, 1H), 4.90 (m, 1H), 4.54-4.49 (m, 3H), 4.22-4.19 (m, 1H), 3.90 (m, 1H), 3.43 (s, 3H), 1.32(d, *J* = 7.2 Hz, 3H), 1.24-1.17(m, 9H). ³¹P NMR (160 MHz, CD₃OD) δ 3.89.



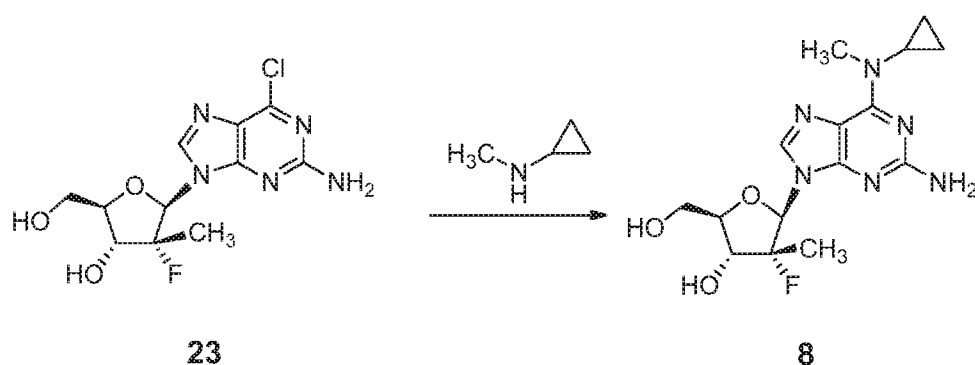
- 20 **Example 13. Preparation of isopropyl (((*R*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(dimethylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate (**26**).**

The compound (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(dimethylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol (3 g, 1.0 eq) and **PPAL-R** (4.17 g, 1 eq) were dissolved in anhydrous THF (60 mL). The mixture was cooled to -5-0 °C and *t*-BuMgCl (11.4 mL, 1.7 M, 2.1 eq) was slowly added under a N₂ atmosphere. After stirring at RT for 16 h, the mixture was quenched with aq. saturated NH₄Cl solution and extracted with EtOAc (50 mL × 3). The combined organic layers were washed with water, brine (30 mL), dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by column chromatography (DCM:MeOH = 50:1) to afford isopropyl (((*R*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(dimethylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate as a white powder (2.2 g, 41%).

¹H NMR (400 MHz, CD₃OD) δ 7.8(s, 1H), 7.35-7.29 (m, 5H), 6.18 (d, *J* = 18.8 Hz, 1H), 4.92 (m, 1H), 4.60 (m, 1H), 4.51-4.23 (m, 3H), 3.90 (m, 1H), 3.44 (s, 6H), 1.29(d, *J* = 6 Hz, 3H), 1.22-1.16(m, 10H). ³¹P NMR (160 MHz, CD₃OD) δ 3.98.



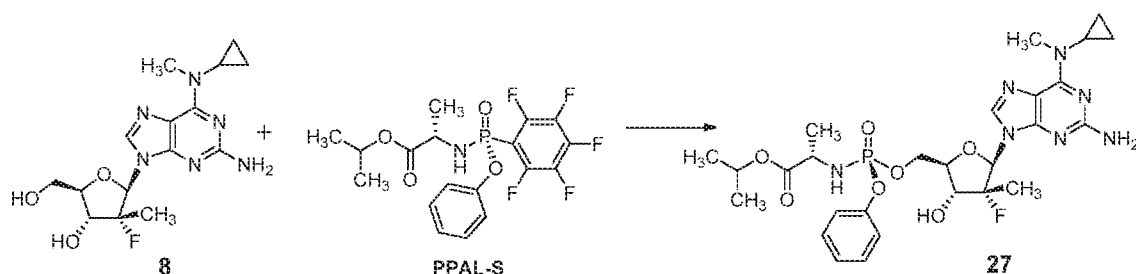
Example 14. Preparation of isopropyl (((*S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylcyclopropanamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate.



Step 1: Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylcyclopropanamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol (**8**).

K_2CO_3 (53 g, 500 mmol) was added to N-methylcyclopropanamine hydrochloride in aqueous solution (100 mL). After stirring at RT for 10 min, a solution of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-chloro-9*H*-purin-9-yl)-2-(hydroxymethyl)-4-fluoro-4-methyl-tetrahydrofuran-3-ol (35 g, 109 mmol) in dioxane (300 mL) was added. The mixture was stirred at RT for 16 h and HPLC indicated that the reaction was complete. The mixture was concentrated and purified by column chromatography (DCM:MeOH = 60:1) to afford (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylcyclopropanamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol (30 g, 82%).

1H NMR (400 MHz, CD_3OD) δ 8.16 (s, 1H), 6.17 (d, $J = 18.0$ Hz, 1H), 4.41 (dd, $J = 9.2, 9.2$ Hz, 1H), 4.06 (m, 2H), 3.90 (m, 1H), 3.37 (s, 3H), 3.16 (m, 1H), 1.18 (d, $J = 22.4$ Hz, 3H), 0.94 (m, 2H), 0.74 (m, 2H). $[M+H]^+ = 353.2$.

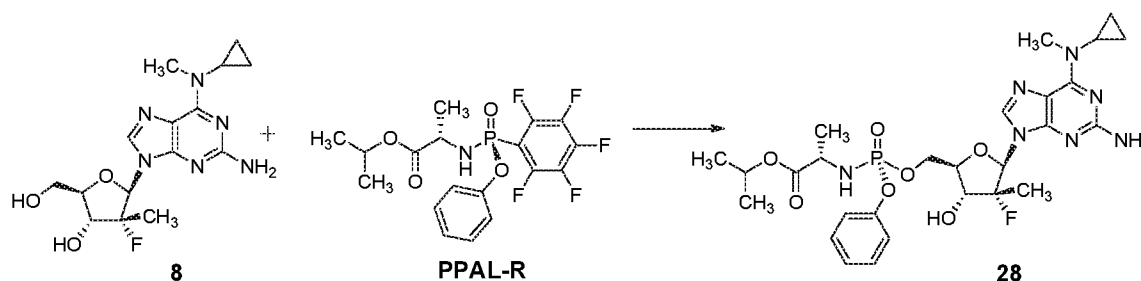


Step 2: Preparation of isopropyl (((*S*)-2-((*S*)-2-((2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylcyclopropanamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy)-phosphoryl)-*L*-alaninate).

The compound (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylcyclopropanamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol (8 g, 1.0 eq) and **PPAL-S** (10.3 g, 1 eq) were

dissolved in anhydrous THF (100 mL). After cooling the mixture to -5-0 °C, *t*-BuMgCl (28 mL, 1.7 M, 2.1 eq) was slowly added under a N₂ atmosphere. The mixture was stirred at RT for 1h, quenched with aq. saturated NH₄Cl solution, and extracted with EtOAc (70 mL × 3). The combined organic layers were washed with water, brine (30 mL), dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by column chromatography (DCM:MeOH = 100:1 to 50:1) to afford isopropyl ((((*S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylcyclopropanamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate as a white powder (9.5 g, 65%).

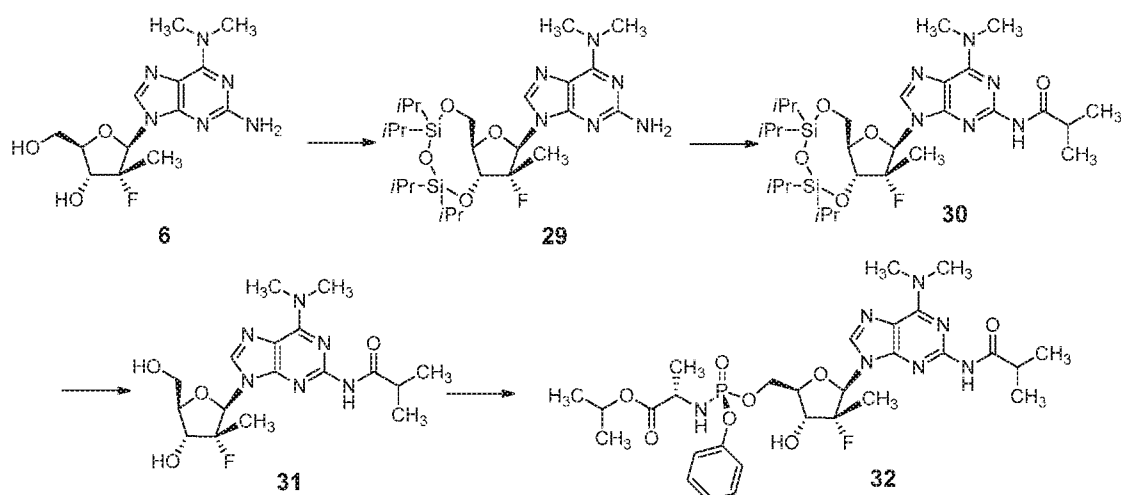
¹H NMR (400 MHz, CD₃OD) δ 7.86 (s, 1H), 7.35-7.19 (m, 5H), 6.17 (d, *J* = 19.2 Hz, 1H), 4.91 (m, 1H), 4.52 (m, 3H), 4.21 (m, 1H), 3.93 (m, 1H), 3.35 (s, 3H), 3.16 (m, 1H), 2.0 (s, 1H), 1.26-1.16 (m, 12H), 0.93 (m, 2H), 0.73 (m, 2H). ³¹P NMR (160 MHz, CD₃OD) δ 3.90



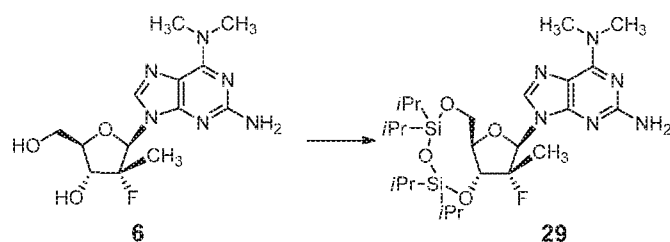
Example 15. Preparation of isopropyl ((((*R*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylcyclopropanamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate.

The compound (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylcyclopropanamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-3-ol (3 g, 1.0 eq) and **PPAL-R** (2.8 g, 1 eq) were dissolved in anhydrous THF (60 mL). After cooling the mixture to -5-0 °C, *t*-BuMgCl (7.6 mL, 1.7 M, 2.1 eq) was slowly added under N₂. Then the mixture was stirred at RT for 1 h and quenched with aq. saturated NH₄Cl solution, and extracted with EtOAc (50 mL × 3). The combined organic layers were washed with water, brine (30 mL), dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by column chromatography (DCM:MeOH = 100:1 to 50:1) to afford the product as a white powder (3 g, 55%).

¹H NMR (400 MHz, CD₃OD) δ 7.81 (s, 1H), 7.30-7.25 (m, 5H), 6.16 (d, *J* = 24.8 Hz, 1H), 4.84 (m, 1H), 4.84-4.50 (m, 3H), 4.22-4.19 (m, 1H), 3.88 (m, 1H), 3.33 (s, 3H), 3.14 (m, 1H), 2.0 (s, 1H), 1.28-1.13 (m, 12H), 0.92 (m, 2H), 0.90 (m, 2H). ³¹P NMR (160 MHz, CD₃OD) δ 3.99.

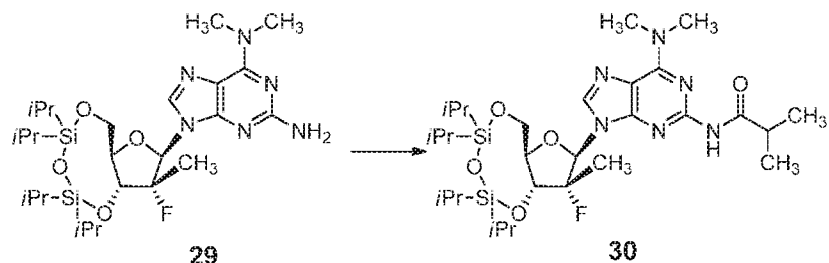


Example 16. Preparation of compound 32.



Step 1. Preparation of compound 29.

To a solution of **6** (3.0 g, 1.0 eq) in pyridine (30 mL) was added TIPDSCl₂ (4.35 g, 1.5 eq) at 0 °C. After stirring at RT for 4 h, TLC showed that starting material was consumed. The mixture was diluted with EtOAc, washed with 1M aq. HCl solution, saturated NaHCO₃ aqueous solution, brine, dried over anhydrous Na₂SO₄ and concentrated to afford **29** as a yellow oil (6.3 g, 100%).

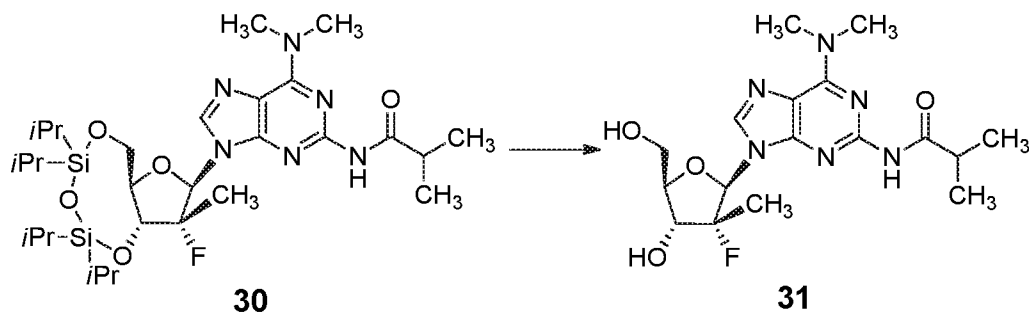


Step 2. Preparation of compound 30.

To a mixture of Compound **29** (800 mg, 1.0 eq), DMAP (16 mg, 0.1 eq), pyridine (1.6 mL) and DCM (10 mL) was added isobutyryl chloride (209 mg, 1.5 eq) at 0 °C. After stirring at RT for 2 h, TLC showed that the starting material was consumed. The mixture was quenched

with water, washed with aq. 1M HCl solution, saturated NaHCO₃ aqueous solution, brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by column chromatography to afford the product, **30**, as a white oil (563 mg, 62.3%).

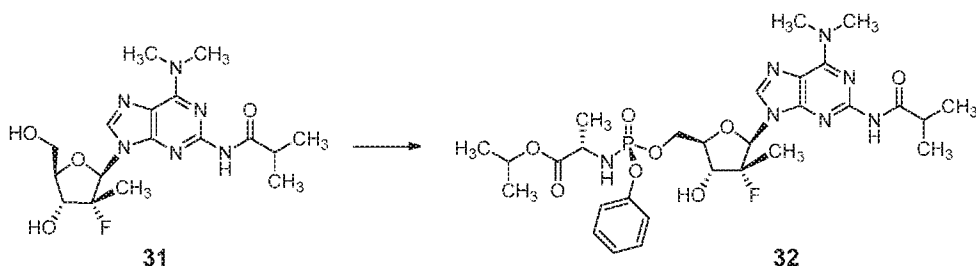
¹H NMR (400 MHz, CDCl₃) δ 7.98 (s, 1H), 7.87 (s, 1H), 6.20 (d, *J* = 16.0 Hz, 1H), 4.32-4.07 (m, 4H), 3.50 (s, 6H), 2.3 (m, 1H), 1.29-1.05 (m, 45H).



Step 3. Preparation of compound **31**.

To a mixture of **30** (560 mg, 1.0 eq) in THF (10 mL) was added Et₃N·3HF (706 mg, 5 eq) and Et₃N (890 mg, 10 eq) at RT. After stirring at RT for 1.5 h, TLC showed that the starting material was consumed. The mixture was concentrated and purified by column chromatography to afford **31** as a white powder (288 mg, 83%).

¹H NMR (400 MHz, CDCl₃) δ 7.72 (s, 1H), 5.96 (d, *J* = 44.0 Hz, 1H), 5.22 (m, 1H), 4.13-3.99 (m, 4H), 3.42 (s, 6H), 2.83-2.63 (m, 2H), 1.29-1.17 (m, 9H).



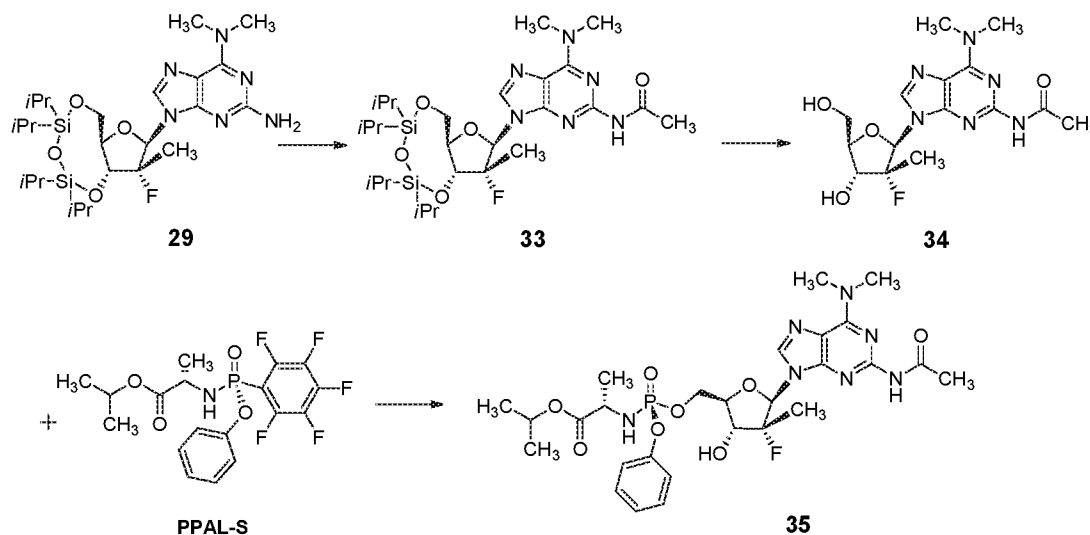
Step 4. Preparation of compound **32**.

Compound **31** (280 mg, 1.0 eq) and **PPAL-S** (320 mg, 1 eq) were dissolved in anhydrous THF (10 mL). After cooling the mixture to -5 °C, *t*-BuMgCl (0.87 mL, 1.7 M, 2.1 eq) was slowly added under a N₂ atmosphere. The mixture was stirred at RT for 2 h, quenched with aq. saturated NH₄Cl solution, and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with water, brine (20 mL), dried over anhydrous Na₂SO₄ and concentrated. The

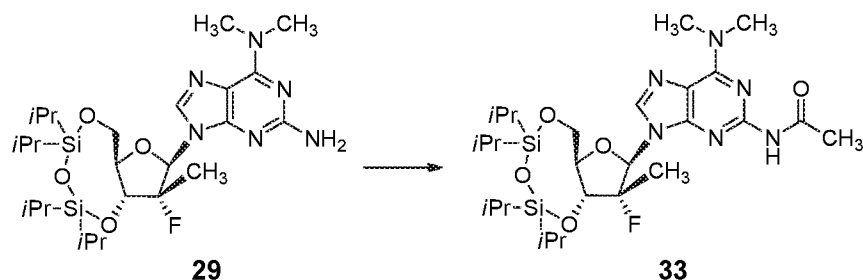
crude product was purified by column chromatography to afford the product as a white powder (260 mg, 50%).

^1H NMR (400 MHz, CD_3OD) δ 7.98 (s, 1H), 7.25 (m, 5H), 6.23 (d, $J = 18.8$ Hz, 1H), 4.52 (m, 3H), 4.38 (m, 1H), 3.81 (m, 1H), 3.75 (m, 1H), 3.48 (s, 6H), 2.81 (m, 1H), 1.32 (m, 18H).

5 $[\text{M}+\text{H}]^+ = 666.9$.



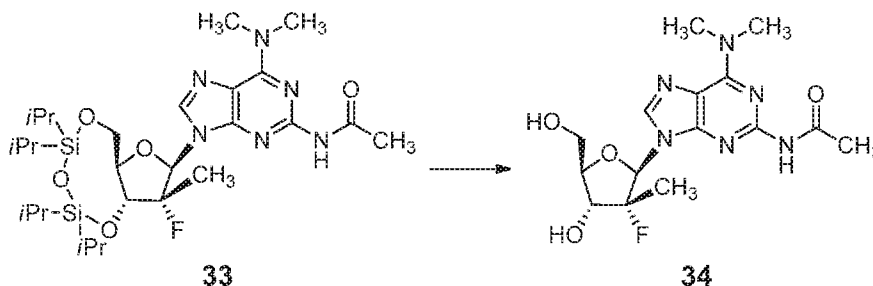
Example 17. Preparation of compound 35.



Step 1. Preparation of compound 33.

To a mixture of **29** (2.0 g, 1.0 eq), DMAP (0.04 g, 0.1 eq), pyridine (4 mL) and DCM (20 mL) was added AcCl (0.414 g, 1.5 eq) at 0 °C. After stirring at RT for 2 h, TLC showed that the starting material was consumed. The mixture was quenched with water, washed with aq. 1M HCl solution, saturated NaHCO_3 aqueous solution then brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by column chromatography to afford the product, **33**, as a white oil (1.73 g, 80.8%).

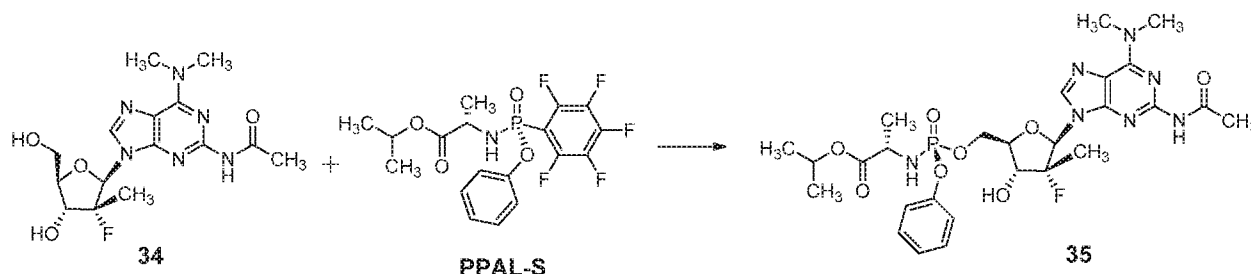
^1H NMR (400 MHz, CDCl_3) δ 7.99 (s, 1H), 7.74 (s, 1H), 6.20 (d, $J = 20.0$ Hz, 1H), 4.33-4.11 (m, 4H), 3.50 (s, 6H), 2.63 (s, 3H), 2.3 (m, 1H), 1.26-1.05 (m, 29H). $[\text{M}+\text{H}]^+ = 611.9$.



Step 2. Preparation of compound **34**.

To a mixture of **33** (1.58 g, 1.0 eq) in THF (20 mL) was added $\text{Et}_3\text{N} \cdot 3\text{HF}$ (2.1 g, 5 eq) and Et_3N (2.6 g, 10 eq) at RT. After stirring at RT for 1.5 h, TLC showed that the starting material was consumed. The mixture was concentrated and purified by column chromatography to afford **34** as a white powder (782 mg, 82%).

$[\text{M}+\text{H}]^+ = 369.6$.



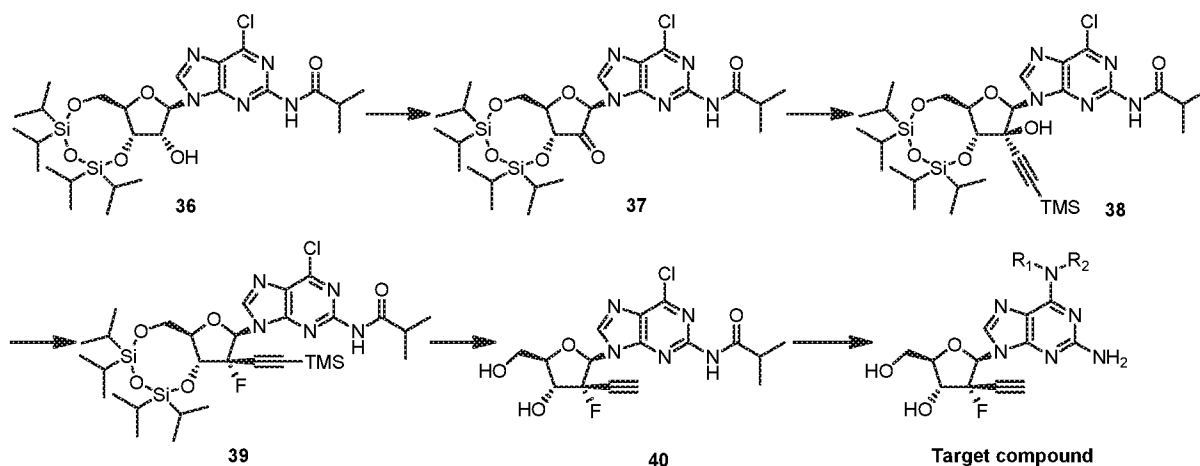
Step 3. Preparation of compound **35**.

Compound **34** (136 mg, 1.0 eq) and **PPAL-S** (184 mg, 1.1 eq) were dissolved in anhydrous THF (3 mL). After cooling the mixture to -5°C , $t\text{-BuMgCl}$ (0.5 mL, 1.7 M, 2.1 eq) was slowly added under a N_2 atmosphere. The mixture was stirred at RT for 30 min, quenched with aq. saturated NH_4Cl solution and extracted with EtOAc (10 mL \times 3). The combined organic layers were washed with water, brine (20 mL), dried over anhydrous and concentrated. The crude product was purified by column chromatography ($\text{DCM}:\text{MeOH} = 50:1-20:1$) to afford the phosphoramidate **35** as a white powder (150 mg, 63.8%).

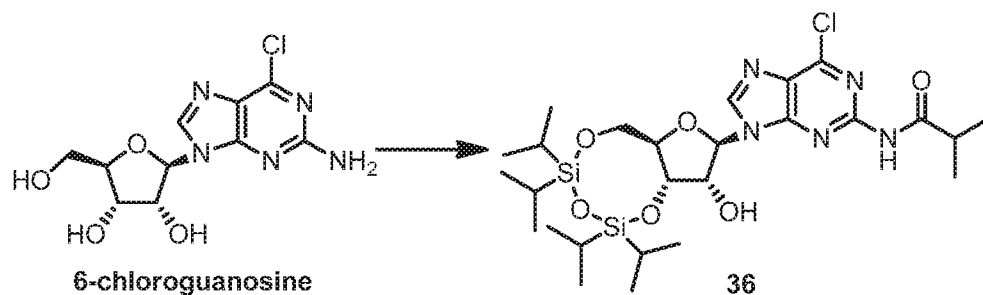
^1H NMR (400 MHz, CD_3OD) δ 7.81 (s, 1H), 7.35-7.16 (m, 5H), 6.10 (d, $J = 18.4$ Hz, 1H), 4.87 (m, 1H), 4.52-4.46 (m, 3H), 4.21 (m, 1H), 3.91-3.87 (m, 1H), 3.03 (s, 3H), 1.30-1.13 (m, 12H).

^{31}P NMR (160 MHz, CD_3OD) δ 3.84. ^{19}F NMR (376 MHz, CD_3OD) δ -162.79.

Synthesis of β -D-2'-deoxy-2'- α -fluoro-2'- β -ethynyl-N⁶-substituted-2,6-diaminopurine nucleotides



Example 18. General route to β -D-2'-deoxy-2'- α -fluoro-2'- β -ethynyl-N⁶-substituted-2,6-diaminopurine nucleotides



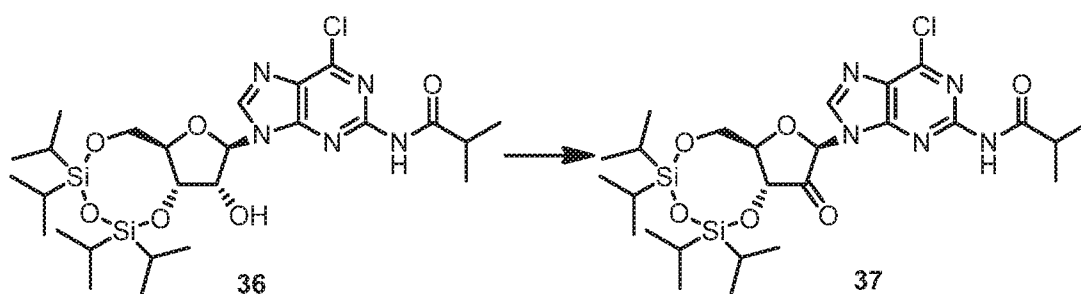
Step 1. Preparation of compound 36.

10 To a solution of 6-chloroguanosine (100 g, 332 mmol) in pyridine (400 mL) was added TPDSCl₂ (110 mL, 1.05 eq.) dropwise at -5~5 °C under a N₂ atmosphere. After stirring at that temperature for 2 h, TLC showed the starting material was consumed. DCM (600 mL) was added, and then TMSCl (85 mL, 2 eq.) was added dropwise at 0-5 °C. After stirring at that temperature for 2 h, TLC showed the intermediate was consumed.

15 Isobutyryl chloride was added dropwise at 0-5 °C. After stirring at that temperature for 2 h, TLC showed the intermediate was consumed. Water was added, and the content was extracted with DCM. The organic phase was then washed with 0.5 N HCl to remove pyridine. After the pH of the content was washed to 5~6, pTSA·H₂O (9.2 g, 484.5 mmol) was added at 0-5 °C. After stirring at that temperature for 1 h, TLC showed the intermediate was consumed.

Water was then added, and the organic phase was washed with water, saturated aqueous NaHCO_3 and brine. After being dried over Na_2SO_4 , the solvent was removed in vacuo. The residue was then purified with column chromatography (PE/EA = 100-10/1) to afford the product as a light yellow solid (82 g, 40%).

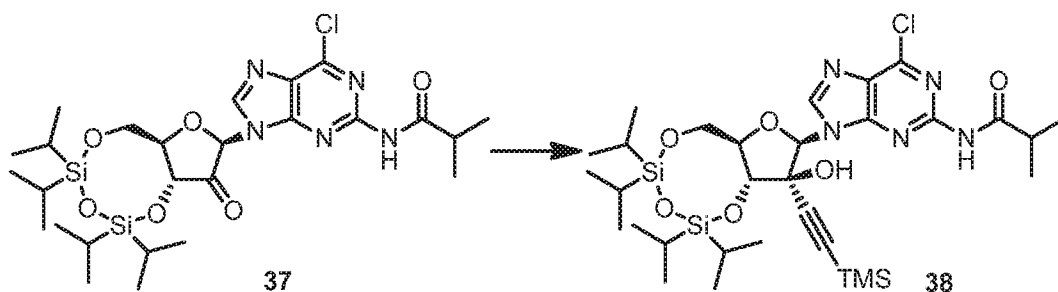
- 5 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.88 (s, 1H), 8.55 (s, 1H), 5.91 (d, J = 1.6 Hz, 1H), 5.53 (d, J = 4.6 Hz, 1H), 4.72 – 4.58 (m, 2H), 4.16 (dd, J = 12.4, 4.8 Hz, 1H), 4.00 (ddd, J = 7.7, 4.8, 2.6 Hz, 1H), 3.93 (dd, J = 12.4, 2.7 Hz, 1H), 2.78 (h, J = 6.9 Hz, 1H), 1.26 – 1.12 (m, 3H), 1.10 (d, J = 6.7 Hz, 6H), 1.09 – 0.88 (m, 24H).



- 10 Step 2. Preparation of compound **37**.

To a solution of **36** (10.0 g, 16.3 mmol) in DCM (100 mL) was added Dess-Martin periodinane at rt and the reaction was stirred for 12 h. TLC showed the starting material was consumed. The reaction mixture was then diluted with DCM (200 mL) and washed with saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ and brine. The organic phase was then dried over Na_2SO_4 and concentrated to afford crude **37** as a light yellow solid (12 g). The crude **53** can be used directly in the next step without purification.

15

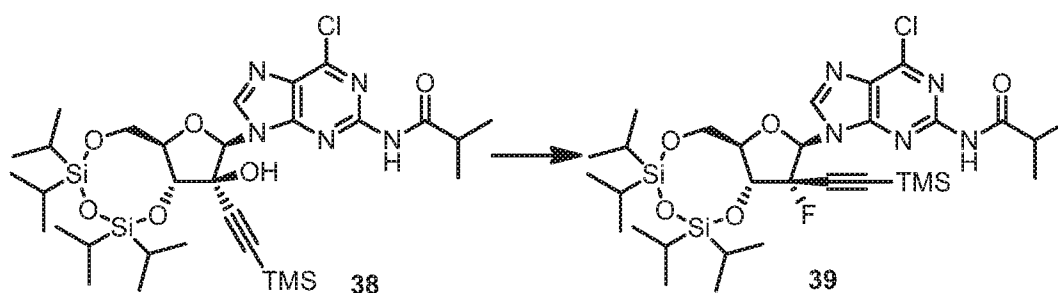


- Step 3. Preparation of compound **38**.

To a solution of ethynyltrimethylsilane (18.6 mL, 142.7 mmol) in THF (240 mL) was added $n\text{-BuLi}$ (46 mL, 2.5 M, 115.0 mmol) dropwise at $-15\sim-20\text{ }^\circ\text{C}$ under a N_2 atmosphere. After stirring for 30 min, the reaction was cooled to $-70\text{ }^\circ\text{C}$, and **37** (crude, 16.3 mmol) in THF (60

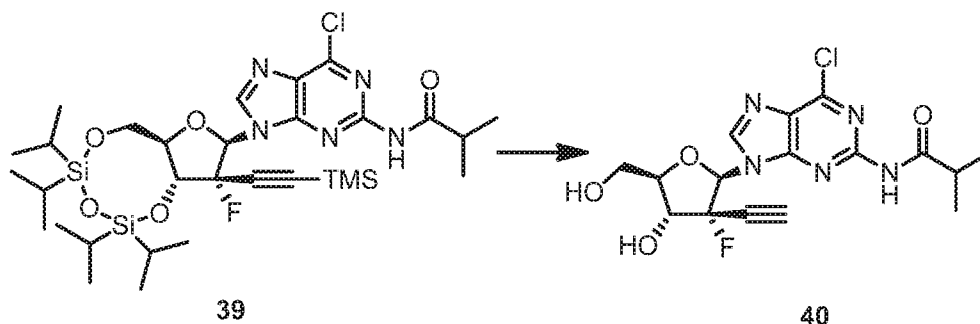
20

mL) was added at that temperature. The content was then warmed to 0 °C. TLC showed the starting material was consumed. Saturated aqueous NH₄Cl was added, and the reaction was extracted with EA (100 mL) three times. The organic phase was combined and then washed with brine, then further dried over Na₂SO₄. After being concentrated in vacuo, the residue was purified by column chromatography (PE/EA = 100->10/1) to afford a light yellow solid (6.0 g, 52%).



Step 4. Preparation of compound **39**.

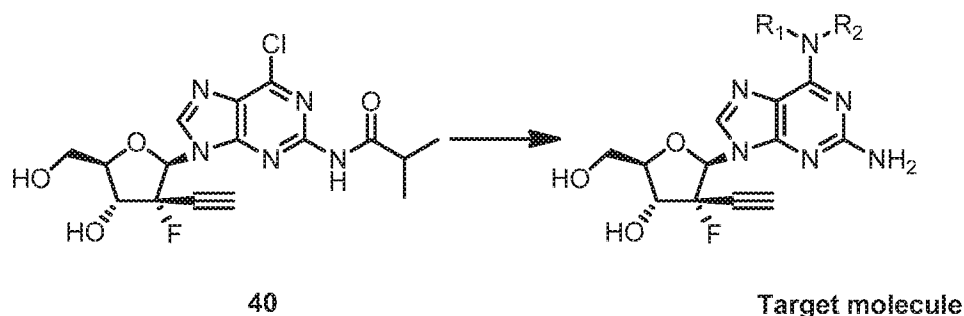
To a solution of **38** (6.0 g, 8.4 mmol) in DCM (240 mL) was added pyridine (4.2 mL, 52.9 mmol) under a N₂ atmosphere. The reaction was cooled to -70 °C, and DAST (12 mL, 90.4 mmol) was added. The content was then warmed to -30 °C. TLC showed that the starting material was consumed. The reaction was poured into saturated aqueous NaHCO₃, and then extracted with DCM (200 mL). The organic phase was washed with brine and dried over Na₂SO₄. After being concentrated in vacuo, the residue was purified with column chromatography (PE/EA = 100->10/1) to afford a light yellow solid (3.8 g, 63%).



Step 5. Preparation of compound **40**.

To a solution of **39** (3.8 g, 5.3 mmol) in THF (120 mL) was added AcOH (1.3 g, 22 mmol) and TBAF (4.2 g, 15.9 mmol) at rt. The reaction was stirred at rt for 30 min. TLC showed the

starting material was consumed. After being concentrated in vacuo, the residue was purified with column chromatography (EA) to afford the product as a white solid (2.0 g, 95%).



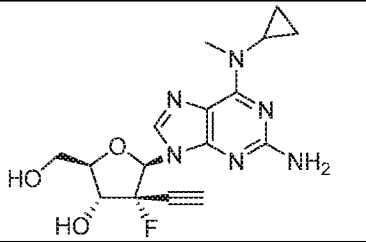
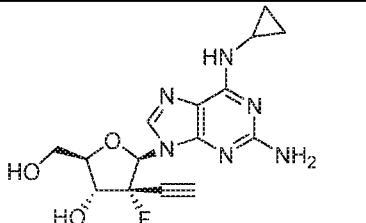
5 General Procedure for Amino Displacement and Deprotection:

To a solution of **40** (350 mg, 0.88 mmol) in dioxane (20 mL) was added the methanol or water solution of the corresponding amine (free base or salt as hydrochloride plus DIEA) at rt. The content was stirred at rt for 1-12 h. TLC showed the starting material was consumed. After being concentrated in vacuo, the residue was used directly in the next step without purification.

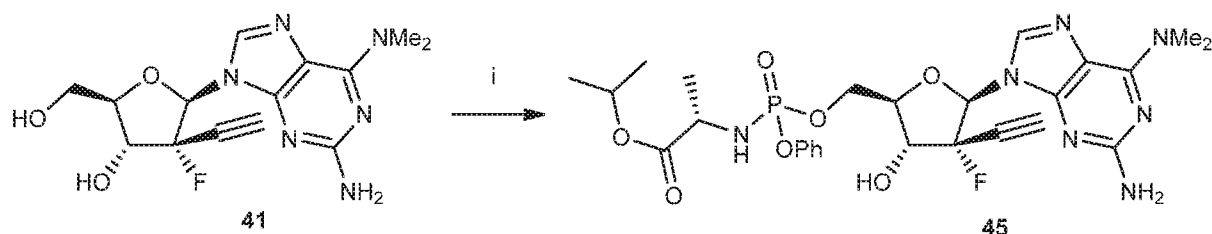
- 10 The above mentioned residue was dissolved in methanol (10 mL). Aqueous NaOH (2.5 N, 10 mL) was added. After stirring overnight at rt, TLC showed that starting material was consumed. The pH of the content was adjusted to 7-8 with 1 N HCl. The solution was concentrated and purified with column chromatography (DCM/MeOH = 100->20/1) to afford the product as an off-white solid (yield: 40-80% over two steps). Table 1 illustrates the structures of compounds
- 15 **57-63** and the corresponding mass spectral and ¹H NMR for the respective compounds.

Table 1.

Compound No.	Structure	¹ H NMR / MS
41		¹ H NMR (400 MHz, Methanol- <i>d</i> ₄) δ 8.05 (s, 1H), 6.27 (d, <i>J</i> = 16.9 Hz, 1H), 4.75 (dd, <i>J</i> = 21.7, 9.1 Hz, 1H), 4.06 (dd, <i>J</i> = 11.0, 2.4 Hz, 2H), 3.87 (dd, <i>J</i> = 13.1, 3.2 Hz, 1H), 3.42 (s, 6H), 3.37 (s, 2H), 3.18 (d, <i>J</i> = 5.4 Hz, 1H). [M+H] ⁺ = 336.9
42		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 7.94 (s, 1H), 7.30 (s, 1H), 6.20 – 6.09 (m, 2H), 5.98 (s, 2H), 5.33 (t, <i>J</i> = 5.3 Hz, 1H), 4.57 (dt, <i>J</i> = 22.1, 8.0 Hz, 1H), 4.12 (q, <i>J</i> = 5.3 Hz, 1H), 3.91 (d, <i>J</i> = 9.3 Hz, 1H), 3.70 (t, <i>J</i> = 8.6 Hz, 1H), 3.36 (s, 1H), 3.18 (d, <i>J</i> = 5.2 Hz, 2H), 2.89 (d, <i>J</i> = 7.0 Hz, 3H). [M+H] ⁺ = 323.0

43		¹ H NMR (400 MHz, Methanol- <i>d</i> ₄) δ 8.11 (s, 1H), 6.29 (d, <i>J</i> = 16.9 Hz, 1H), 4.76 (dd, <i>J</i> = 21.7, 9.0 Hz, 1H), 4.10 – 4.01 (m, 2H), 3.87 (dd, <i>J</i> = 13.1, 3.1 Hz, 1H), 3.37 (s, 1H), 3.24 – 3.11 (m, 2H), 1.00 – 0.87 (m, 2H), 0.74 (td, <i>J</i> = 4.6, 2.8 Hz, 2H). [M+H] ⁺ = 363.0
44		¹ H NMR (400 MHz, Methanol- <i>d</i> ₄) δ 8.07 (s, 1H), 6.26 (d, <i>J</i> = 16.9 Hz, 1H), 4.76 (dd, <i>J</i> = 21.8, 9.3 Hz, 1H), 4.11 – 4.01 (m, 2H), 3.89 (d, <i>J</i> = 3.0 Hz, 1H), 3.89 – 3.75 (m, 1H), 3.37 (s, 2H), 3.21 (d, <i>J</i> = 5.4 Hz, 1H), 2.97 – 2.86 (m, 1H), 1.00 – 0.77 (m, 2H), 0.67 – 0.46 (m, 2H). [M+H] ⁺ = 348.8

Example 19. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-dimethylamino-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-ethynyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate



5 i) Isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, *t*BuMgCl, THF, 0 °C.

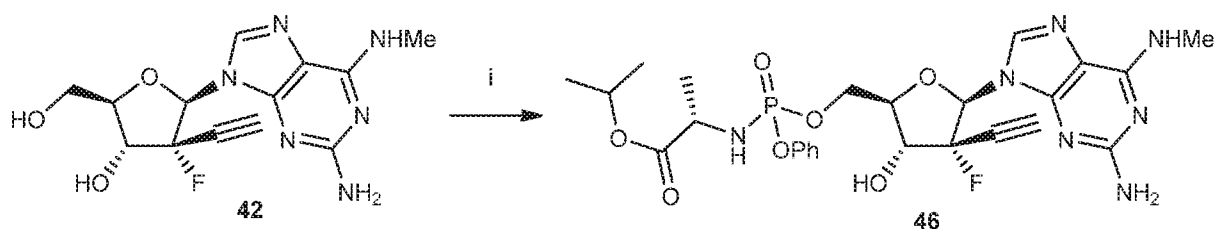
Step 1. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-dimethylamino-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-ethynyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate.

To a solution of compound **41** (30 mg, 0.09 mmol) in dry THF (2 mL) at 0 °C was added *tert*-butyl magnesium chloride (1.0 M in THF, 125 μL, 0.13 mmol) dropwise over 10 min. The reaction mixture was stirred 15 min at 0 °C then another 15 min at room temperature. The reaction mixture was cooled down to 0 °C and a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate (49 mg, 0.11 mmol) dissolved in dry THF (2 mL) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30 min and 18 h at room temperature. The reaction was quenched with a saturated aq. NH₄Cl solution (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient

DCM/MeOH 100:0 to 90:10) to afford the product (mixture of 2 diastereoisomers, 12 mg, 0.02 mmol, 24%) as a white solid.

¹H NMR (300 MHz, CD₃OD) δ 7.79 (s, 0.45H), 7.77 (s, 0.55H), 7.36-7.14 (m, 5H), 6.28 (d, J = 17.4 Hz) and 6.26 (d, J = 17.5 Hz, 1H), 5.00-4.44 (m, 5H), 4.23-4.16 (m, 1H), 3.69-3.81 (m, 1H), 3.42 (bs, 3H), 3.40 (bs, 3H), 1.32-1.26 (m, 3H), 1.20-1.15 (m, 6H). ³¹P NMR (121 MHz, CD₃OD) δ 4.04 (s), 3.98 (s). MS (ESI) m/z calcd. for C₂₆H₃₄FN₇O₇P [M+H]⁺ 606.2; found 606.2.

Example 20. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-methylamino-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-ethynyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate.



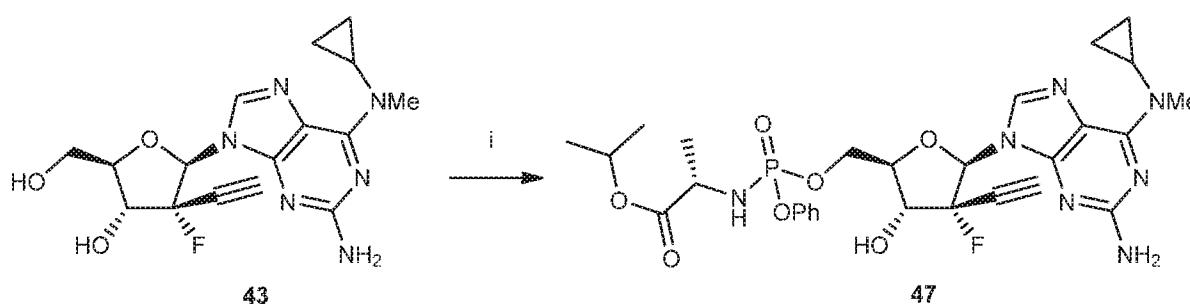
i) Isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, *t*BuMgCl, THF, 0 °C.

Step 1. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-methylamino-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-ethynyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate.

To a solution of compound **42** (30 mg, 0.09 mmol) in dry THF (2 mL) at 0 °C was added *tert*-butyl magnesium chloride (1.0 M in THF, 125 μ L, 0.13 mmol) dropwise over 10 min. The reaction mixture was stirred 15 min at 0 °C then another 15 min at room temperature. The reaction mixture was cooled down to 0 °C and a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate (49 mg, 0.11 mmol) dissolved in dry THF (2 mL) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30 min and 18 h at room temperature. The reaction was quenched with a saturated aq. NH₄Cl solution (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) to afford the product (mixture of 2 diastereoisomers, 9 mg, 0.02 mmol, 18%) as a white solid.

¹H NMR (300 MHz, CD₃OD) δ 7.81, 7.79 (0.9s+0.1s, 1H), 7.36-7.14 (m, 5H), 6.26 (d, *J* = 17.4 Hz, 0.1H) and 6.24 (d, *J* = 17.4 Hz, 0.9H), 4.93-4.89 (overlapped with H₂O, m, 1H), 4.80-4.78 (m, 1H), 4.53-4.49 (m, 2H), 4.21-4.18 (m, 1H), 3.95-3.84 (m, 1H), 3.23-3.20 (m, 1H), 3.04 (bs, 1H), 1.31-1.14 (m, 9H). ³¹P NMR (121 MHz, CD₃OD) δ 4.06 (s), 3.97 (s). MS (ESI) *m/z* calcd. for C₂₅H₃₂N₇O₇P [M+H]⁺ 592.2; found 592.2.

Example 21. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methylcyclopropylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-ethynyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate



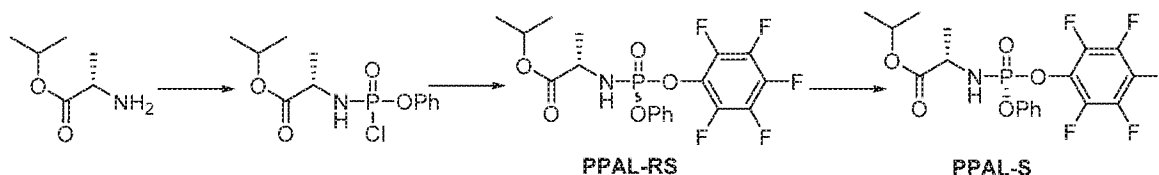
i) Isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate, *t*BuMgCl, THF, 0 °C.

Step 1. Preparation of isopropyl ((((*R,S*)-(2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(*N*-methylcyclopropylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-ethynyltetrahydrofuran-2-yl)methoxy)-phenoxy-phosphoryl)-*L*-alaninate.

To a solution of compound **43** (40 mg, 0.11 mmol) in dry THF (2 mL) at 0 °C was added *tert*-butyl magnesium chloride (1.0 M in THF, 160 μL, 0.16 mmol) dropwise over 10 min. The reaction mixture was stirred 15 min at 0 °C then another 15 min at room temperature. The reaction mixture was cooled down to 0 °C and a solution of isopropyl ((*R,S*)-(pentafluorophenoxy)-phenoxy-phosphoryl)-*L*-alaninate (55 mg, 0.12 mmol) dissolved in dry THF (2 mL) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30 min and 18 h at room temperature. The reaction was quenched with a saturated aq. NH₄Cl solution (4 mL) and extracted with EtOAc (3 x 5 mL). The combined organics were dried over Na₂SO₄ and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 100:0 to 90:10) to afford the product (mixture of 2 diastereoisomers, 18 mg, 0.03 mmol, 26%) as a white solid.

¹H NMR (300 MHz, CD₃OD) δ 7.84, 7.82 (s+s, 1H), 7.35-7.14 (m, 5H), 6.30 (d, *J* = 17.4 Hz) and 6.26 (d, *J* = 17.6 Hz, 1H), 4.99-4.89 (overlapped with H₂O, m, 1H), 4.82-4.69 (m, 1H), 4.59-4.46 (m, 2H), 4.21 (m, 1H), 3.96-3.82 (m, 1H), 3.24-3.22 (m, 1H), 3.17-3.11 (m, 1H) 1.31-1.26 (m, 3H), 1.20-1.15 (m, 6H), 0.93-0.89 (m, 2H), 0.75-0.68 (m, 2H). ³¹P NMR (121 MHz, CD₃OD) δ 4.06 (s), 3.98 (s). MS (ESI) *m/z* calcd. for C₂₈H₃₆FN₇O₇P [M+H]⁺ 632.2; found 632.2.

Example 22. Preparation of PPAL-S



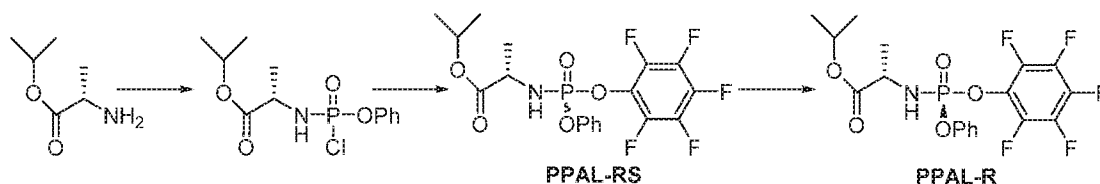
Step 1. Preparation of racemic PPAL

To a stirred solution of phenyl dichlorophosphate (250 g) in EtOAc (800 mL) was added isopropyl L-alaninate (200 g) in triethylamine (120 g) at -10 °C. The reaction was stirred at -10 °C for 1 h. The compound 2,3,4,5,6-pentafluorophenol (220 g) in triethylamine (120 g) and EtOAc (400 mL) was added at -5 °C and stirred at that temperature for 0.5 h. The reaction mixture was allowed to warm to 25 °C and stirred at that temperature for 2 h. The solution was filtrated and washed with EtOAc (2 × 200 mL), and the combined organic phases were evaporated under vacuum to afford the solid **PPAL-RS** (racemate).

Step 2. Preparation of PPAL-RS

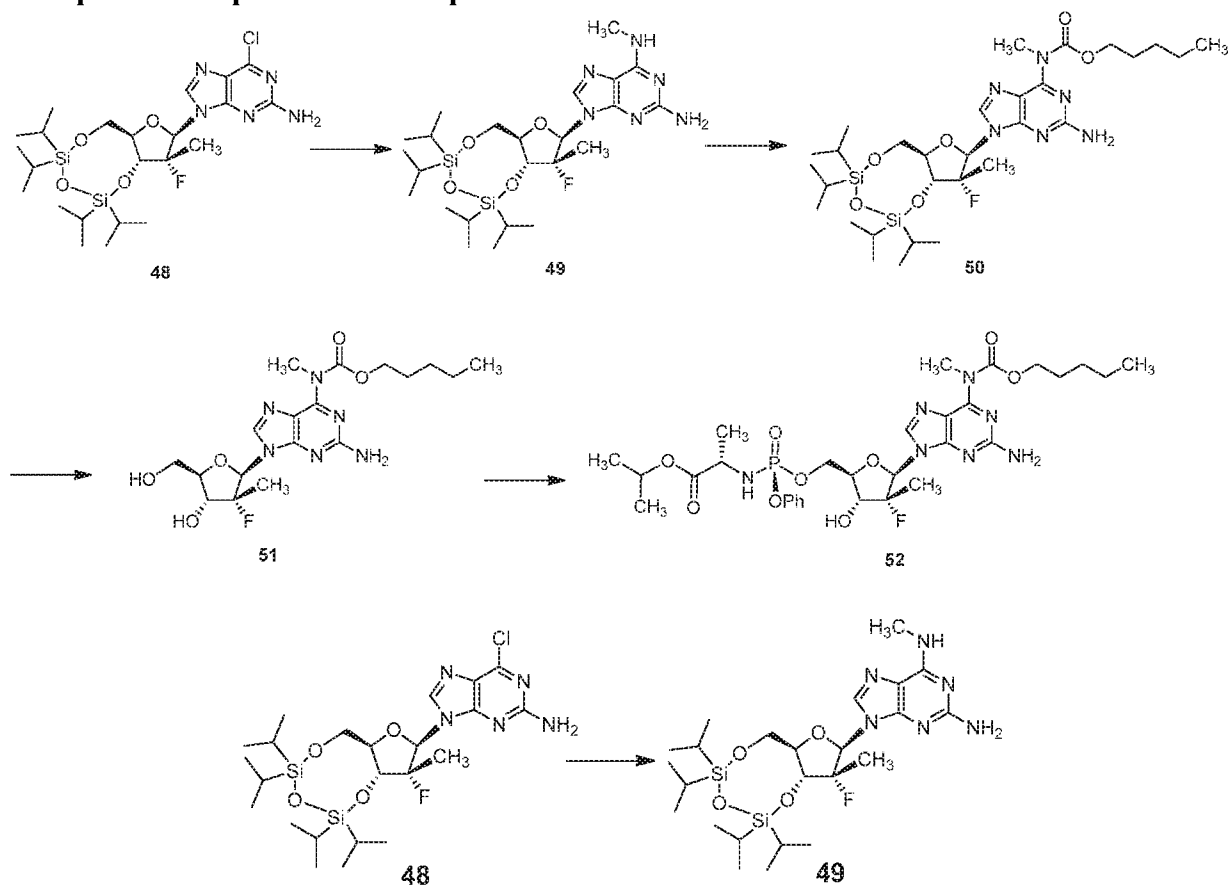
To a stirred solution of **PPAL-RS** in EtOAc (200 mL) and *n*-heptane (1.4 L) was added 2,3,4,5,6-pentafluorophenol (10.1 g) in triethylamine (6 g), and stirring was continued for about 4-8 h. After the R-isomer of the solid was less than 0.5%, the solid was filtered. The solid was dissolved in EtOAc (4 L), washed with water (2 × 100 mL), brine (1 L), dried over anhydrous Na₂SO₄, and filtered. The solvent was removed under vacuum to afford the **PPAL-S** (350 g).

¹H NMR (400 MHz, DMSO- d₆) δ = 7.42 – 7.40 (m, 2H), 7.24 – 7.22 (m, 3H), 6.87 (dd, *J* = 14.1, 9.9 Hz, 1H), 4.90 – 4.84 (m, 1H), 3.94 – 3.88 (m, 1H), 1.27 (dd, *J* = 7.1, 1.1 Hz, 3H), 1.15 (dd, *J* = 6.2, 1.2 Hz, 6H) ppm. ¹³P NMR (160 MHz, DMSO- d₆) δ = 0.37 ppm.

Example 23. Preparation of PPAL-R

To a three-necked round bottom flask fitted with a mechanic stirrer were added phenyl
 5 dichlorophosphate (189.6 g, 0.90 mol) and anhydrous EtOAc (750 mL). The solution was
 cooled to -10 °C under a nitrogen atmosphere. Iso-propyl L-alaninate (118 g, 0.90 mmol) and
 triethylamine (100 g, 1.1eq) were added to the above solution. A pre-cooled (below 10 °C)
 mixture of 2,3,4,5,6-pentafluorophenol (165 g, 1 eq) and triethylamine (90.5 g, 1 eq) in EtOAc
 (300 mL) was added to the mixture via an addition funnel at -5 °C and the resulting mixture was
 10 stirred between 20-25 °C for 1 hour. The white precipitate (TEA·HCl) was filtered off and rinsed
 with EtOAc. The filtrate was concentrated under reduced pressure to yield **PPAL-RS** about 280
 g (S/R=1/1) as a white solid. **PPAL-RS** (280 g) was triturated in 300 mL of heptane/EtOAc
 (20:1) at room temperature for 5 min. The white suspension was filtered and the solid was rinsed
 with a mixture of heptane/EtOAc (20:1). The filtrate was cooled to 8 °C and the solid was
 15 collected by filtration. Crude **PPAL-R** (10 g) was obtained with 95% chiral purity. The crude
 product was purified following above step. **PPAL-R** (5 g) was obtained in NLT 98% chiral
 purity.

¹H NMR (400 MHz, DMSO- d₆) δ = 7.43 – 7.39 (m, 2H), 7.27 – 7.22 (m, 3H), 6.87 (dd, *J* =
 14.1, 9.9 Hz, 1H), 4.89 – 4.85 (m, 1H), 3.95 – 3.90 (m, 1H), 1.27 (dd, *J* = 7.1, 1.1 Hz, 3H), 1.14
 20 (dd, *J* = 6.2, 1.2 Hz, 6H). ¹³P NMR (160 MHz, DMSO- d₆) δ = 0.35.

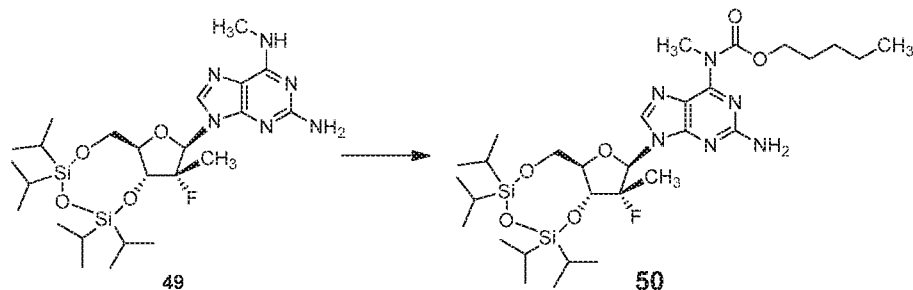
Example 24: Preparation of compound 52.

5

Step 1. Preparation of compound 49.

To a solution of **48** (1.81 g, 3.23 mmol) in dioxane (18 mL) was added 40% aqueous CH_3NH_2 solution (16.2 mmol). The reaction was stirred at 40 °C for 2 h. The mixture was concentrated, diluted with EtOAc (50 mL), washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated to afford a white solid **49** (1.66 g, 92%).

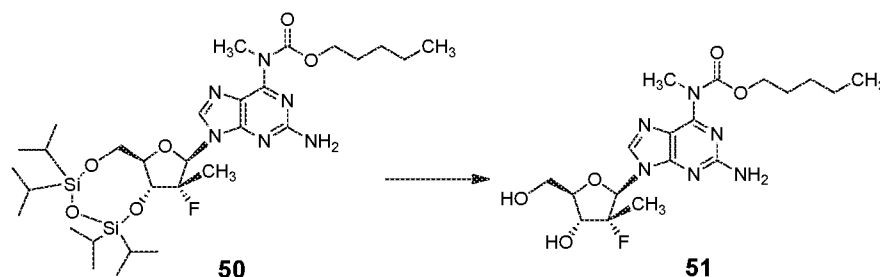
10

**Step 2. Preparation of compound 50.**

To a solution of **49** (1.34 g, 2.42 mmol) and 1-methylimidazole (794 mg, 9.68 mmol) in DCM (14 mL) was slowly added pentyl chloroformate (547 mg, 3.63 mmol) at 0 °C. The

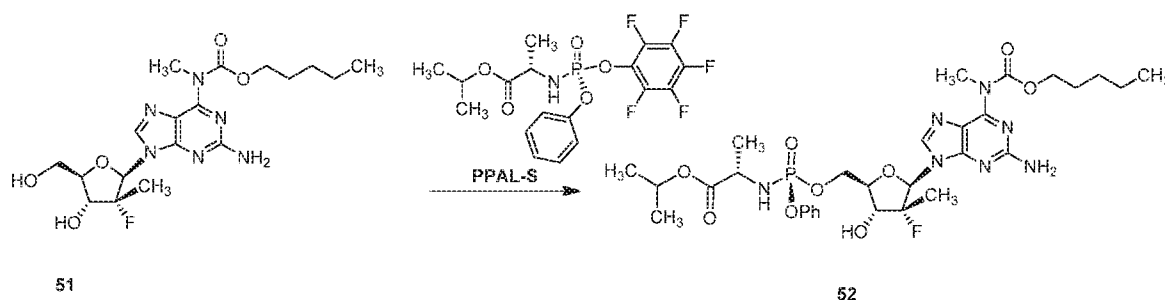
reaction was stirred at r.t overnight. The mixture was concentrated, and purified by column chromatography (PE: EtOAc = 5:1 - 2:1) to afford **50** (1.01 g, 62%) as a white solid.

¹H NMR (400 MHz, DMSO) δ 7.96 (s, 1H), 6.73 (s, 1H), 6.06-6.10 (d, J= 16.0 Hz, 1H), 4.09-4.30 (m, 2H), 3.97-4.09 (m, 4H), 3.28 (s, 3H), 1.39-1.46 (m, 2H), 1.0-1.2 (m, 35H), 0.73-0.76 (t, J= 8.0 Hz, 3H).



Step 3. Preparation of compound **51**.

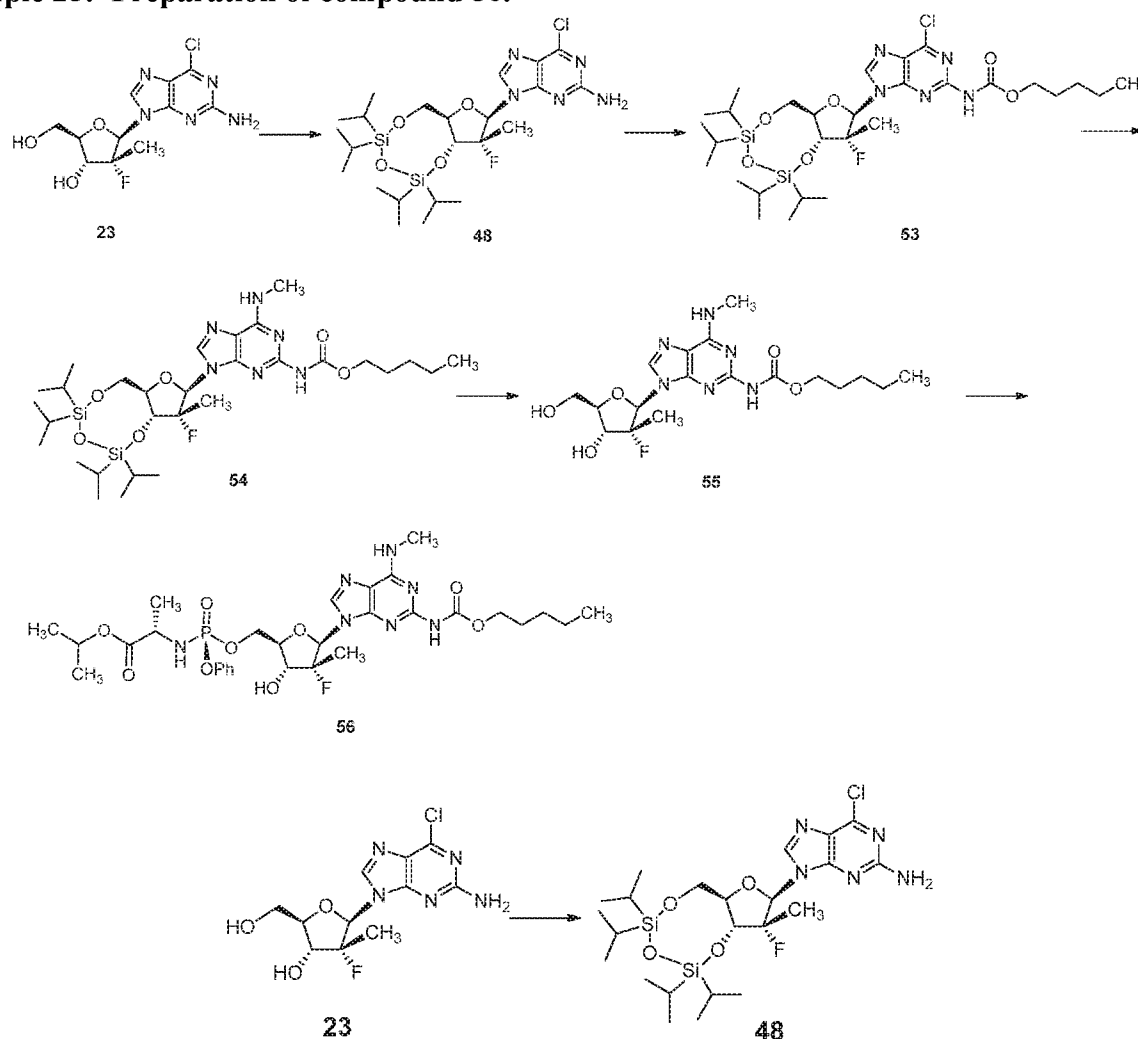
To a solution of **50** (1.00 g, 1.5 mmol) in THF (11 mL) was added Et₃N (2.0 mL, 15 mmol) and Et₃N·3HF (1.21 g, 7.5 mmol) at 0 °C. The reaction was stirred at r.t for 1.5 h. The mixture was concentrated, and purified by column chromatography (MeOH: CH₂Cl₂ = 50:1) to afford **75** (460 mg, 72.2%) as a white powder.



Step 4. Preparation of compound **52**.

To a solution of **51** (460 mg, 1.08 mmol) and **PPAL-S** (538 mg, 1.19 mmol) in anhydrous THF (9 mL) was slowly added *t*-BuMgCl (2.27 mmol) at 5-10 °C under N₂. The reaction was stirred at r.t for 40 min. The mixture was quenched with aq. saturated NH₄Cl solution, extracted with EtOAc, washed with aq. 5% K₂CO₃ solution and brine, dried over anhydrous Na₂SO₄, filtered and concentrated. The crude product was purified by column chromatography (CH₂Cl₂: MeOH = 15:1) to afford **52** (280 mg, 37.3%) as a white solid.

¹H NMR (400 MHz, DMSO) δ 8.12 (s, 1H), 7.34-7.38 (m, 2H), 7.18-7.23 (m, 3H), 6.74 (s, 2H), 6.11-6.16 (d, J= 16.0 Hz, 1H), 5.99-6.05 (m, 1H), 5.84 (m, 1H), 4.77-4.81 (m, 1H), 4.30-4.41 (m, 3H), 4.03-4.11 (m, 3H), 3.78-3.80 (m, 1H), 3.3 (s, 3H), 1.44-1.51 (m, 2H), 1.00-1.21 (m, 16H), 0.76-0.80 (t, J= 8.0 Hz, 3H). [M+H]⁺ = 696.6.

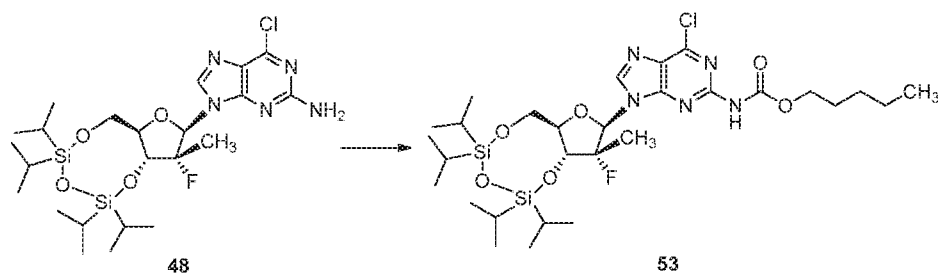
Example 25: Preparation of compound 56.

5

Step 1. Preparation of compound 48.

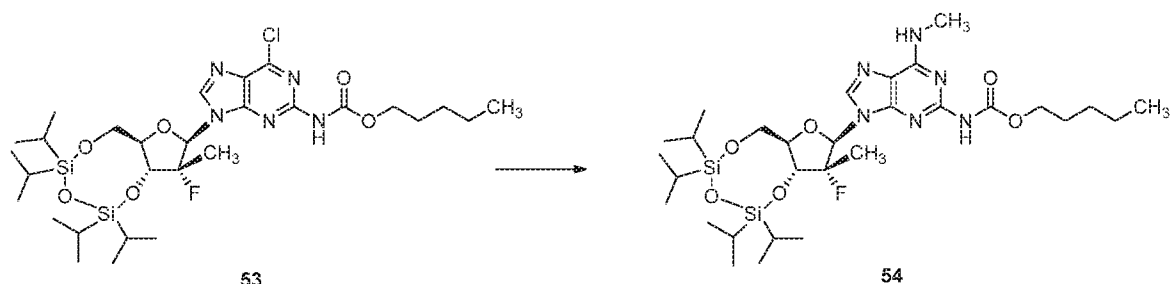
To a solution of **23** (600 mg, 1 eq) in pyridine (30 mL) was added TIPDSCl₂ (1.5eq) at 0 °C. The resulting solution was allowed to stand at room temperature for 2 h. The mixture was quenched with ice water and extracted with EtOAc. The organic layer was washed with 1M *aq.* HCl solution, saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, and concentrated to yield the crude residue. The residue was purified by chromatography (MeOH: CH₂Cl₂ = 1:50) to afford **48** (998 mg, 94.4%) as a white solid foam.

10



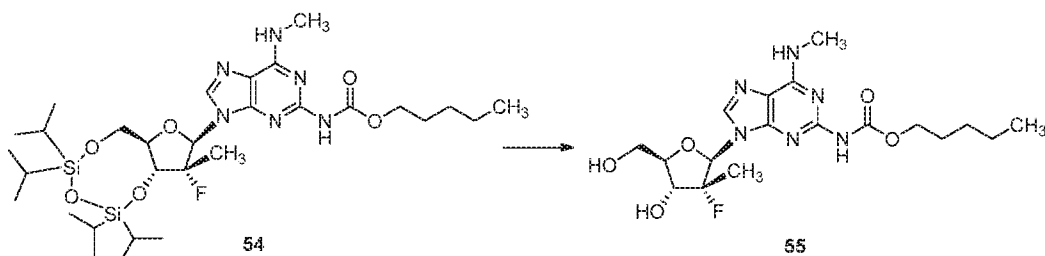
Step 2. Preparation of compound **53**.

A mixture of **48** (800 mg, 1 eq), pyridine (3.2 mL), DMAP (34.9 mg, 0.2 eq) in DCM (20 mL) was stirred at room temperature. N-amyloxy carbonyl chloride (3.2 mL) was added dropwise at 0 °C, and the mixture was stirred at room temperature for 1 day. The organic layer was washed with 1M aqueous HCl solution, saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, and evaporated in vacuo. The residue was purified by chromatography on silica gel (MeOH: CH₂Cl₂ = 1:50) to afford **53** (255 mg, 26%) as a white solid foam.



Step 3. Preparation of compound **54**.

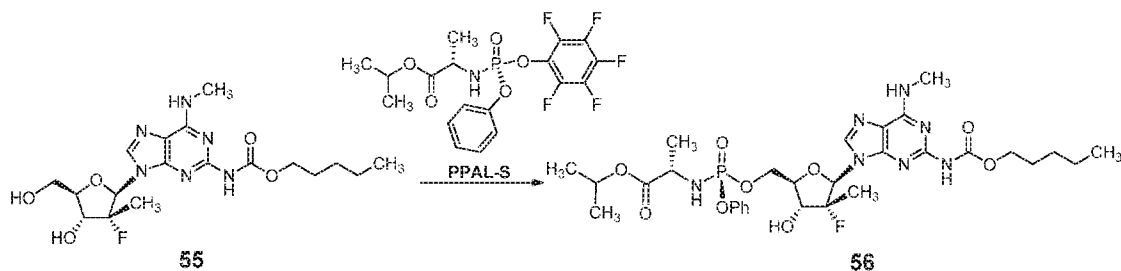
To the solution of **53** (270 mg, 1 eq) in 1,4-dioxane (10 mL), was dropwise added 40% aqueous CH₃NH₂ solution (225.7 mg, 5 eq). The mixture was stirred for 2 h at room temperature and then concentrated in vacuo. The residue was chromatographed on silica gel (methanol: dichloromethane= 1:40) to afford **54** (220 mg, 81.7%) as a white solid foam.



Step 4. Preparation of compound **55**.

Triethylamine (1011.9 mg, 10 eq) and Et₃N·3HF (806.05 mg, 5 eq) were added to an ice-cooled solution of **54** (668 mg, 1 eq) in THF (10 mL), the mixture was stirred for 2 h at room

temperature. The mixture was concentrated and chromatographed on silica gel (MeOH: CH₂Cl₂ = 1:30) to afford **55** (492 mg, 84%) as a white solid foam.



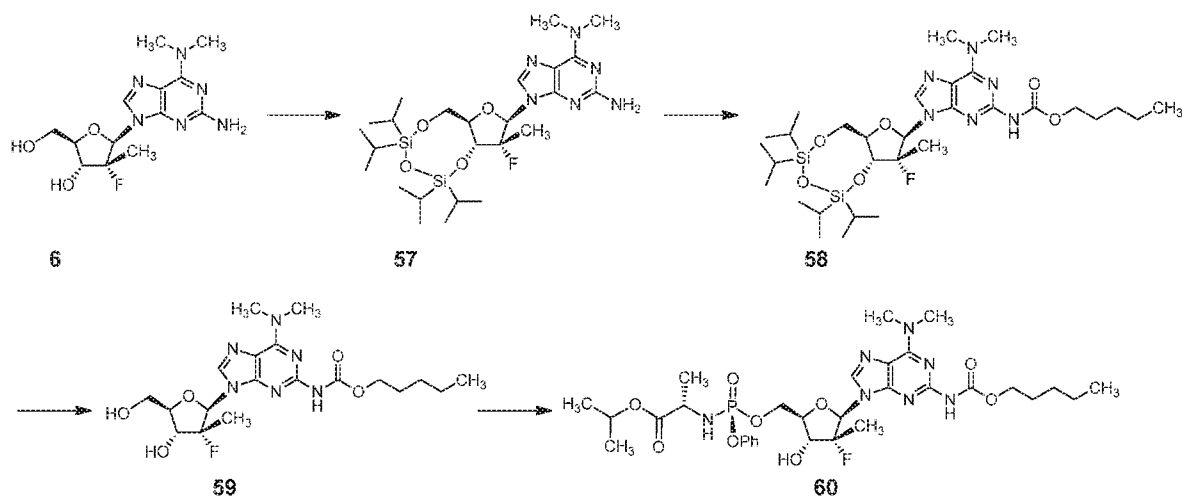
5 Step 5. Preparation of compound **56**.

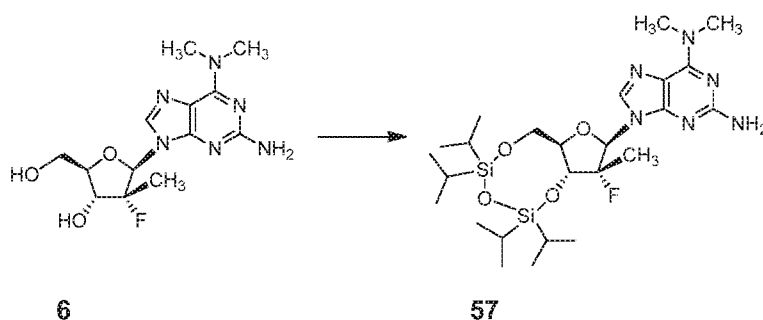
To the mixture of **55** (113 mg, 1 eq) and **PPAL-S** (120 mg, 1 eq) in THF (4 mL) was dropwise added 1.7 M *t*-BuMgCl in THF (0.327 mL, 2.1 eq) at -10 °C. The mixture was stirred at room temperature for 1 h, and then quenched with saturated *aq.* NH₄Cl solution. The aqueous phase was extracted with EtOAc and the organic phase was washed with brine, dried and concentrated to obtain crude residue. The residue was subjected to flash chromatography to afford **56** (126 mg, 68.5%) as a white solid.

¹H NMR (400 MHz, DMSO) δ 8.00 (s, 1H), 7.10-7.45 (m, 5H), 6.15-6.20 (d, J= 20.0 Hz, 1H), 5.00-5.25 (s, 1H), 4.80-4.86 (m, 1H), 4.45-4.70 (m, 2H), 4.12-4.19 (m, 3H), 3.80-3.85 (m, 1H), 3.04 (s, 3H), 1.60-1.75 (m, 2H), 1.10-1.40 (m, 16H), 0.76-0.80 (t, J= 8.0 Hz, 3H).

³¹P NMR (160 MHz, DMSO) δ 3.57. [M+H]⁺= 696.5.

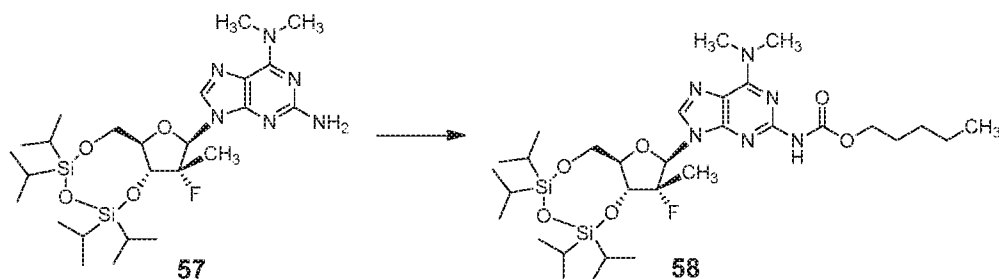
Example 26: Preparation of compound **60**.





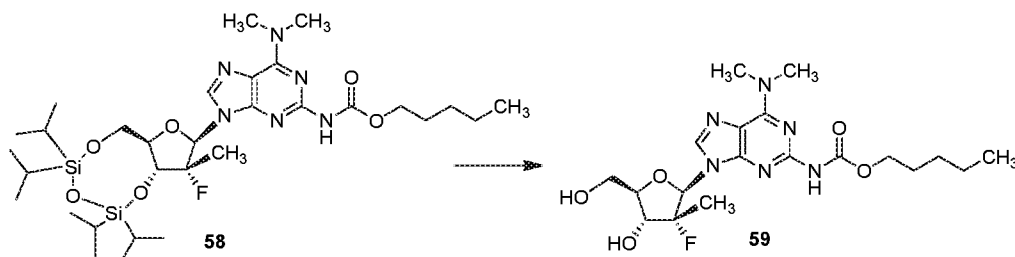
Step 1. Preparation of compound **57**.

To a solution of **6** (20 g, 1 eq) in CH₃CN (100 mL) was added imidazole (16.6 g), TIPDSCl₂ (28.9 g, 1.5 eq) in sequence at 5±5 °C. The resulting solution was allowed to stand at room temperature for 4 h. The mixture was quenched with ice water and extracted with EtOAc. The organic layer was washed with water, saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, and concentrated to afford the crude residue (32 g).



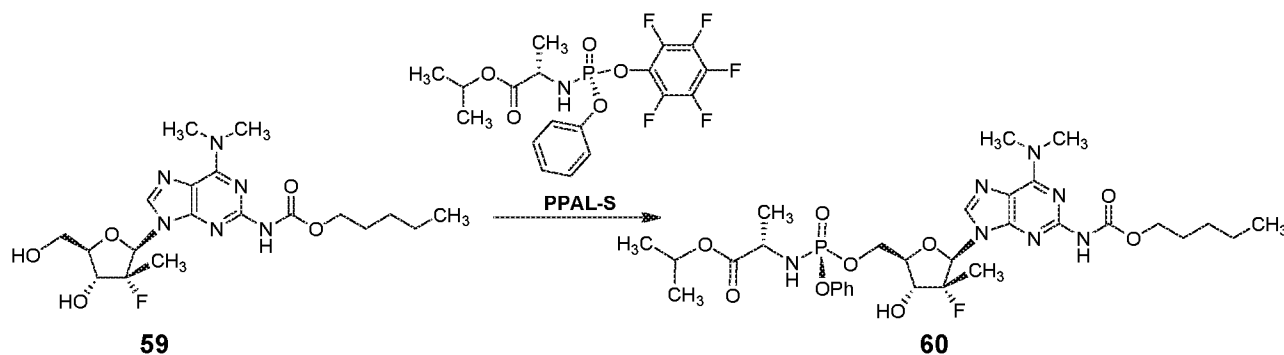
Step 2. Preparation of compound **58**.

To the solution of **57** (9.8 g, 1 eq) in THF (4 mL) was dropwise added 1.7 M *t*-BuMgCl in THF (50 mL, 4.8 eq) at 0-5 °C. The mixture was stirred at room temperature for 0.5 h, and *n*-amyl chloroformate (2.7 g, 1.05 eq) was slowly added. The mixture was stirred at 0-5 °C for 3-4 h. The mixture was quenched with saturated *aq.* NH₄Cl solution. The aqueous phase was extracted with EtOAc (200 mL) and the organic phase was washed with brine, dried and concentrated to obtain **58** (10.7 g) as oil.



Step 3. Preparation of compound **59**.

Triethylamine (10.119 g) and Et₃N·3HF (8.6 g, 5 eq) were added to an ice-cooled solution of **58** (7.3 g, 1 eq) in THF (100 mL) and the mixture was stirred for 1 h at room temperature. The mixture was concentrated and chromatographed on silica gel (MeOH: CH₂Cl₂ = 1:30) to afford **59** (4.3 g, 91%) as a white solid.



Step 4. Preparation of compound **60**.

To the mixture of **59** (2 g, 1 eq) and **PPAL-S** (2.3 g, 1.1 eq) in THF (40 mL) was dropwise added 1.7 M *t*-BuMgCl in THF (5.6 mL, 2.1 eq) at -5 °C. The mixture was stirred at -20±5 °C for 1 h, and then quenched with saturated *aq.* NH₄Cl solution. The aqueous phase was extracted with EtOAc and the organic phase was washed with brine, dried and concentrated to obtain crude residue. The residue was subjected to flash chromatography to afford **60** (1.5 g, 47%) as a white solid.

¹H NMR (400 MHz, CD₃OD) δ 7.9 (s, 1H), 7.1~7.2 (m, 5H), 6.2 (d, J = 20 Hz, 1H), 5.1 (br, 1H), 4.84 (m, 1H), 4.49 (m, 2H), 4.16 (m, 1H), 4.13 (m, 2H), 3.86 (m, 1H), 3.45 (br, 6H), 1.70 (m, 2H), 1.26 (m, 4H), 1.20 (m, 6H), 1.14 (m, 6H), 0.93 (m, 3H). [M+H]⁺ = 710.5.

Biological Data

Example 27. Assay Methodology and Additional Biological Data

Huh-7 luc/neo ET cells bearing a discistronic HCV genotype 1b luciferase reporter replicon were plated at 7.5 x 10³ cells/ml in duplicate 96-well plates for the parallel determination of antiviral efficacy (EC₅₀) and cytotoxicity (TC₅₀). The plates were cultured for 24 hours prior to the addition of compounds. Six serial one half log dilutions of the test articles (high test concentration of 100.0 μM or high test concentration of 1.0 μM) and human interferon-alpha2b (high test 10.0 U/ml) were prepared in cell culture medium and added to the cultured cells in triplicate wells for each dilution. Six wells in the test plates received medium alone as an

untreated control. Following 72 hours of culture in the presence of compound, one of the plates was used for the determination of cytotoxicity by staining with XTT and the other for antiviral efficacy by determination of luciferase reporter activity. Cytotoxicity and efficacy data were collected and imported into a customized Excel workbook for determination of the TC₅₀ and EC₅₀ values. Data for compounds of Formula I-VII are illustrated in Table 7 below. In addition, Figure 2 illustrates the HCV replication inhibition curves for Compound 5-2 and Sofosbuvir. As can be seen in Figure 2, Compound 5-2 has an EC₅₀ = 4 nM, while Sofosbuvir has an EC₅₀ = 53 nM. The y-axis is the percent of virus control and the x-axis is the concentration of drug in μ M. Figure 3 illustrates the HCV replication inhibition curves for Compound 25 and Sofosbuvir. Compound 25 has an EC₅₀ = 4 nM and Sofosbuvir has an EC₅₀ = 53 nM. The y-axis is the percent of virus control and the x-axis is the concentration of drug in μ M. Figure 4 illustrates an intra-assay comparison of the anti-HCV activity for Compounds 5-2, 25, 27 and Sofosbuvir. The y-axis is the percent of virus control and the x-axis is the concentration of drug in μ M.

Various patient-derived HCV genotypes containing wild-type and resistance-associated variants were used to determine their relative replication sensitivity to test compounds. Replicon resistance test vectors (RTVs) containing the NS5B genomic regions were prepared using viral RNA isolated from plasma of HCV patients. Each NS5B region was amplified by reverse-transcription polymerase chain reaction and cloned into an HCV replicon RTV which was then transferred by electroporation into Huh-7 cells. After incubation in the absence and presence of serially diluted test compounds for 72-96 hr, viral replication was measured by luciferase activity and 50% inhibitory concentrations (IC₅₀ values) were determined.

Table 2 reports the IC₅₀ and IC₉₅ values for compound 25, 27, 5-2 and Sofosbuvir against various clinical isolates containing wild-type and resistance-associated variants.

All compounds were significantly more effective against HCV replication than sofosbuvir and neither 25, 27 nor 5-2 compound showed any evidence of cross-resistance to L159F, L159F and S282T, and C316N mutants.

Table 2: Antiviral Activity of Test Compounds in Patient-derived HCV Genotypes

HCV	NS5B	Test	IC ₅₀ Value	IC ₉₅ Value	Fold Change in IC ₅₀	Fold Change in IC ₉₅
Genotype	Mutation	Compound	(nM)	(nM)	from Sofosbuvir	from Sofosbuvir
1a	none	sofosbuvir	62.7	507.7		

		25	4.4	31.3	14.2	16.2
		27	4.2	26.4	15.0	19.3
		5-2	10.5	60.8	6.0	8.4
1b	none	sofosbuvir	86.0	642.2	1.0	
		25	5.9	32.0	1.0	20.0
		27	5.0	28.9	0.9	22.2
		5-2	10.6	72.4	0.8	8.9
2a	none	sofosbuvir	22.5	195.1		
		25	2.7	22.2	8.4	8.8
		27	2.9	16.2	7.9	12.0
		5-2	6.2	45.4	3.6	4.3
2b	none	sofosbuvir	44.8	295.3		
		25	3.0	14.9	15.2	19.9
		27	3.1	14.7	14.4	20.1
		5-2	6.3	32.5	7.1	9.1
3a-1	none	sofosbuvir	125.9	689.8		
		25	5.1	27.8	24.5	24.8
		27	4.4	25.4	28.4	27.2
		5-2	11.8	59.3	10.7	11.6
3a-2	none	sofosbuvir	123.5	808.1		
		25	4.7	24.2	26.3	33.4
		27	4.5	23.3	27.5	34.6
		5-2	10.4	56.5	11.9	14.3
4a	none	sofosbuvir	74.9	681.4		
		25	4.6	33.0	16.2	20.7
		27	3.6	38.1	20.7	17.9
		5-2	9.9	74.4	7.5	9.2
4d	none	sofosbuvir	93.7	1019.7		
		25	5.9	44.2	16.0	23.1
		27	5.6	38.4	16.7	26.6
		5-2	14.0	79.9	6.7	12.8
1a	L159F	sofosbuvir	114.7	1067.5		
		25	5.2	40.4	22.0	26.4
		27	5.1	36.2	22.3	29.5
		5-2	13.0	95.3	8.8	11.2
1a	L159F and S282T	sofosbuvir	1619.9	16950.9		
		25	17.2	158.5	94.0	107.0
		27	14.9	141.6	108.4	119.7
		5-2	38.7	313.5	41.9	54.1
1b	C316N	sofosbuvir	73.9	472.8		
		25	3.2	18.1	23.1	26.2
		27	3.1	16.5	23.5	28.7

		5-2	7.7	42.7	9.6	11.1
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A transient transfection assay was performed to determine the sensitivity of the wild type S282T mutant of HCV to test compounds. Huh-7 cells were electroporated in the presence of RNA transcribed from wild type or S282T HCV replicon plasmids from the T7 promoter. The transfected cells were seeded in to 96-well plates at 7.5×10^3 cells per well in Dulbecco's Modified Eagle's medium. After 24 hr of incubation, medium was removed and replaced with fresh medium containing no or various concentrations of test compounds. Following an additional 96-hr incubation, the anti-HCV activity was measured by luciferase endpoint with Britelite™ Plus luminescence reporter gene kit (Perkin Elmer, Shelton, CT). Duplicate plates were treated and incubated in parallel for assessment of cellular toxicity by staining with the tetrazolium dye XTT.

Table 3 reports the IC₅₀ and IC₉₅ values for compounds 25, 27, 5-2 and Sofosbuvir against HCV wild type and S282T replicons.

All compounds were significantly more effective against HCV replication than sofosbuvir and neither 25, 27, nor 5-2 compounds showed any evidence of cross-resistance to S282T variant.

Table 3: Antiviral Activity of Test Compounds in a HCV Transient Infection Assay

Compound	NS5B Mutation	IC ₅₀ Value (nM)	IC ₉₅ value (nM)	Fold change in IC ₅₀ from Sofosbuvir	Fold change in IC ₉₅ from Sofosbuvir
5-2	None S282T	1.4 2.8	9.98 20.6	26 99.3	22.2 > 48.5
25	None S282T	< 1 < 1	2.7 9.4	> 36.4 > 278	80.7 > 106.4
27	None S282T	< 1 < 1	4.1 11.8	> 36.4 > 278	53.2 > 84.7
Sofosbuvir	None S282T	36.4 278	218 >1000		

The stability of selected compounds in fresh human whole blood and in human liver S9 fraction was determined in incubations containing 10 μ M test compound. After incubations of 0, 30, 60 min, and up to 120 min, aliquots were removed and immediately extracted with 3 volumes

of ice-cold methanol/acetonitrile (1:1, v/v). Extracts were centrifuged and supernatants were analyzed by LC-MS/MS for concentrations of unchanged test compound and potential metabolites.

Figure 5 illustrates the excellent stability of compound 5-2 and all 2-amino derivatives in human blood.

Interestingly, Figure 6 illustrates the in vitro time course dealkylation of the 2'-deoxy-2'- α -fluoro-2'- β -methyl-N²-methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate to 2'-deoxy-2'- α -fluoro-2'- β -methyl-N⁶-methyl-2,6-diaminopurine nucleoside phosphoramidate with a human liver S9 fraction. Furthermore, unexpected, faster, and a more extensive rate of cleavage of the carbamate moiety by human liver S9 fraction was observed as compared to compound 5-2 and its other 2-amino derivatives (Figure 7).

Example 28. HCV (gt1b) NS5B Polymerase Assay

Inhibition of HCV (gt1b) NS5B polymerase was determined in triplicate by measuring de novo polymerization in reaction mixtures containing serial dilutions of TA, in vitro transcribed viral RNA complementary to the HCV (-) strand 3'UTR region, polymerase, radiolabeled ribonucleotide, 250 μ M non-competing rNTPs, and 1 μ M competing rNTP. TA concentrations that produced 50% inhibition (IC₅₀) were determined from resulting inhibition curves.

Example 29. Human Bone Marrow Progenitor Cell Assay

Fresh human bone marrow progenitor cells (Invitrogen) suspended in either BFU-E or GM-CSF-specific culture medium were added, at 10⁵ cells/well, to triplicate serial dilutions of TA in 6-well plates. After 14-day incubations, colony counts were used to determine CC₅₀ values. BFU-E colonies were confirmed using the benzidine technique.

Compounds 25, 27 and 5-2 show no cytotoxicity against bone marrow stem cells in vitro.

Example 30. iPS Cardiomyocyte Assay

iPS Cardiomyocytes (Cellular Dynamics) were seeded in microliter plates at 1.5 x 10⁴ cells per well. After 48-hr incubation, cells were washed and maintenance medium containing serially diluted TA was added in triplicate. After incubating for an additional 3 days, cell viability was measured by staining with XTT and CC₅₀ values were calculated.

Compounds 25, 27 and 5-2 show no cytotoxicity against iPS cardiomyocytes in vitro.

Example 31. Human DNA Polymerase Assays

Inhibition of human DNA polymerases α , β and γ (CHIMERx) was determined in triplicate in reaction mixtures of serially diluted TA, 0.05 mM dCTP, dTTP, and dATP, 10 μ Ci [32 P]- α -dGTP (800 Ci/mmol), 20 μ g activated calf thymus DNA and additional reagents specific for each polymerase. After 30-min incubations, incorporation of [α - 32 P]-GTP was measured and resulting incubation curves were used to calculate IC₅₀ values.

The triphosphate, β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-guanine triphosphate, as well as the triphosphate analogs of compounds 25, 27 and 5-2 do not inhibit human DNA polymerases α , β or γ .

Example 32. Human Hepatocyte Co-Cultures

Cytotoxicity and hepatocyte health were assessed in triplicate by measuring ALT leakage, urea production, albumin secretion and cellular ATP contents in micro-patterned human hepatocyte co-cultures (HepatoPac®, Hepregan Corporation) prepared by seeding cryopreserved female human hepatocytes (single donor) and 3T3 J2 mouse fibroblasts in microtiter plates according to procedures established by Hepregan. Culture media was replaced with fresh media containing TA, test article, (0, 1, 10 or 30 μ M) every 2 or 3 days through day 16. Spent culture media was assayed for ALT and urea content on days 2, 5, 7, 9, 12, 16 and 21 and for albumin content on days 2, 5, 7 and 9. Cellular ATP levels were measured on days 9 and 21. ATP signals in stromal-only control cultures (murine 3T3 fibroblasts) were subtracted from those of human HepatoPac co-cultures to obtain hepatocyte-specific effects. See, Table 4, 5 and 6 below.

Compound 5-2 at concentrations up to 30 μ M, showed no signs of cytotoxicity as measured by ALT leakage, albumin secretion, urea production and cellular ATP content when incubated for up to 12 days with micro-patterned co-cultured human hepatocytes. The minor indications of cytotoxicity detected with extended exposure (up to 21 days of culture) were significantly less than those observed with sofosbuvir. See, Table 4, 5 and 6 below.

INX-189 was highly cytotoxic to human co-cultured hepatocytes, showing decreased albumin secretion as early as day 2 and cytotoxicity by all measures. Sofosbuvir showed more cytotoxicity than AT-511 under the same conditions.

Table 4. Effect of Test Article on Cellular ATP Concentrations

Test Article	50% Inhibitory Concentration (IC ₅₀) - μ M	
	Day 9	Day 21
Cmpd 5-2	>30	12.8
Sofosbuvir	8.6	2.3
INX-189	8.1	0.1

Table 5. Effect of Test Articles on Albumin Secretion

Test Article	50% Inhibitory Concentration (IC ₅₀) - μ M			
	Day 2	Day 5	Day 7	Day 9
Cmpd 5-2	>30	>30	>30	>30
Sofosbuvir	>30	19.5	10.9	9.3
INX-189	13.6	3.1	3.2	2.4

5

Table 6. Effect of Test Articles on Albumin Secretion

Test Article	50% Inhibitory Concentration (IC ₅₀) - μ M						
	Day 2	Day 5	Day 7	Day 9	Day 12	Day 16	Day 21
Cmpd 5-2	>30	>30	>30	>30	>30	24.2	14.5
Sofosbuvir	>30	>30	>30	12.1	6.8	2.7	2.3
INX-189	>30	4.2	1.8	1.8	1.3	<<1	<<1

Example 33. Metabolic Studies

10 The metabolism of compounds 25, 27 and 5-2, at a concentration of 10 μ M, were investigated in fresh primary cultures of human, dog and mouse hepatocytes. Plated hepatocytes from humans (XenoTech, mixed gender, pooled from 10 donors), male Beagle dog (BioreclamationIVT), and male ICR/CD-1 mice (BioreclamationIVT, 8 donors) in 6-well plates with matrigel overlay were incubated in singlet with 10 μ M TA. After 2, 4, 6, 8 or 24 hr, intracellular levels of nucleotide prodrugs and their potential metabolites (prodrugs, 15 monophosphates, triphosphates and nucleosides) were quantitated by LC-MS/MS. Concentrations below the lower limit of quantitation (1.5 pmol/10⁶ cells for prodrugs,

monophosphates and nucleosides and 12 pmol/10⁶ cells for triphosphates) were extrapolated from the standard curves.

The compound β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-guanine triphosphate is the predominant metabolite of compounds 25, 27 and 5-2 observed in cultured human hepatocytes and is a potent inhibitor of the HCV (gt1b) NS5B polymerase, with an IC₅₀ of 0.15 μ M.

Figure 8 shows the predominant Compound 25 metabolites in human hepatocytes.

Figure 9 shows the predominant Compound 27 metabolites in human hepatocytes.

Figure 10 shows the predominant Compound 5-2 metabolites in human hepatocytes.

Figure 11 illustrates the activation pathways for Compounds 25, 27 and 5-2. As can be seen, Compounds 25, 27 and 5-2 are converted to their corresponding monophosphate analogs which are subsequently metabolized to a common MP analog; β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-guanine monophosphate (Compound 61). The monophosphate is then stepwise phosphorylated to the active triphosphate: β -D-2'-deoxy-2'- α -fluoro-2'- β -methyl-guanine triphosphate (Compound 62).

Example 34. Controls

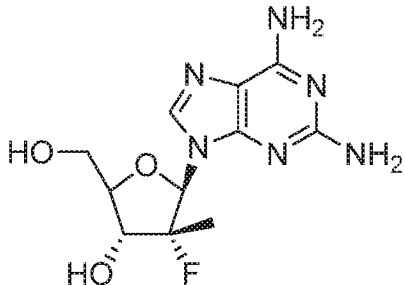
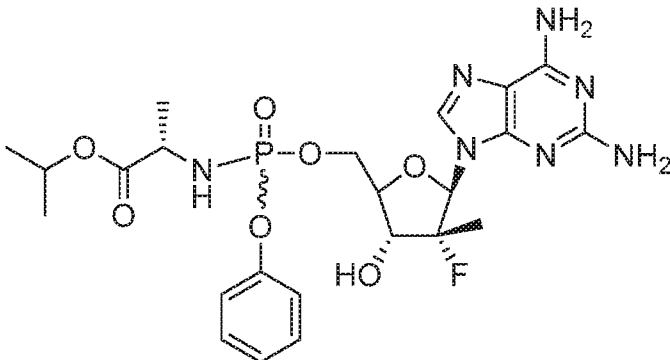
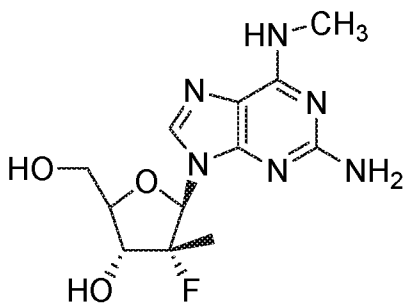
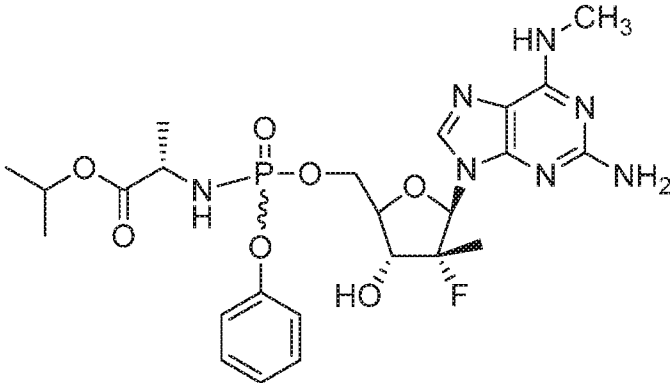
INX-189 (INX-08189/BMS-986094) and sofosbuvir were used as controls in the Examples above.

The two most potent nucleotide prodrugs, Compounds 25 and 27, demonstrated excellent selectivity, with CC₅₀ values greater than 100 μ M in Huh-7 cells, human bone marrow stem cells and human cardiomyocytes. No inhibition of human DNA polymerase α , β or γ , no activity against other RNA or DNA viruses, and no toxicity in all host cell lines was observed at concentrations up to 100 μ M.

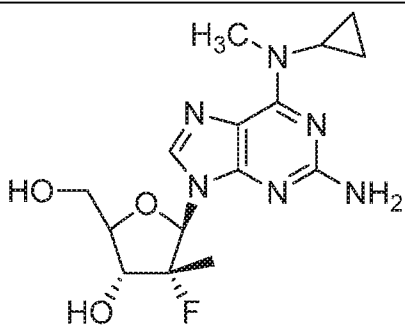
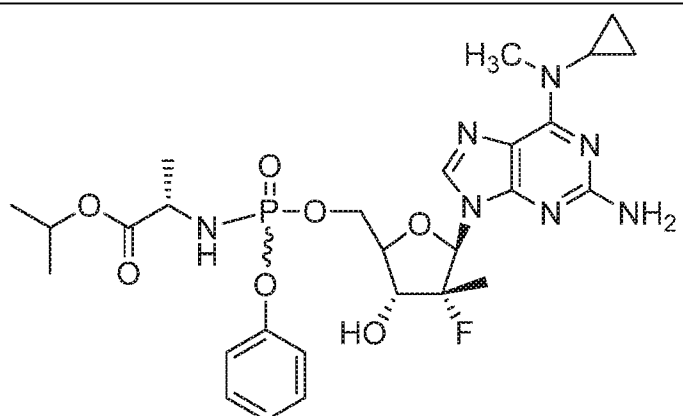
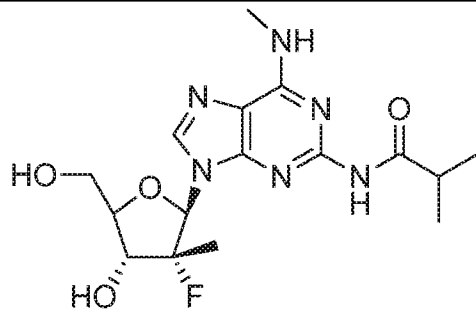
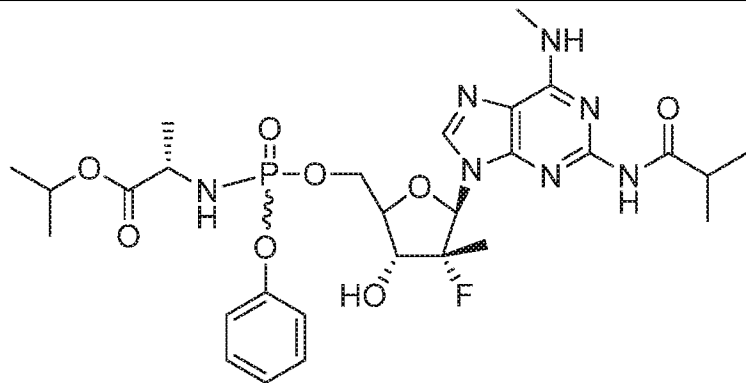
Table 7 is a table illustrating the compounds tested in a HCV Replicon Assay along with the EC₅₀/EC₉₅ (μ M) and CC₅₀ (μ M) results.

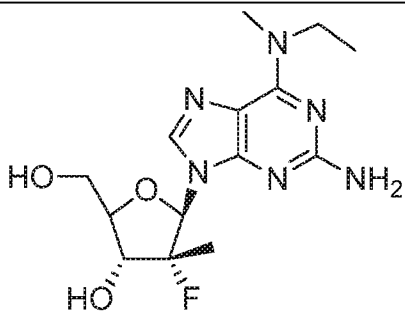
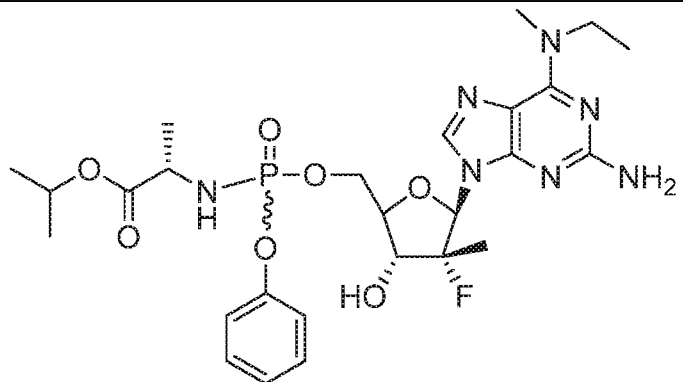
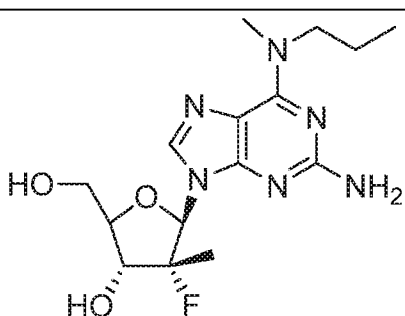
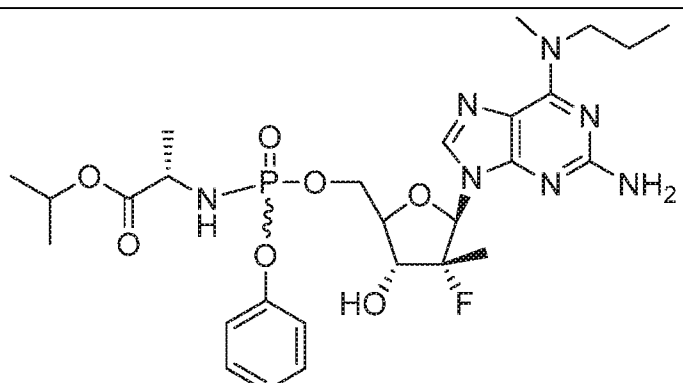
Table 7. Replicon Assay Results for Compounds Tested.

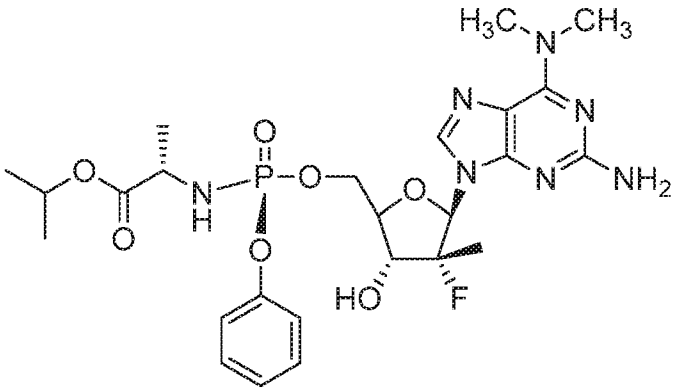
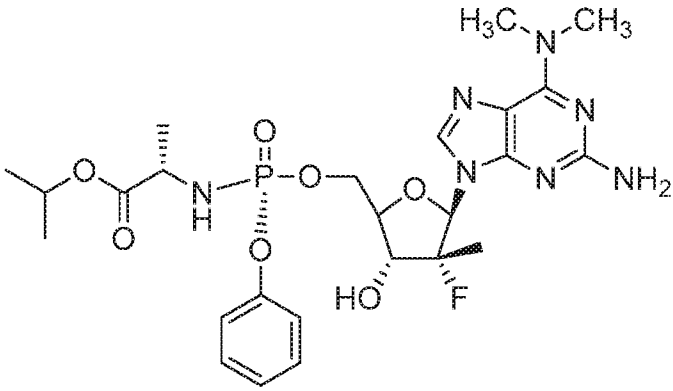
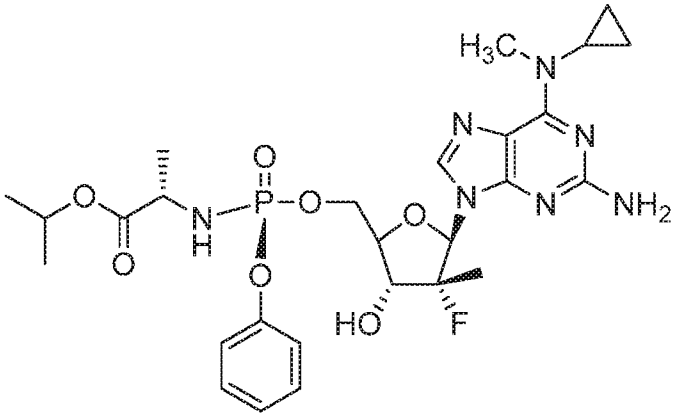
Cmpd No.	Structure	HCV Replicon	HCV Replicon	Fold increase
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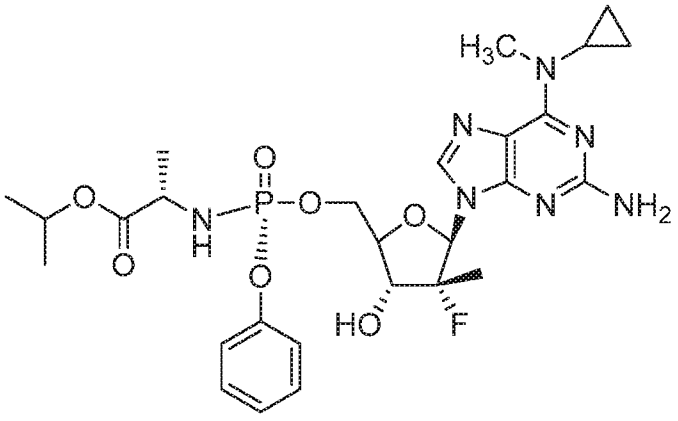
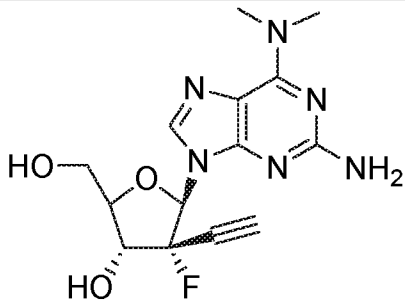
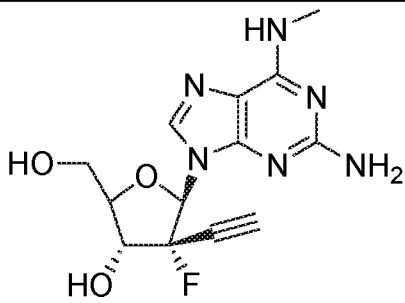
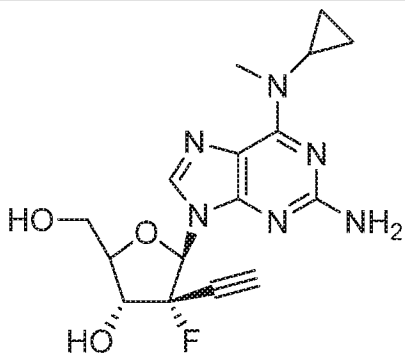
		EC ₅₀ /EC ₉₅ (μM)	CC ₅₀ (μM)	in activity compared to parent nucleoside
		6.7	>100	
		2.1/9.04	>100	3
4		15.7	>100	
5		0.026/0.124	>100	> 600

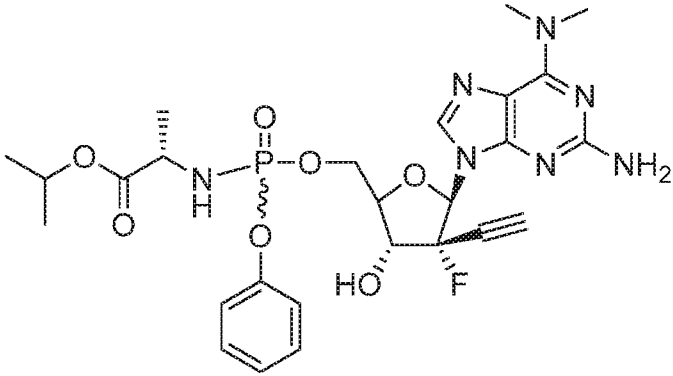
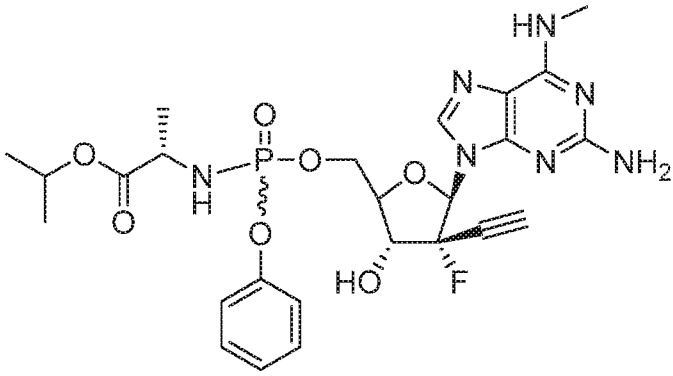
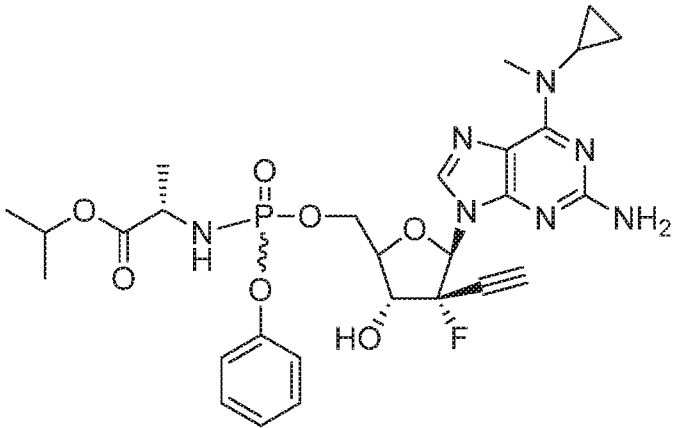
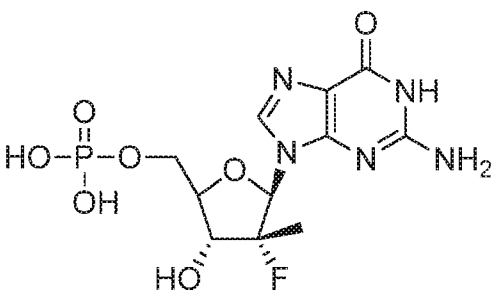
5-1		0.0551/0.282	>100	>280
5-2		0.004/0.028	>100	>3,900
6		10.7	>100	
7		0.0121/0.071	>100	>890

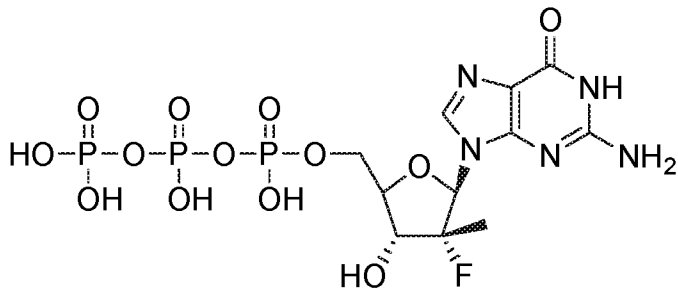
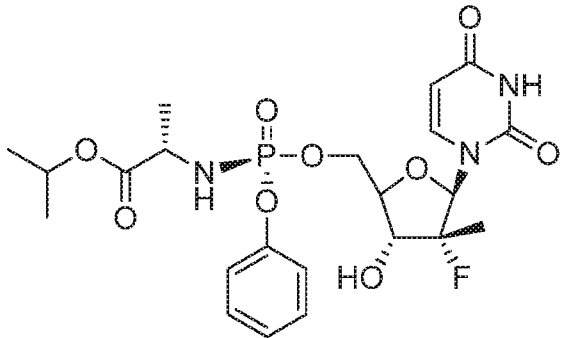
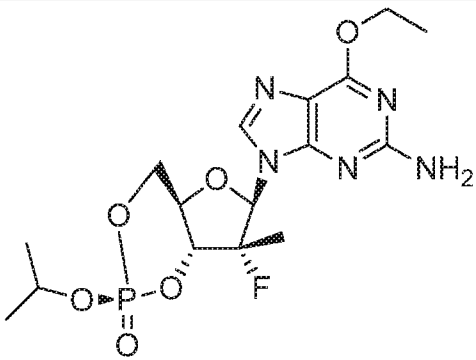
8		5.56	>100	
9		0.0091/0.054	>100	>600
15		>100	>100	
16		0.576/3.69	>100	

17		11.5/65.4	>100	
18		0.048/0.219	90.0	
19		7.47	>100	
20		0.073/0.315	>100	

25		0.004/0.019	>100	>2,600
26		0.0351/0.057	>100	
27		0.005/0.025	>100	>1,100

28		0.014/0.076	>100	
41		0.508/25.1	21.8	
42		4.18/20.4	>100	
43		6.43/24.7	21.6	

45		0.16/0.876	0.68	
46		0.224/0.961	>100	
47		0.338/1.72	1.68	
61				

62				
	 Sofosbuvir	0.052/0.310	>100	
	 PSI-352938	0.045/0.259	>100	

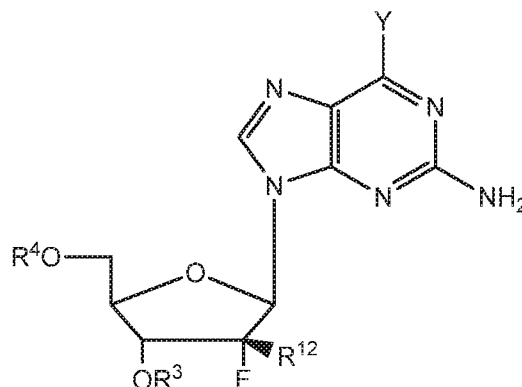
The β -D-2'-D-2'- α -fluoro-2'- β -C-substituted-2-modified-N⁶-substituted purine nucleotides described herein exhibit significant activity against the HCV virus. Compounds according to the present invention are assayed for desired relative activity using well-known and conventional assays found in the literature.

For example, anti-HCV activity and cytotoxicity of the compounds may be measured in the HCV subgenomic RNA replicon assay system in Huh7 ET cells. (See, Korba, et al., *Antiviral Research* **2008**, 77, 56). The results can be summarized in comparison to a positive control, 2'-C-Me-cytosine {2'-C-Me-C}(Pierra, et al., *Journal of Medicinal Chemistry* **2006**, 49, 6614).

Another in-vitro assay for anti-hepatitis C virus activity is described in U.S. Patent No. 7,718,790 by Stuyver, et al., and assigned to Pharmasset, Inc.

This specification has been described with reference to embodiments of the invention. Given the teaching herein, one of ordinary skill in the art will be able to modify the invention for
5 a desired purpose and such variations are considered within the scope of the invention.

1. A compound of Formula I:



Formula I

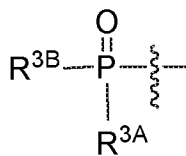
wherein:

Y is NR^1R^2 ;

R^1 is C_1 - C_5 alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C_1 - C_5 haloalkyl (including CH_2F , CHF_2 , CF_3 , CH_2CF_3 , CF_2CH_3 and CF_2CF_3), C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{C}_3\text{-C}_6\text{cycloalkyl})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{heterocycle})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{aryl})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{heteroaryl})$, $-\text{OR}^{25}$, $-\text{C}(\text{O})\text{R}^{3\text{C}}$ (including $-\text{C}(\text{O})\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{OCH}_3$, $-\text{C}(\text{O})\text{OC}_2\text{H}_5$, $-\text{C}(\text{O})\text{OC}_3\text{H}_7$, $-\text{C}(\text{O})\text{OC}_4\text{H}_9$, and $-\text{C}(\text{O})\text{OC}_5\text{H}_{11}$), $-\text{C}(\text{S})\text{R}^{3\text{D}}$, or $-\text{SO}_2\text{R}^{28}$ each of which can be optionally substituted;

R^2 is hydrogen, optionally substituted C_1 - C_5 alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C_1 - C_5 haloalkyl (including CHF_2 , CHF_2 , CF_3 , CH_2CF_3 and CF_2CF_3), optionally substituted $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{C}_3\text{-C}_6\text{cycloalkyl})$, optionally substituted $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{heterocycle})$, optionally substituted $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{aryl})$, optionally substituted $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{heteroaryl})$, $-\text{C}(\text{O})\text{R}^{3\text{C}}$ (including $-\text{C}(\text{O})\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{OCH}_3$, $-\text{C}(\text{O})\text{OC}_2\text{H}_5$, $-\text{C}(\text{O})\text{OC}_3\text{H}_7$, $-\text{C}(\text{O})\text{OC}_4\text{H}_9$, and $-\text{C}(\text{O})\text{OC}_5\text{H}_{11}$), $-\text{C}(\text{S})\text{R}^{3\text{D}}$, or $-\text{SO}_2\text{R}^{28}$; and

wherein at least one of R^1 and R^2 is methyl, CH_2F , CHF_2 or CF_3 ;



R^3 is hydrogen, R^{3A} , diphosphate, triphosphate, an optionally substituted carbonyl linked amino acid, or $-C(O)R^{3C}$;

R^{3A} can be selected from O^- , OH , an $-O$ -optionally substituted aryl, an $-O$ -optionally substituted heteroaryl, or an optionally substituted heterocyclyl;

R^{3B} can be selected from O^- , OH , an optionally substituted N-linked amino acid or an optionally substituted N-linked amino acid ester;

R^{3C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$, $-O\text{-alkyl}$, $-O\text{-alkenyl}$, $-O\text{-alkynyl}$, $-O-(C_0-C_2)(\text{cycloalkyl})$, $-O-(C_0-C_2)(\text{heterocyclo})$, $-O-(C_0-C_2)(\text{aryl})$, or $-O-(C_0-C_2)(\text{heteroaryl})$, each of which can be optionally substituted;

R^4 is a monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, including but not limited to a phosphoramidate, a thiophosphoramidate, or any other moiety that is metabolized to a monophosphate, diphosphate or triphosphate *in vivo* in the host human or animal; or

R^3 and R^4 together with the oxygens that they are bonded to can form a 3',5'-cyclic prodrug;

R^{12} is CH_3 , CH_2F , CHF_2 , CF_3 , or ethynyl;

R^{25} is hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, $-(C_0-C_2\text{alkyl})(C_3-C_6\text{cycloalkyl})$, $-(C_0-C_2\text{alkyl})(C_3-C_6\text{heterocycle})$, $-(C_0-C_2\text{alkyl})(\text{aryl})$ or $-(C_0-C_2\text{alkyl})(\text{heteroaryl})$ wherein except for the hydrogen each of which can be optionally substituted;

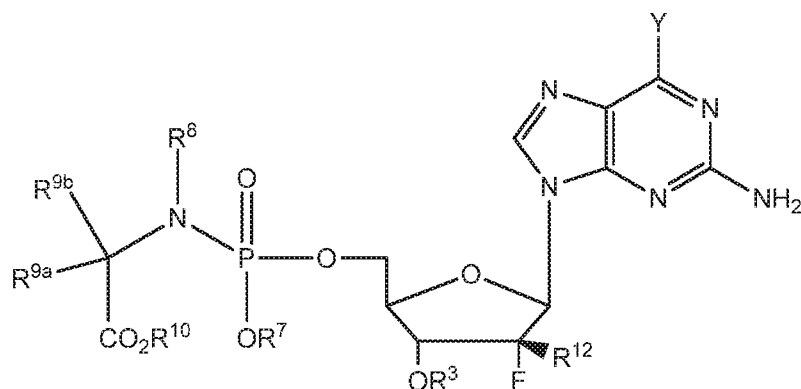
R^{28} is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, $-(C_0-C_2\text{alkyl})(C_3-C_6\text{cycloalkyl})$, $-(C_0-C_2\text{alkyl})(C_3-C_6\text{heterocycle})$, $-(C_0-C_2\text{alkyl})(\text{aryl})$ or $-(C_0-C_2\text{alkyl})(\text{heteroaryl})$ each of which can be optionally substituted;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein:

R^4 is a stabilized phosphate prodrug and R^{12} is CH_3 , CH_2F , CF_2H or CF_3 .

3. The compound of claim 2, wherein:
 R^4 is a phosphoramidate and R^{12} is CH_3 , CH_2F , CF_2H or CF_3 .
4. The compound of claim 2, wherein:
 Y is NR^1R^2 ; and
 R^1 is methyl, R^2 is hydrogen and R^{12} is CH_3 , CH_2F , CF_2H or CF_3 .
5. The compound of claim 2, wherein:
 Y is NR^1R^2 ; and
 R^1 is methyl, R^2 is methyl and R^{12} is CH_3 , CH_2F , CF_2H or CF_3 .
6. The compound of claim 2, wherein:
 Y is NR^1R^2 ; and
 R^1 is methyl, R^2 is cyclopropyl and R^{12} is CH_3 , CH_2F , CF_2H or CF_3 .
7. The compound of claim 1, wherein R^{12} is CH_3 .
8. The compound of claim 1, wherein R^{12} is ethynyl.
9. A compound of the formula:



wherein:

R^7 is hydrogen, C_{1-6} alkyl; C_{3-7} cycloalkyl; heteroaryl, heterocyclic, or aryl, where phenyl or naphthyl are optionally substituted with C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-}

alkoxy, F, Cl, Br, I, nitro, cyano, C₁₋₆haloalkyl, $-(CH_2)_{1-6}COOH$, $-(CH_2)_{1-6}COOC_{1-6}alkyl$, $-N(R^7)_2$, C₁₋₆acylamino, $NHSO_2C_{1-6}alkyl$, $-SO_2N(R^7)_2$, $COR^{7'}$, and $-SO_2C_{1-6}alkyl$; (R^7 is independently hydrogen or C₁₋₆alkyl; $R^{7'}$ is $-OR^{11}$ or $-N(R^7)_2$);

R^8 is hydrogen, C₁₋₆alkyl, or R^{9a} or R^{9b} and R^8 together are $(CH_2)_n$ so as to form a cyclic ring that includes the adjoining N and C atoms; where n is 2 to 4;

R^{9a} and R^{9b} are (i) independently selected from hydrogen, C₁₋₆alkyl, cycloalkyl, $-(CH_2)_c(NR^{9'})_2$, C₁₋₆hydroxyalkyl, $-CH_2SH$, $-(CH_2)_2S(O)(Me)$, $-(CH_2)_3NHC(=NH)NH_2$, (1H-indol-3-yl)methyl, (1H-imidazol-4-yl)methyl, $-(CH_2)_cCOR^{9''}$, aryl and aryl(C₁₋₃alkyl)-, the aryl groups can be optionally substituted with a group selected from hydroxyl, C₁₋₆alkyl, C₁₋₆alkoxy, halogen, nitro and cyano; (ii) R^{9a} and R^{9b} both are C₁₋₆alkyl; (iii) R^{9a} and R^{9b} together are $(CH_2)_r$ so as to form a spiro ring; (iv) R^{9a} is hydrogen and R^{9b} and R^8 together are $(CH_2)_n$ so as to form a cyclic ring that includes the adjoining N and C atoms (v) R^{9b} is hydrogen and R^{9a} and R^8 together are $(CH_2)_n$ so as to form a cyclic ring that includes the adjoining N and C atoms, where c is 1 to 6, n is 2 to 4, r is 2 to 5 and where $R^{9'}$ is independently hydrogen or C₁₋₆ alkyl and $R^{9''}$ is $-OR^{11}$ or $-N(R^{11'})_2$); (vi) R^{9a} is hydrogen and R^{9b} is hydrogen, CH_3 , CH_2CH_3 , $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph , CH_2 -indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H , $CH_2C(O)NH_2$, CH_2CH_2COOH , $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, CH_2 -imidazol-4-yl, CH_2OH , $CH(OH)CH_3$, $CH_2((4'-OH)-Ph)$, CH_2SH , or lower cycloalkyl; or (vii) R^{9a} is CH_3 , CH_2CH_3 , $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph , CH_2 -indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H , $CH_2C(O)NH_2$, CH_2CH_2COOH , $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, CH_2 -imidazol-4-yl, CH_2OH , $CH(OH)CH_3$, $CH_2((4'-OH)-Ph)$, CH_2SH , or lower cycloalkyl and R^{9b} is hydrogen;

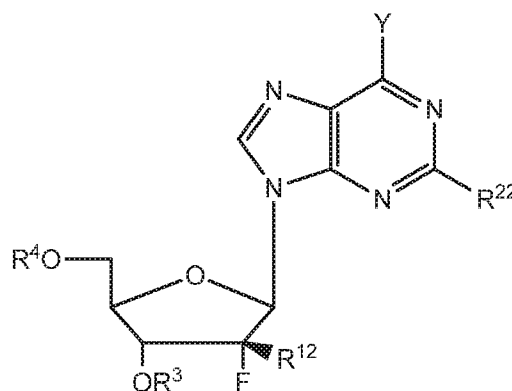
R^{10} is hydrogen, C₁₋₆alkyl optionally substituted with an alkoxy, di(lower alkyl)-amino, or halogen, C₁₋₆haloalkyl, C₃₋₇cycloalkyl, heterocycloalkyl, aminoacyl, aryl, such as phenyl, heteroaryl, such as, pyridinyl, substituted aryl, or substituted heteroaryl;

R^{11} is an optionally substituted C₁₋₆alkyl, an optionally substituted cycloalkyl; an optionally substituted C₂₋₆alkynyl, an optionally substituted C₂₋₆alkenyl, or optionally substituted acyl, which includes but is not limited to $C(O)(C_{1-6}alkyl)$; and

Y, R^3 and R^{12} are as defined in claim 1;

or a pharmaceutically acceptable salt thereof.

10. The compound of claim 9, wherein:
Y is NR^1R^2 , R^1 is methyl, R^2 is hydrogen or methyl, and R^{12} is CH_3 .
11. The compound of claim 9, wherein:
Y is NR^1R^2 , R^1 is methyl, R^2 is methyl or hydrogen, R^3 is hydrogen, R^7 is phenyl, R^8 is hydrogen, R^{9a} is hydrogen, R^{9b} is methyl, R^{10} is isopropyl and R^{12} is CH_3 .
12. The compound of claim 9, wherein:
Y is NR^1R^2 , R^1 is methyl, R^2 is cyclopropyl and R^{12} is CH_3 .
13. The compound of claim 9, wherein:
Y is NR^1R^2 , R^1 is CH_3 , R^2 is $\text{C}(\text{O})\text{OR}^3$, R^3 is hydrogen and R^{12} is CH_3 .
14. A compound of Formula II:



Formula II

wherein:

Y is NR^1R^2 ;

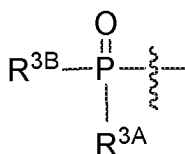
R^1 is C_1 - C_5 alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C_1 - C_5 haloalkyl (including CH_2F , CHF_2 , CF_3 , CH_2CF_3 , CF_2CH_3 and CF_2CF_3), C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{C}_3\text{-C}_6\text{cycloalkyl})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{heterocycle})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{aryl})$, $-(\text{C}_0\text{-C}_2\text{alkyl})(\text{heteroaryl})$, $-\text{OR}^{25}$, $-\text{C}(\text{O})\text{R}^{3C}$ (including $-\text{C}(\text{O})\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{OCH}_3$, $-$

C(O)OC₂H₅, -C(O)OC₃H₇, -C(O)OC₄H₉, and -C(O)OC₅H₁₁), -C(S)R^{3D}, or -SO₂R²⁸ each of which can be optionally substituted;

R² is hydrogen, optionally substituted C₁-C₅alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C₁-C₅haloalkyl (including CHF₂, CHCl₂, CF₃, CH₂CF₃ and CF₂CF₃), optionally substituted -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), optionally substituted -(C₀-C₂alkyl)(heterocycle), optionally substituted -(C₀-C₂alkyl)(aryl),

optionally substituted -(C₀-C₂alkyl)(heteroaryl), -C(O)R^{3C} (including -C(O)CH₃, -C(O)CH₂CH₃, -C(O)CH(CH₃)₂, -C(O)OCH₃, -C(O)OC₂H₅, -C(O)OC₃H₇, -C(O)OC₄H₉, and -C(O)OC₅H₁₁), -C(S)R^{3D}, or -SO₂R²⁸; and

wherein at least one of R¹ and R² is methyl, CH₂F, CHF₂ or CF₃;



R³ is hydrogen, substituted carbonyl linked amino acid, or -C(O)R^{3C};

R^{3A} can be selected from O⁻, OH, an -O-optionally substituted aryl, an -O-optionally substituted heteroaryl, or an optionally substituted heterocyclyl;

R^{3B} can be selected from O⁻, OH, an optionally substituted N-linked amino acid or an optionally substituted N-linked amino acid ester;

R^{3C} is alkyl, alkenyl, alkynyl, -(C₀-C₂)(cycloalkyl), -(C₀-C₂)(heterocyclo), -(C₀-C₂)(aryl), -(C₀-C₂)(heteroaryl), -O-alkyl, -O-alkenyl, -O-alkynyl, -O-(C₀-C₂)(cycloalkyl), -O-(C₀-C₂)(heterocyclo), -O-(C₀-C₂)(aryl), -O-(C₀-C₂)(heteroaryl), -S-alkyl, -S-alkenyl, -S-alkynyl, -S-(C₀-C₂)(cycloalkyl), -S-(C₀-C₂)(heterocyclo), -S-(C₀-C₂)(aryl), or -S-(C₀-C₂)(heteroaryl) each of which can be optionally substituted;

R^{3D} is alkyl, alkenyl, alkynyl, -(C₀-C₂)(cycloalkyl), -(C₀-C₂)(heterocyclo), -(C₀-C₂)(aryl), -(C₀-C₂)(heteroaryl), -O-alkyl, -O-alkenyl, -O-alkynyl, -O-(C₀-C₂)(cycloalkyl), -O-(C₀-C₂)(heterocyclo), -O-(C₀-C₂)(aryl), or -O-(C₀-C₂)(heteroaryl), each of which can be optionally substituted;

R⁴ is a monophosphate, diphosphate, triphosphate, or a stabilized phosphate prodrug, including but not limited to a phosphoramidate, a thiophosphoramidate, or any

other moiety that is metabolized to a monophosphate, diphosphate or triphosphate *in vivo* in the host human or animal; or

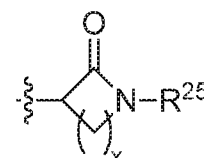
R^3 and R^4 together with the oxygens that they are bonded to can form a 3',5'-cyclic prodrug, including but not limited to, a 3',5'-cyclic phosphate prodrug;

R^5 is C_1 - C_5 alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C_1 - C_5 haloalkyl (including CHF_2 , CHF_2 , CF_3 , CH_2CF_3 and CF_2CF_3), C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, $-(C_0-C_2alkyl)(C_3-C_6cycloalkyl)$, $-(C_0-C_2alkyl)(heterocycle)$, $-(C_0-C_2alkyl)(aryl)$, $-(C_0-C_2alkyl)(heteroaryl)$, $-OR^{25}$, $-C(O)R^{3C}$ (including $-C(O)CH_3$, $-C(O)CH_2CH_3-C(O)CH(CH_3)_2$, $-C(O)OCH_3$, $-C(O)OC_2H_5$, $-C(O)OC_3H_7$, $-C(O)OC_4H_9$, and $-C(O)OC_5H_{11}$), $-C(S)R^{3D}$, or $-SO_2R^{28}$ each of which can be optionally substituted;

R^6 is hydrogen, optionally substituted C_1 - C_5 alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and pentyl), C_1 - C_5 haloalkyl (including CHF_2 , CHF_2 , CF_3 , CH_2CF_3 and CF_2CF_3), optionally substituted $-(C_0-C_2alkyl)(C_3-C_6cycloalkyl)$, optionally substituted $-(C_0-C_2alkyl)(heterocycle)$, optionally substituted $-(C_0-C_2alkyl)(aryl)$, optionally substituted $-(C_0-C_2alkyl)(heteroaryl)$, $-C(O)R^{3C}$ (including $-C(O)CH_3$, $-C(O)CH_2CH_3-C(O)CH(CH_3)_2$, $-C(O)OCH_3$, $-C(O)OC_2H_5$, $-C(O)OC_3H_7$, $-C(O)OC_4H_9$, and $-C(O)OC_5H_{11}$), $-C(S)R^{3D}$, or $-SO_2R^{28}$; or R^5 and R^6 together with the nitrogen that they are bonded to can form a heterocyclic ring;

R^{12} is CH_3 , CH_2F , CHF_2 , CF_3 , or ethynyl;

R^{22} is F, Cl, Br, CN, N_3 , C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, $-(C_1-C_2alkyl)(C_3-C_6cycloalkyl)$, $-(C_0-C_2alkyl)(C_3-C_6heterocycle)$, $-(C_0-C_2alkyl)(aryl)$, $-(C_0-C_2alkyl)(heteroaryl)$; $-ONHC(=O)OR^{23}$, $-NHOR^{24}$, $-OR^{25}$, $-SR^{25}$, $-NH(CH_2)_{1-4}N(R^{26})_2$, $-NHNHR^{26}$, $-N=NR^{27}$, $-NHC(O)NHNHR^{27}$, $-NHC(S)NHNHR^{27}$, $-C(O)NHNHR^{27}$, $-$



$NR^{27}SO_2R^{28}$, $-SO_2NR^{27}R^{29}$, $-C(O)NR^{27}R^{29}$, $-CO_2R^{29}$, $-SO_2R^{29}$, $-P(O)H(OR^{29})$, $-P(O)(OR^{29})(OR^{30})$, $-P(O)(OR^{29})(NR^{29}R^{30})$ or $-NR^5R^6$;

R^{23} is C_1 - C_5 alkyl, $-(C_0-C_2alkyl)(C_3-C_6cycloalkyl)$, $-(C_0-C_2alkyl)(heterocycle)$, $-(C_0-C_2alkyl)(aryl)$ or $-(C_0-C_2alkyl)(heteroaryl)$ each of which can be optionally substituted;

R^{24} is hydrogen, C_1 - C_6 alkyl, $-(C_1-C_2alkyl)(C_3-C_6cycloalkyl)$,

-(C₁-C₂alkyl)(C₃-C₆heterocycle) -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted;

R²⁵ is hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted;

R²⁶ is independently selected from hydrogen, C₁-C₆alkyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(heterocycle), -(C₀-C₂alkyl)(aryl), or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted;

R²⁷ hydrogen or optionally substituted C₁-C₆ alkyl;

R²⁸ is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) each of which can be optionally substituted;

R²⁹ is hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted; or

R²⁷ and R²⁹ together with the nitrogen that they are bonded to can form a heterocyclic ring;

R³⁰ is hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₀-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl) or -(C₀-C₂alkyl)(heteroaryl) wherein except for the hydrogen each of which can be optionally substituted; or

R²⁹ and R³⁰ can be bonded together to form a heterocyclic ring;

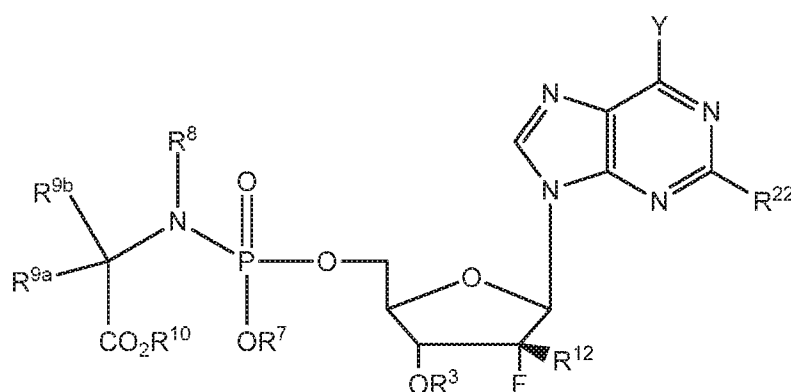
x is 1, 2 or 3;

or a pharmaceutically acceptable salt thereof.

15. The compound of claim 14, wherein:

R⁴ is a stabilized phosphate prodrug and R¹² is CH₃, CH₂F, CF₂H or CF₃.

16. The compound of claim 15, wherein:
R⁴ is a phosphoramidate and R¹² is CH₃, CH₂F, CF₂H or CF₃.
17. The compound of claim 15, wherein:
Y is NR¹R² and R¹ is methyl and R² is hydrogen or methyl.
18. The compound of claim 15, wherein R²² is F.
19. The compound of claim 15, wherein: R²² is OR²⁵.
20. The compound of claim 15, wherein:
R²² is NR⁵R⁶ and R⁶ is hydrogen.
21. The compound of claim 15, wherein:
R²² is NR⁵R⁶.
22. The compound of claim 15, wherein:
R²² is selected from Cl, Br, CN, N₃, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₁-C₂alkyl)(C₃-C₆cycloalkyl), -(C₀-C₂alkyl)(C₃-C₆heterocycle), -(C₀-C₂alkyl)(aryl), -(C₀-C₂alkyl)(heteroaryl); -ONHC(=O)OR²³, -NHOR²⁴, -OR²⁵ and -SR²⁵
23. The compound of claim 15, wherein:
R²² is NR⁵R⁶; and
R⁵ and R⁶ is hydrogen.
24. A compound of Formula III:



Formula III

wherein:

R^7 is hydrogen, C_{1-6} alkyl; C_{3-7} cycloalkyl; heteroaryl, heterocyclic, or aryl, where phenyl or naphthyl are optionally substituted with C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} alkoxy, F, Cl, Br, I, nitro, cyano, C_{1-6} haloalkyl, $-(CH_2)_{1-6}COOH$, $-(CH_2)_{1-6}COOC_{1-6}$ alkyl, $-N(R^7)_2$, C_{1-6} acylamino, $NHSO_2C_{1-6}$ alkyl, $-SO_2N(R^7)_2$, $COR^{7'}$, and $-SO_2C_{1-6}$ alkyl; (R^7 is independently hydrogen or C_{1-6} alkyl; $R^{7'}$ is $-OR^{11}$ or $-N(R^7)_2$);

R^8 is hydrogen, C_{1-6} alkyl, or R^{9a} or R^{9b} and R^8 together are $(CH_2)_n$ so as to form a cyclic ring that includes the adjoining N and C atoms; where n is 2 to 4;

R^{9a} and R^{9b} are (i) independently selected from hydrogen, C_{1-6} alkyl, cycloalkyl, $-(CH_2)_c(NR^9)_2$, C_{1-6} hydroxyalkyl, $-CH_2SH$, $-(CH_2)_2S(O)(Me)$, $-(CH_2)_3NHC(=NH)NH_2$, (1H-indol-3-yl)methyl, (1H-imidazol-4-yl)methyl, $-(CH_2)_cCOR^{9'}$, aryl and aryl(C_{1-3} alkyl)-, the aryl groups can be optionally substituted with a group selected from hydroxyl, C_{1-6} alkyl, C_{1-6} alkoxy, halogen, nitro and cyano; (ii) R^{9a} and R^{9b} both are C_{1-6} alkyl; (iii) R^{9a} and R^{9b} together are $(CH_2)_r$ so as to form a spiro ring; (iv) R^{9a} is hydrogen and R^{9b} and R^8 together are $(CH_2)_n$ so as to form a cyclic ring that includes the adjoining N and C atoms (v) R^{9b} is hydrogen and R^{9a} and R^8 together are $(CH_2)_n$ so as to form a cyclic ring that includes the adjoining N and C atoms, where c is 1 to 6, n is 2 to 4, r is 2 to 5 and where R^9 is independently hydrogen or C_{1-6} alkyl and $R^{9'}$ is $-OR^{11}$ or $-N(R^{11})_2$); (vi) R^{9a} is hydrogen and R^{9b} is hydrogen, CH_3 , CH_2CH_3 , $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph , CH_2 -indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H , $CH_2C(O)NH_2$, CH_2CH_2COOH , $CH_2CH_2C(O)NH_2$, $CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, CH_2 -imidazol-4-yl, CH_2OH , $CH(OH)CH_3$, $CH_2((4'-OH)-Ph)$, CH_2SH , or lower cycloalkyl; or (vii) R^{9a} is CH_3 , CH_2CH_3 , $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph , CH_2 -indol-3-yl, $-CH_2CH_2SCH_3$, CH_2CO_2H , $CH_2C(O)NH_2$, CH_2CH_2COOH , $CH_2CH_2C(O)NH_2$,

CH₂CH₂CH₂CH₂NH₂, -CH₂CH₂CH₂NHC(NH)NH₂, CH₂-imidazol-4-yl, CH₂OH, CH(OH)CH₃, CH₂((4'-OH)-Ph), CH₂SH, or lower cycloalkyl and R^{9b} is hydrogen;

R¹⁰ is hydrogen, C₁₋₆alkyl optionally substituted with an alkoxy, di(lower alkyl)-amino, or halogen, C₁₋₆haloalkyl, C₃₋₇cycloalkyl, heterocycloalkyl, aminoacyl, aryl, such as phenyl, heteroaryl, such as, pyridinyl, substituted aryl, or substituted heteroaryl;

R¹¹ is an optionally substituted C₁₋₆alkyl, an optionally substituted cycloalkyl; an optionally substituted C₂₋₆alkynyl, an optionally substituted C₂₋₆alkenyl, or optionally substituted acyl, which includes but is not limited to C(O)(C₁₋₆ alkyl);

wherein Y, R³, R¹² and R²² are defined in claim 14; or a pharmaceutically acceptable salt thereof.

25. The compound of claim 24, wherein:

Y is NR¹R², R¹ is methyl, R² is hydrogen and R¹² is CH₃.

26. The compound of claim 24, wherein:

Y is NR¹R², R¹ is methyl, R² is methyl and R¹² is CH₃.

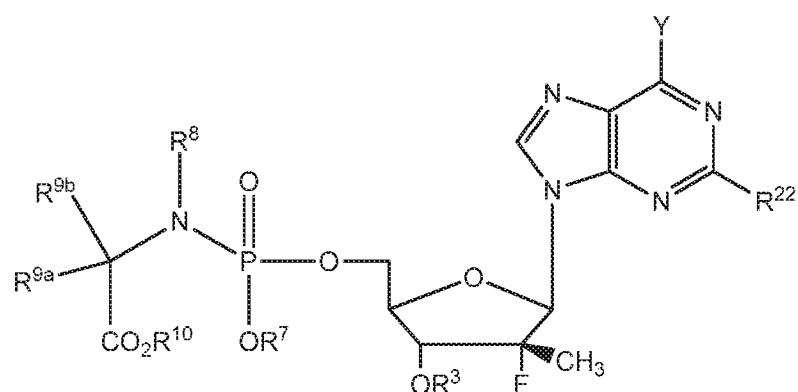
27. The compound of claim 24, wherein:

Y is NR¹R², R¹ is methyl, R² is cyclopropyl, R³ is hydrogen, R⁷ is phenyl, R⁸ is hydrogen, R^{9a} is hydrogen, R^{9b} is methyl, R¹⁰ is isopropyl and R¹² is CH₃.

28. The compound of claim 24, wherein:

Y is NR¹R², R¹ is CH₃, R¹² is CH₃ and R²² is F.

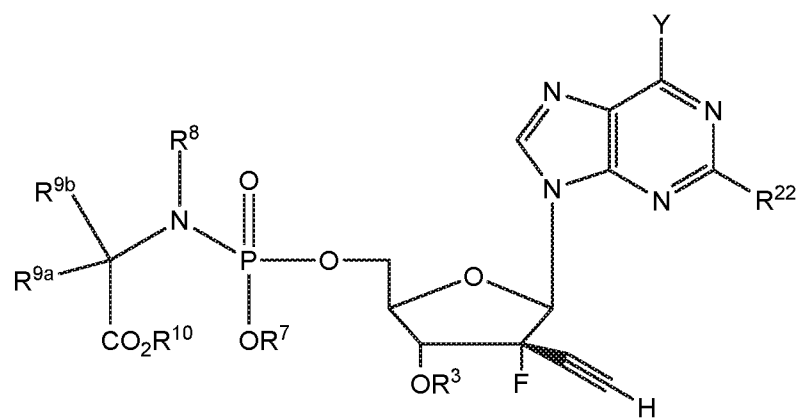
29. A compound of Formula IV:



Formula IV

wherein the variables Y, R³, R⁷, R⁸, R^{9a}, R^{9b}, R¹⁰ and R²² are defined in claim 24;
or a pharmaceutically acceptable salt thereof.

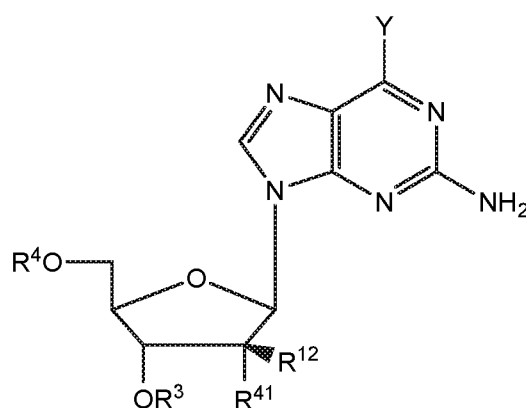
30. A compound of Formula V:



Formula V

wherein the variables Y, R³, R⁷, R⁸, R^{9a}, R^{9b}, R¹⁰ and R²² are defined in claim 24;
or a pharmaceutically acceptable salt thereof.

31. A compound of Formula VI:



Formula VI

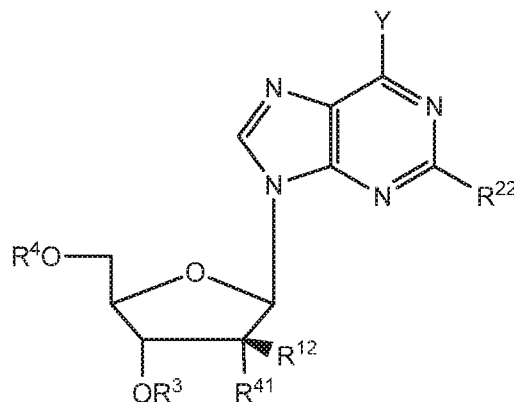
wherein:

Y, R³, R⁴ and R¹² are defined in claim 24;

R⁴¹ is F, Cl, OR³, N₃, NH₂ or CN; or

a pharmaceutically acceptable salt thereof.

32. A compound of Formula VII:



Formula VII

wherein:

Y, R³, R⁴, R¹², R²² and R⁴¹ are defined in claims 1, 24 and 31;

or a pharmaceutically acceptable salt thereof.

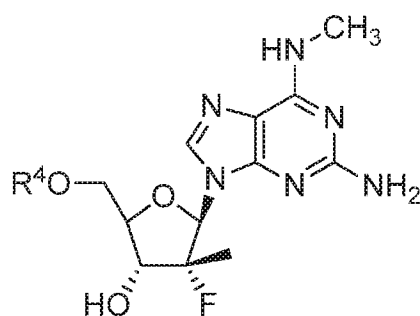
33. A pharmaceutical composition comprising an effective amount of the compound of claim 1 to treat HCV in a host in a pharmaceutically acceptable carrier.
34. A pharmaceutical composition comprising an effective amount of the compound of claim 9 to treat HCV in a host in a pharmaceutically acceptable carrier.

35. A pharmaceutical composition comprising an effective amount of the compound of claim 14 to treat HCV in a host in a pharmaceutically acceptable carrier.
36. A pharmaceutical composition comprising an effective amount of the compound of any of claims 24 or 29-32 to treat HCV in a host in a pharmaceutically acceptable carrier.
37. The pharmaceutical composition of claim 33, wherein the composition is suitable for oral delivery.
38. The pharmaceutical composition of claim 34, wherein the composition is suitable for oral delivery.
39. The pharmaceutical composition of claim 35, wherein the composition is suitable for oral delivery.
40. The pharmaceutical composition of claim 36, wherein the composition is suitable for oral delivery.
41. A method for the treatment of a hepatitis C infection or a condition resulting from a hepatitis C infection, in a host in need thereof, comprising administering an effective amount of a compound of claim 1, optionally in a pharmaceutically acceptable carrier.
42. A method for the treatment of a hepatitis C infection or a condition resulting from a hepatitis C infection, in a host in need thereof, comprising administering an effective amount of a compound of claim 9, optionally in a pharmaceutically acceptable carrier.
43. A method for the treatment of a hepatitis C infection or a condition resulting from a hepatitis C infection, in a host in need thereof, comprising administering an effective amount of a compound of claim 14, optionally in a pharmaceutically acceptable carrier.
44. A method for the treatment of a hepatitis C infection or a condition resulting from a hepatitis C infection, in a host in need thereof, comprising administering an effective

amount of a compound of any of claims 24 or 29-32 optionally in a pharmaceutically acceptable carrier.

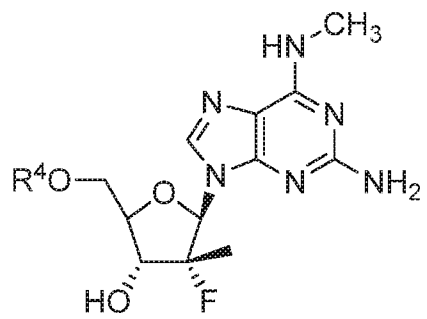
45. The method of claim 41, wherein the compound is administered transdermally.
46. The method of claim 41, wherein the compound is administered via controlled release.
47. The method of claim 41, wherein the compound is administered intravenously.
48. The method of claim 41, wherein the condition resulting from a hepatitis C infection is an antibody positive and antigen positive condition, viral-based chronic liver inflammation, liver cancer resulting from advanced hepatitis C, cirrhosis, or fatigue.
49. The method of claim 41, further comprising administering the compound in combination with another anti-HCV agent.
50. The method of claim 49, wherein the additional anti-HCV agent is selected from the group consisting of a protease inhibitor; an NS5A inhibitor; another NS5B polymerase inhibitor; a non-substrate (allosteric) inhibitor; interferon alfa-2a, which may be pegylated; ribavirin; a helicase inhibitor; an antisense oligodeoxynucleotide (S-ODN); an aptamer; a nuclease-resistant ribozyme; iRNA; an antibody to HCV; a partial antibody to HCV; and a domain antibody to HCV.
51. The method of claim 50, wherein the protease inhibitor is selected from the group consisting of telaprevir, boceprevir, simeprevir and paritaprevir.
52. The method of claims 41-51, wherein the host is a human.

53. A compound of the formula:



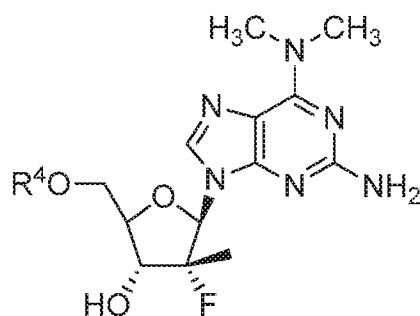
wherein R^4 is a stabilized phosphate prodrug or a pharmaceutically acceptable salt thereof.

54. A pharmaceutical composition comprising the compound:



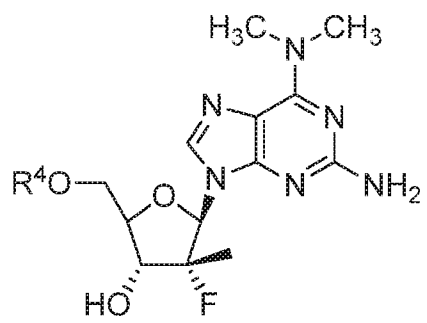
wherein R^4 is a stabilized phosphate prodrug; or a pharmaceutically acceptable salt thereof.

55. A compound of the formula:



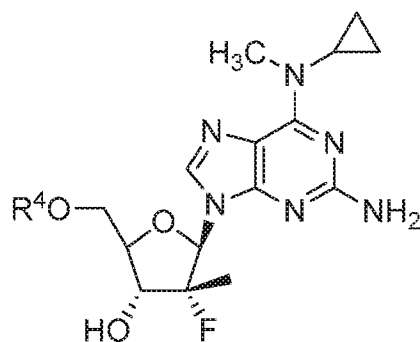
wherein R^4 is a stabilized phosphate prodrug or a pharmaceutically acceptable salt thereof.

56. A pharmaceutical composition comprising the compound:



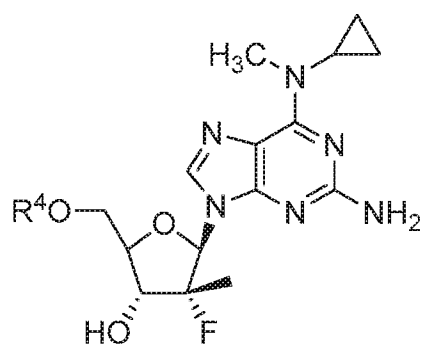
wherein R^4 is a stabilized phosphate prodrug or a pharmaceutically acceptable salt thereof.

57. A compound of the structure:



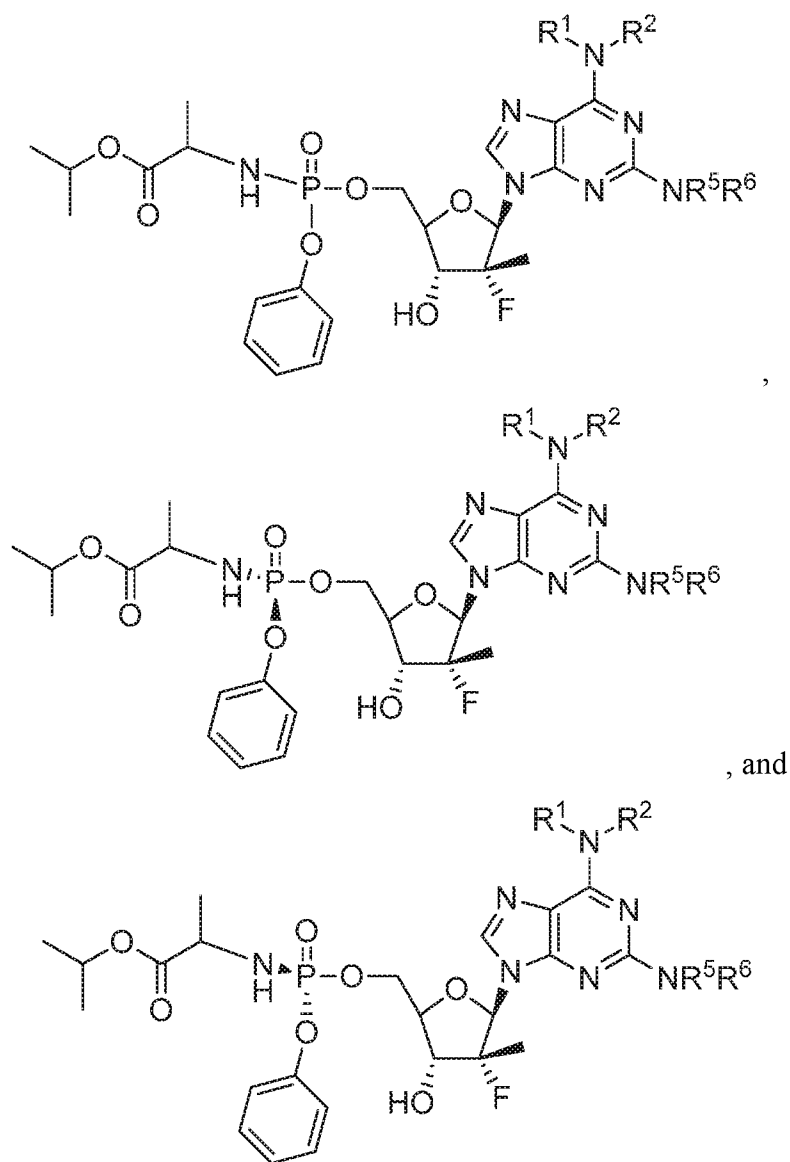
wherein R^4 is a stabilized phosphate prodrug or a pharmaceutically acceptable salt thereof.

58. A pharmaceutical composition comprising the compound:



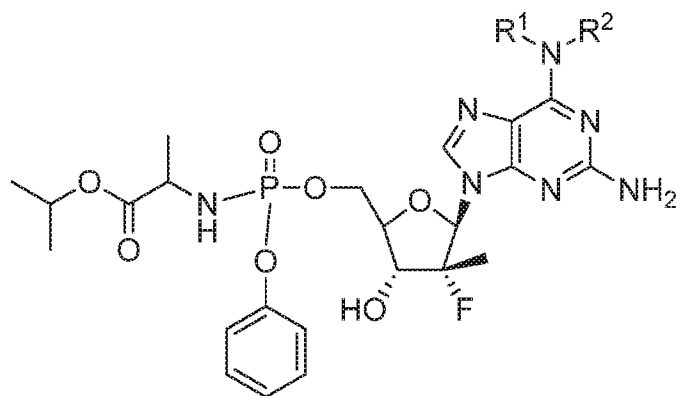
wherein R^4 is a stabilized phosphate prodrug or a pharmaceutically acceptable salt thereof.

59. A compound of the structure selected from the group consisting of:



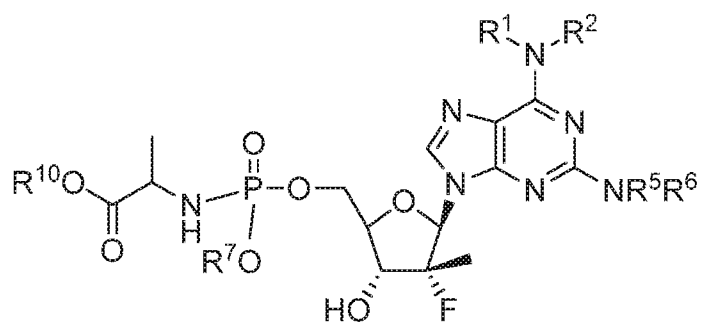
in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof;
 wherein R^1 , R^2 , R^5 and R^6 are as defined in claim 14;
 or a pharmaceutically acceptable salt thereof.

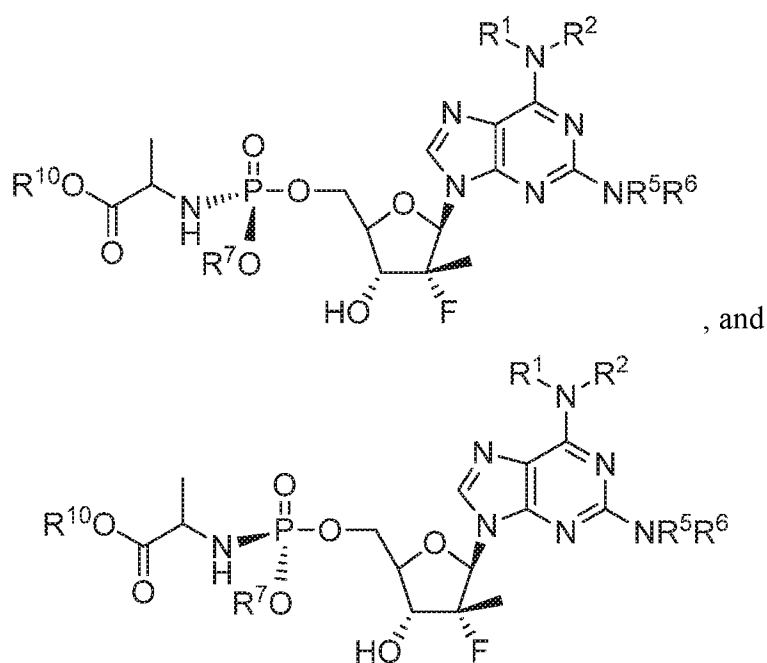
60. A pharmaceutical composition comprising the compound:



wherein R^1 and R^2 are as defined in claim 1; or a pharmaceutically acceptable salt thereof.

61. A compound of the structure selected from the group consisting of:

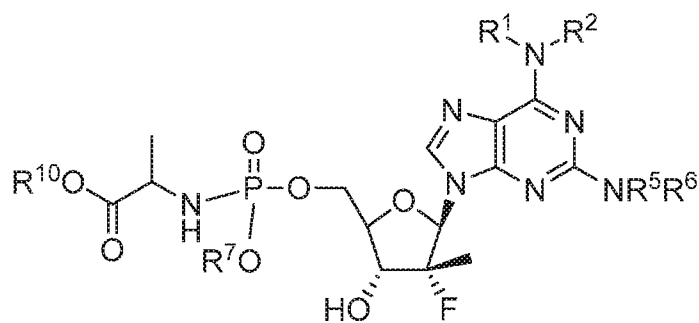




wherein R^1 , R^2 , R^5 and R^6 are as defined in claim 14;

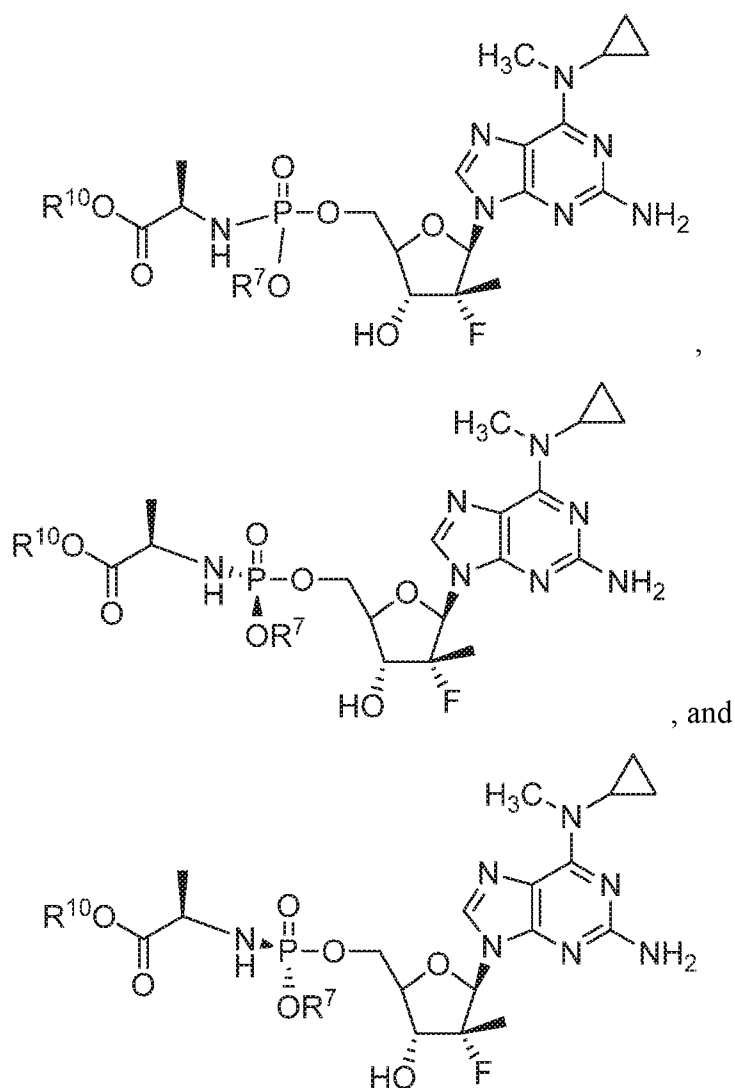
in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

62. A pharmaceutical composition comprising the compound:



wherein R^1 , R^2 , R^5 , R^6 , R^7 and R^{10} are as defined in claims 9 and 14; or a pharmaceutically acceptable salt thereof.

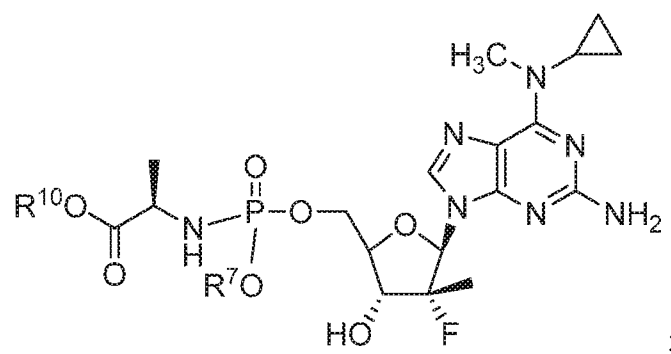
63. A compound of the structure selected from the group consisting of:



wherein R⁷ and R¹⁰ are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

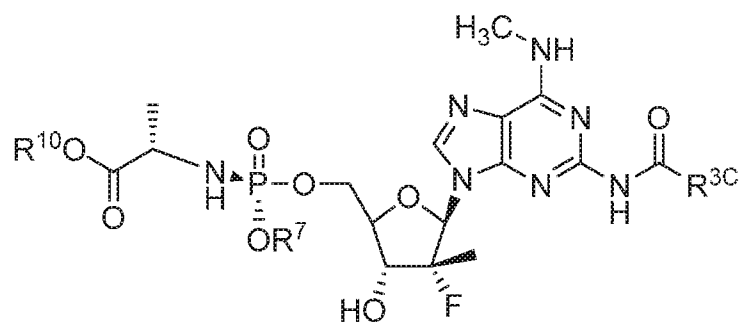
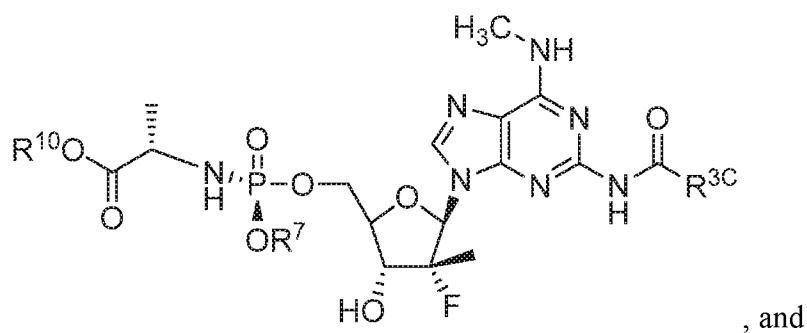
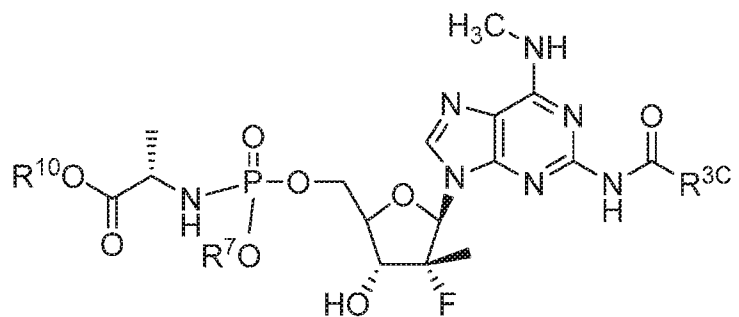
64. A pharmaceutical composition comprising the structure:



wherein R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

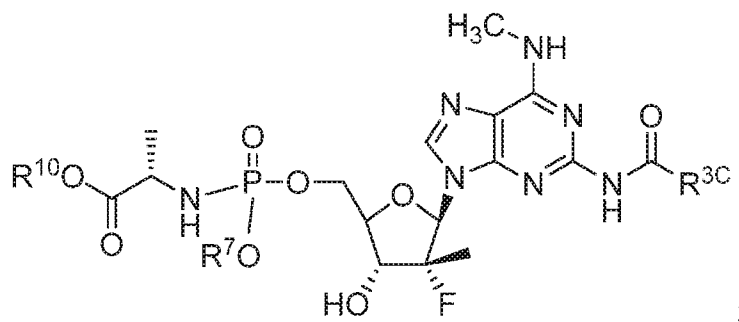
65. A compound of the structure selected from the group consisting of:



wherein R^{3C} , R^7 and R^{10} are defined in claims 1 and 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof, or a pharmaceutically acceptable salt thereof.

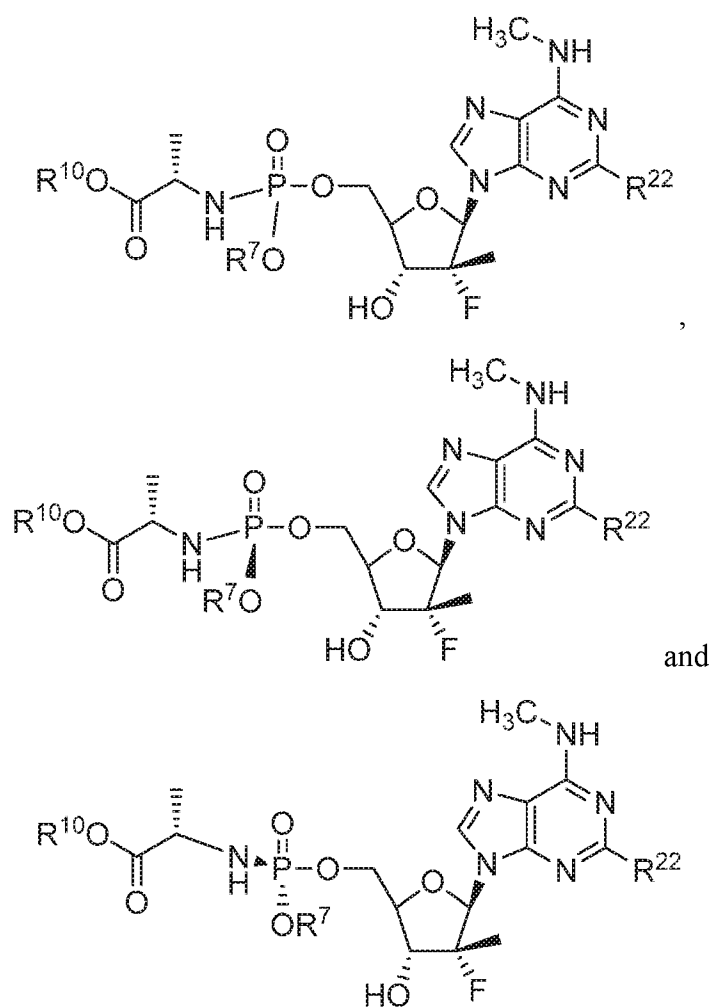
66. A pharmaceutical composition comprising the structure:



wherein R^{3C} , R^7 and R^{10} are defined in claims 1 and 9;

or a pharmaceutically acceptable salt thereof.

67. A compound of the structure selected from the group consisting of:



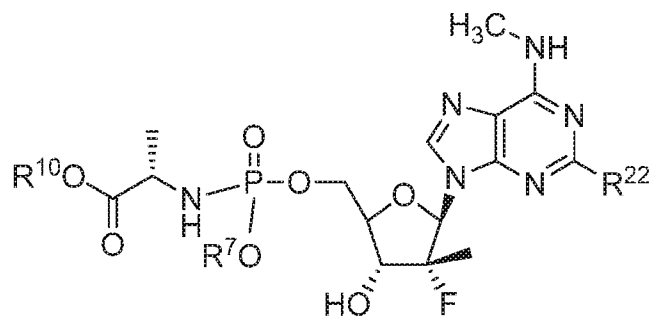
wherein

R²² is selected from F and OR²⁵;

R⁷ and R¹⁰ are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

68. A pharmaceutical composition comprising the compound:



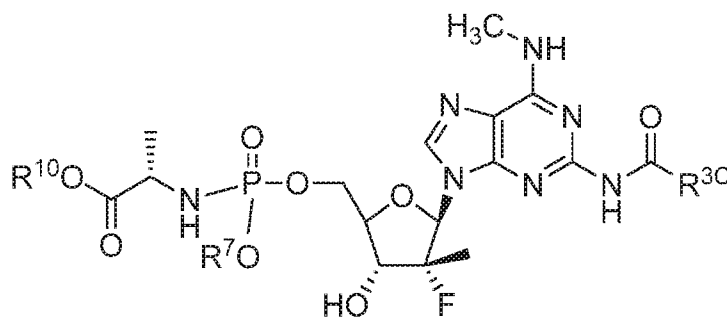
wherein

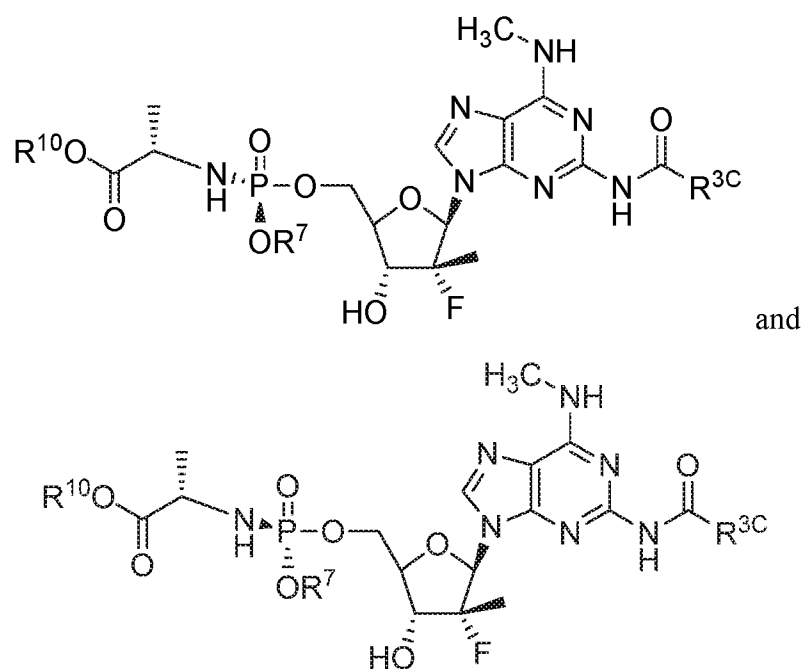
R^{22} is selected from F and OR^{25} ;

R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

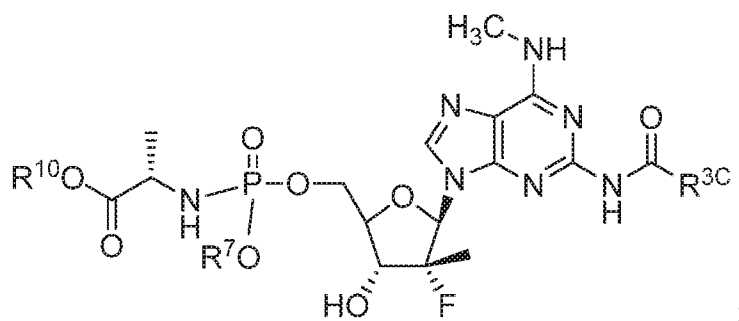
69. A compound of the structure selected from the group consisting of:





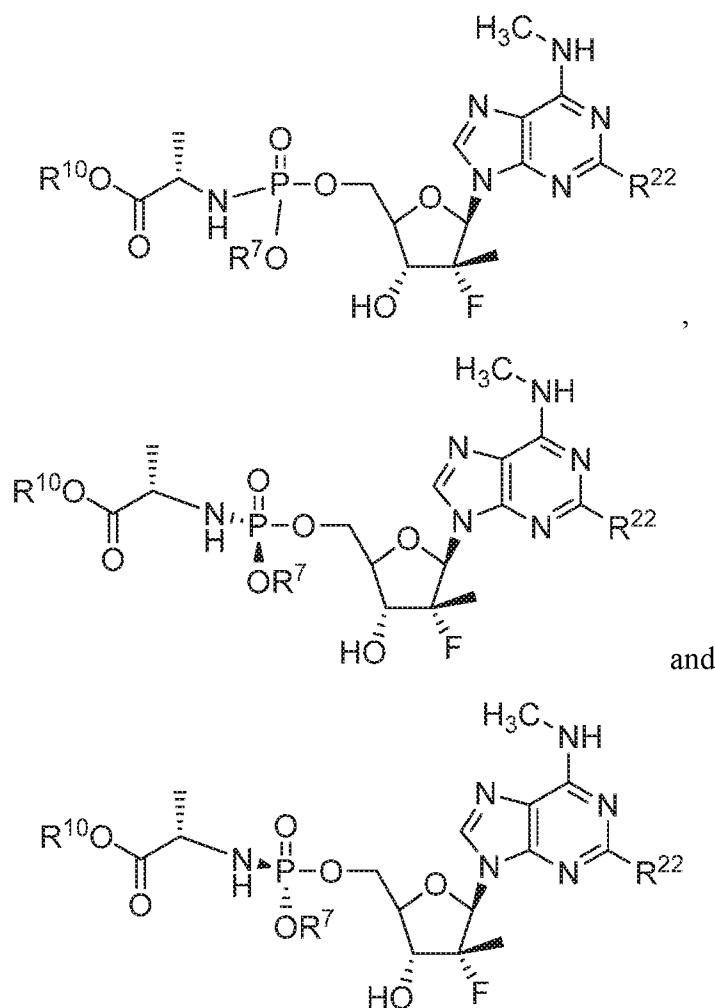
wherein R^7 and R^{10} are defined in claims 1 and 9 and wherein R^{3C} is selected from alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$ and $-O\text{-alkyl}$ in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

70. A pharmaceutical composition comprising the compound:



wherein R^{3C} , R^7 and R^{10} are defined in claims 1 and 9;
or a pharmaceutically acceptable salt thereof.

71. A compound of the structure selected from the group consisting of:



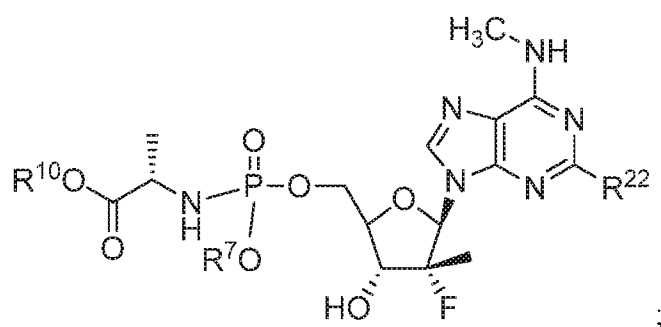
wherein

R²² is selected from F, OR²⁵, N₃ or CN;

R⁷ and R¹⁰ are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

72. A pharmaceutical composition comprising the compound:



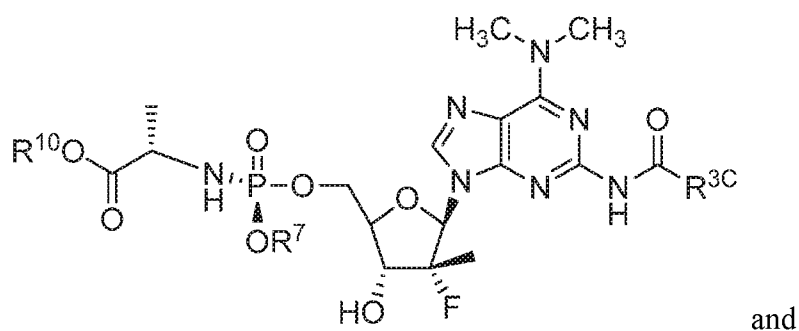
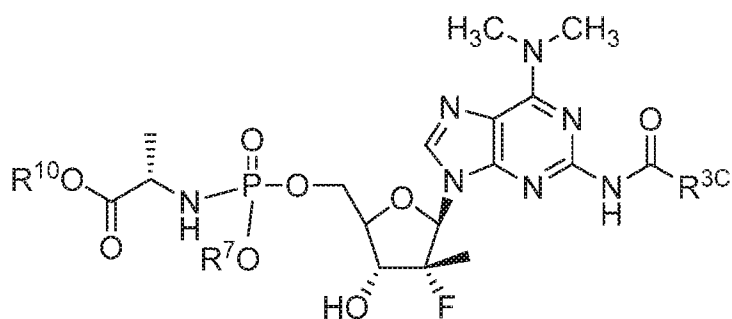
wherein

R^{22} is selected from F, OR^{25} , N_3 or CN;

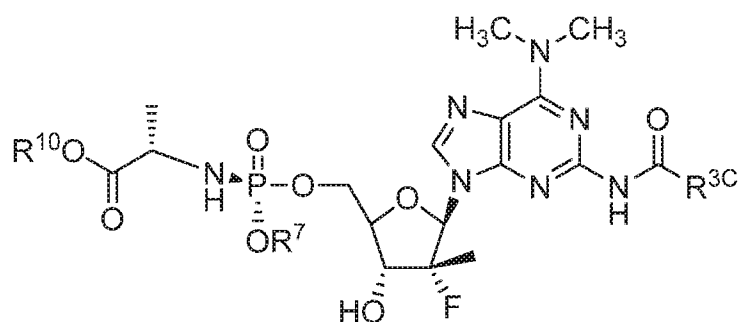
R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

73. A compound of the structure selected from the group consisting of:



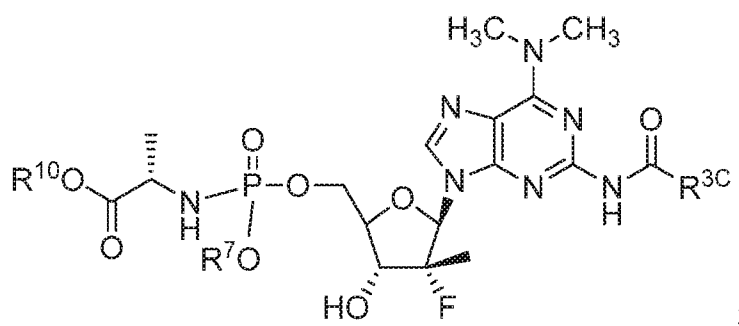
and



wherein R^{3C} , R^7 and R^{10} are defined in claims 1 and 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

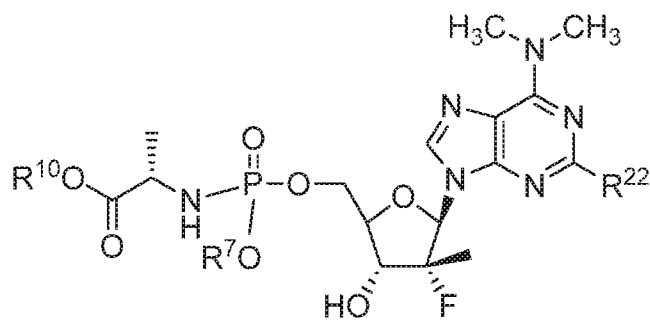
74. A pharmaceutical composition comprising the compound:

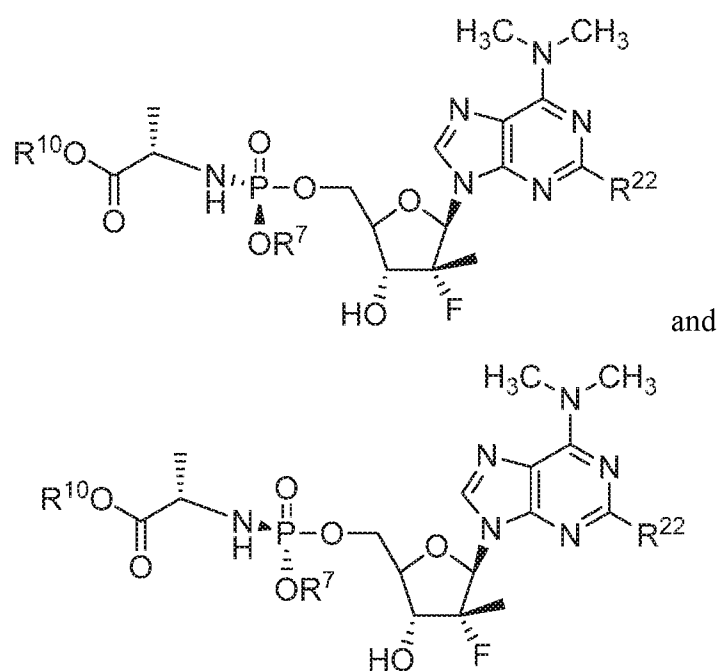


wherein R^{3C} , R^7 and R^{10} are defined in claims 1 and 9;

or a pharmaceutically acceptable salt thereof.

75. A compound of the structure selected from the group consisting of:





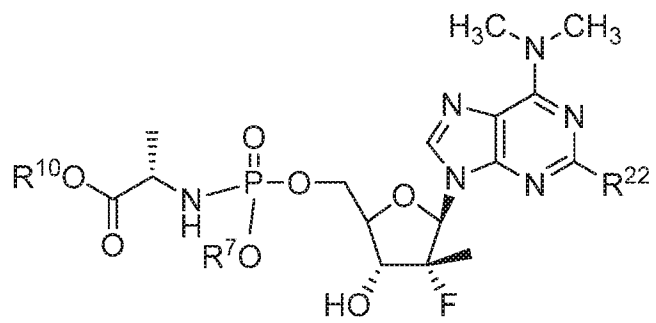
wherein

R^{22} is selected from F, OR^{25} , N_3 or CN;

R^7 and R^{10} are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

76. A pharmaceutical composition comprising the structure:



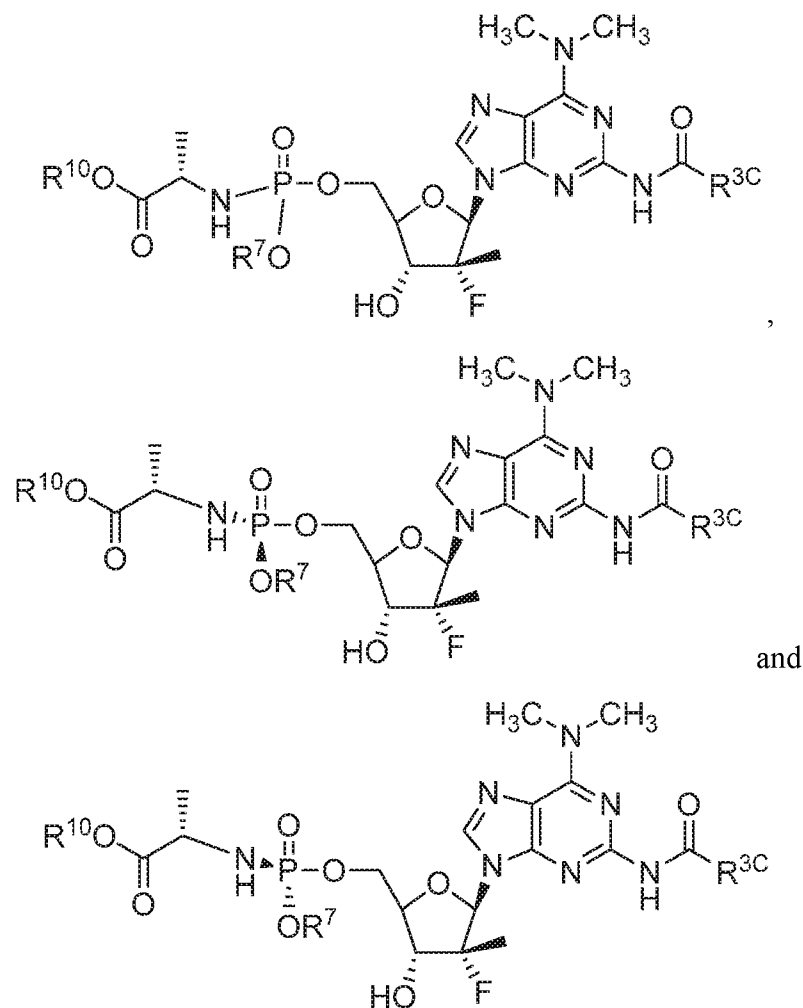
wherein

R^{22} is selected from F, OR^{25} , N_3 or CN;

R^7 and R^{10} are defined in claim 9;

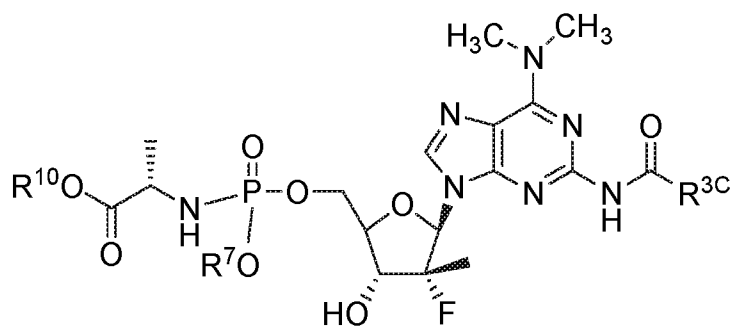
or a pharmaceutically acceptable salt thereof.

77. A compound of the structure selected from the group consisting of:



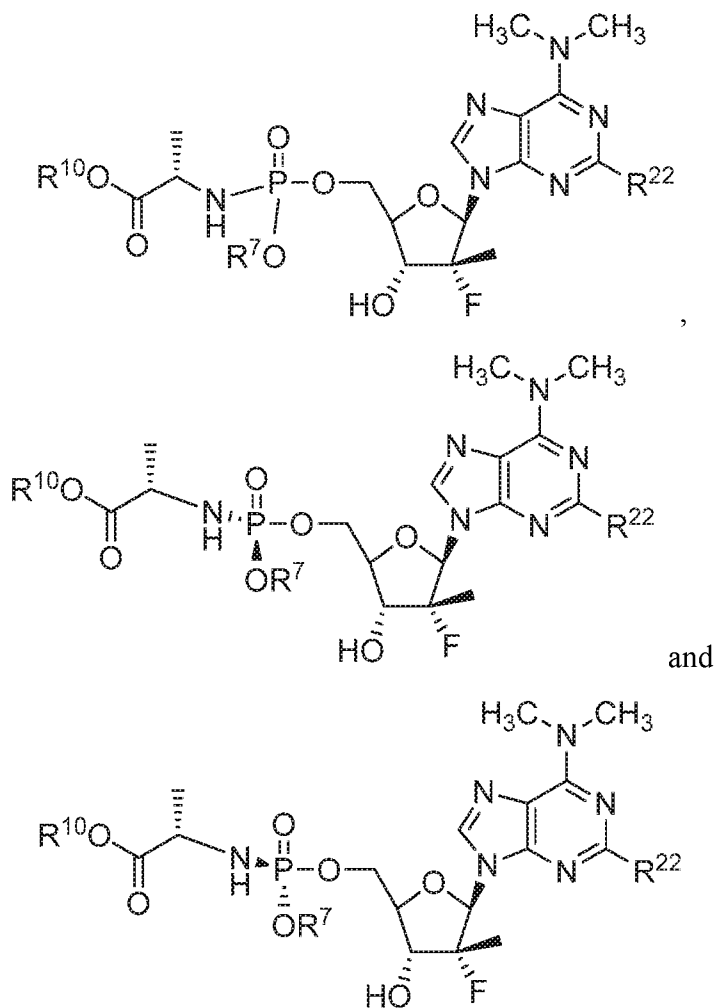
wherein R⁷ and R¹⁰ are defined in claims 1 and 9 and R^{3C} is alkyl, alkenyl, alkynyl, -(C₀-C₂)(cycloalkyl), -(C₀-C₂)(heterocyclo), -(C₀-C₂)(aryl), -(C₀-C₂)(heteroaryl), -O-alkyl, alkyl, alkenyl, alkynyl, -(C₀-C₂)(cycloalkyl), -(C₀-C₂)(heterocyclo), -(C₀-C₂)(aryl), -(C₀-C₂)(heteroaryl), and -O-alkyl, in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

78. A pharmaceutical composition comprising the structure:



wherein R^7 and R^{10} are defined in claims 1 and 9; and R^{3C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$ and $-O\text{-alkyl}$ or a pharmaceutically acceptable salt thereof.

79. A compound of the structure selected from the group consisting of:



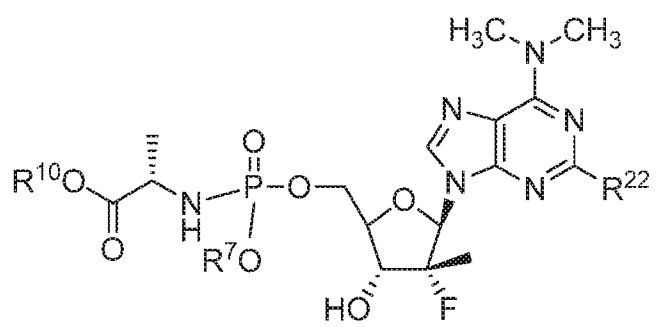
wherein

R^{22} is selected from F and OR^{25} ;

R^7 and R^{10} are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

80. A pharmaceutical composition comprising the compound:



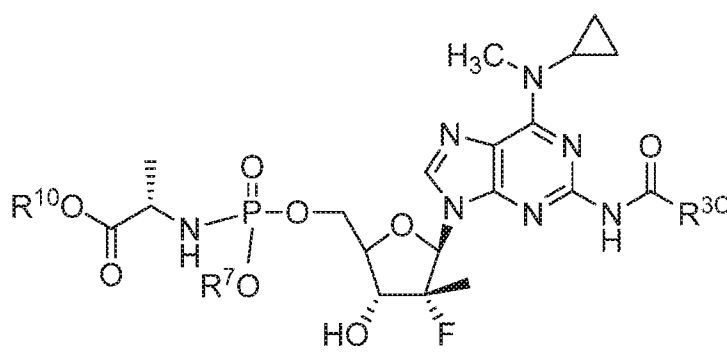
wherein

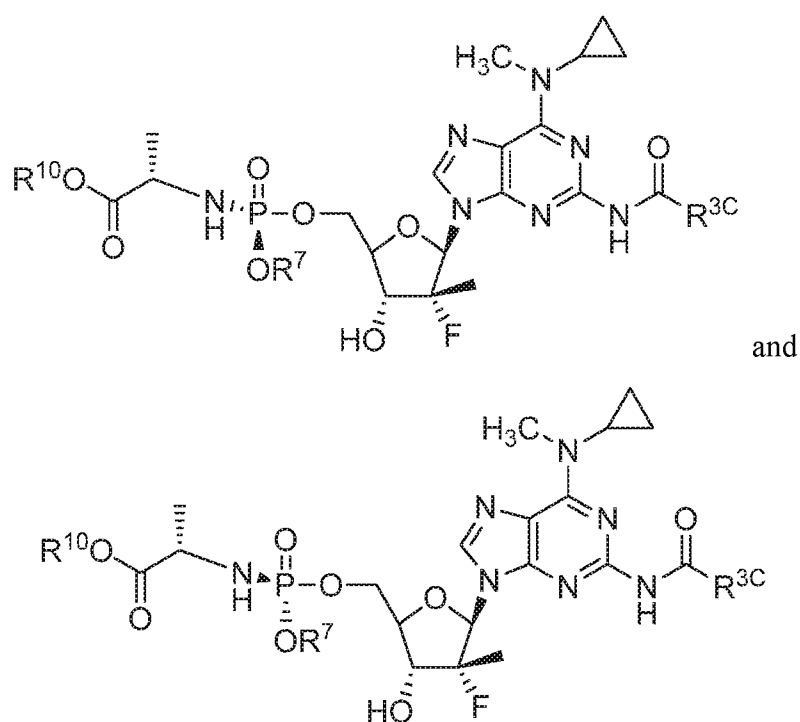
R^{22} is selected from F and OR^{25} ;

R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

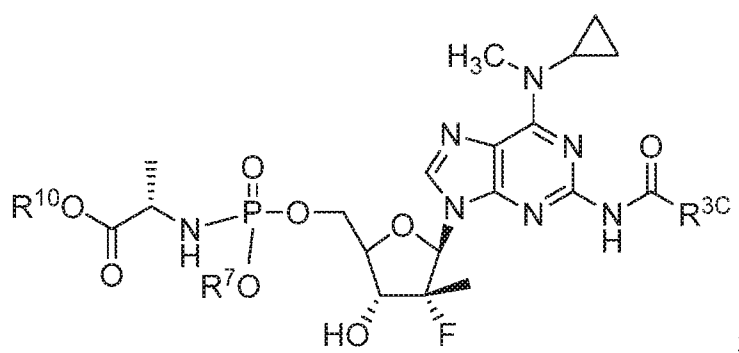
81. A compound of the structure selected from the group consisting of:





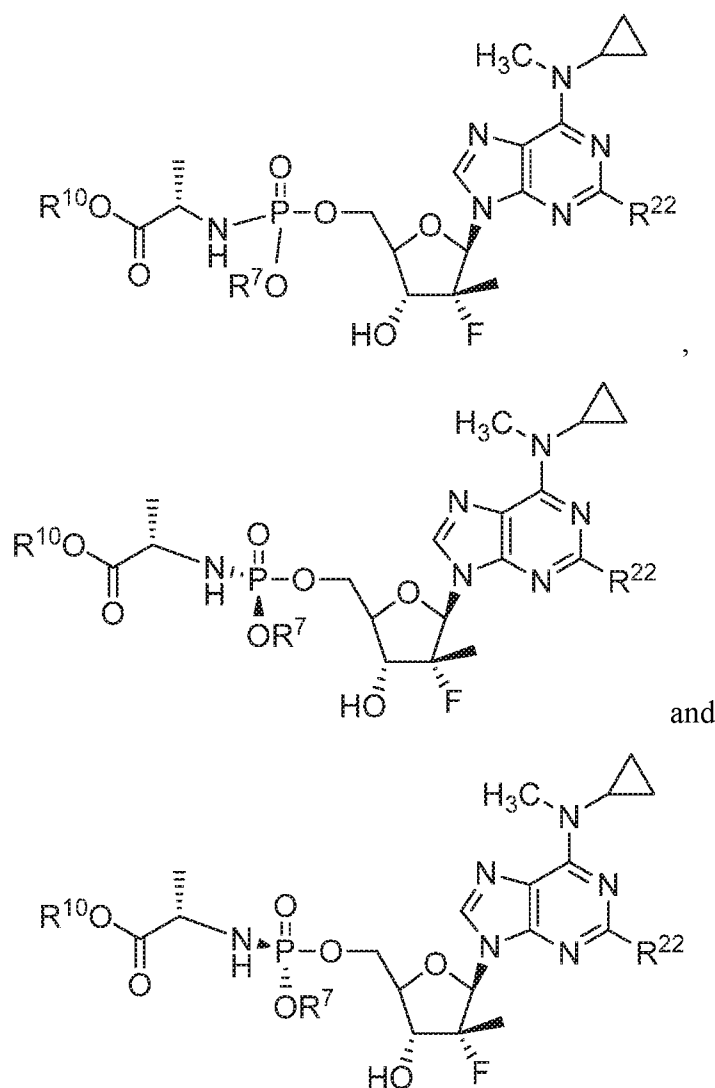
wherein R^7 and R^{10} are defined in claims 1 and 9; and R^{3C} is alkyl, alkenyl, alkynyl, -(C₀-C₂)(cycloalkyl), -(C₀-C₂)(heterocyclo), -(C₀-C₂)(aryl), -(C₀-C₂)(heteroaryl) and -O-alkyl or a pharmaceutically acceptable salt thereof.

82. A pharmaceutical composition comprising the compound:



wherein R^7 and R^{10} are defined in claims 1 and 9 and R^{3C} is alkyl, alkenyl, alkynyl, -(C₀-C₂)(cycloalkyl), -(C₀-C₂)(heterocyclo), -(C₀-C₂)(aryl), -(C₀-C₂)(heteroaryl) and -O-alkyl or a pharmaceutically acceptable salt thereof.

83. A compound of the structure selected from the group consisting of:



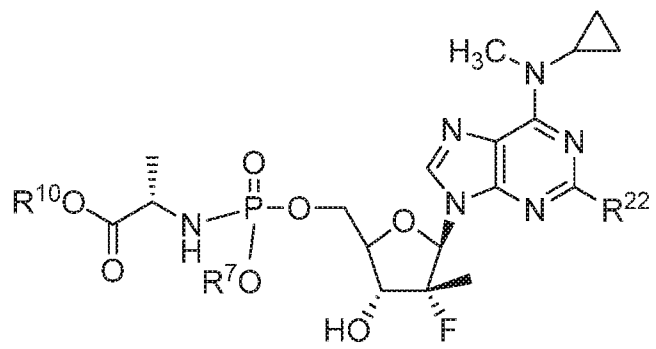
wherein

R^{22} is selected from F and OR²⁵;

R^7 and R^{10} are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof, or a pharmaceutically acceptable salt thereof.

84. A pharmaceutical composition comprising the compound:



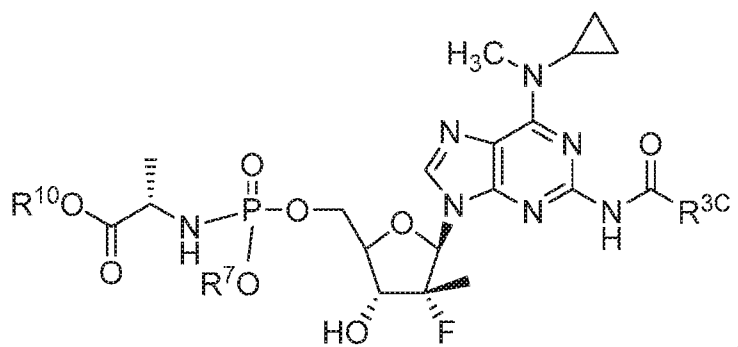
wherein

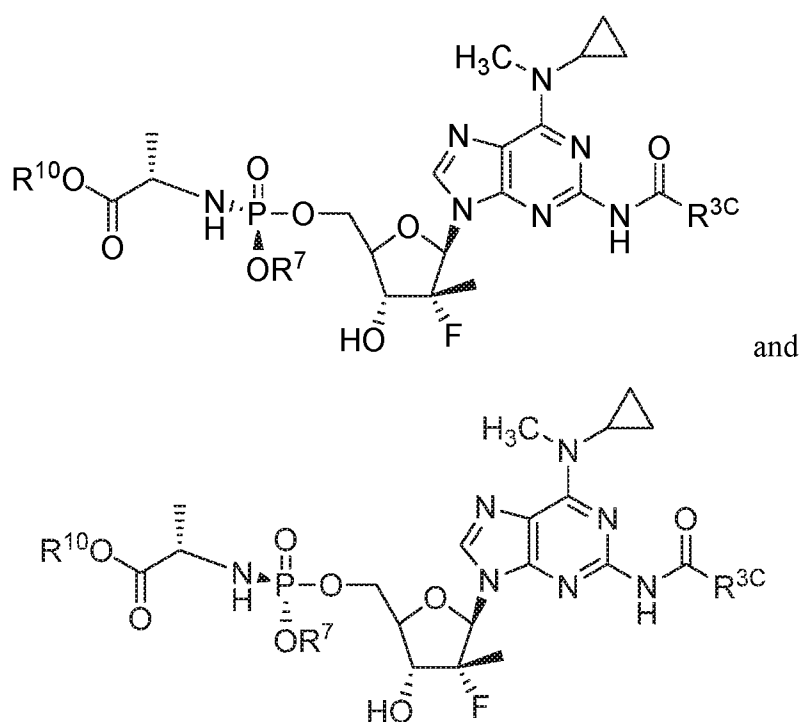
R^{22} is selected from F and OR^{25} ;

R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

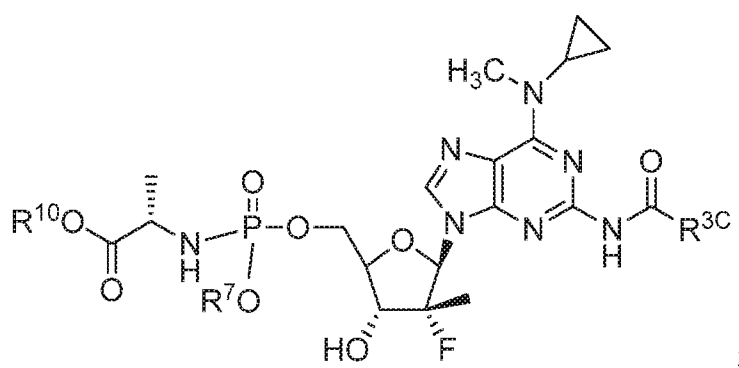
85. A compound of the structure selected from the group consisting of:





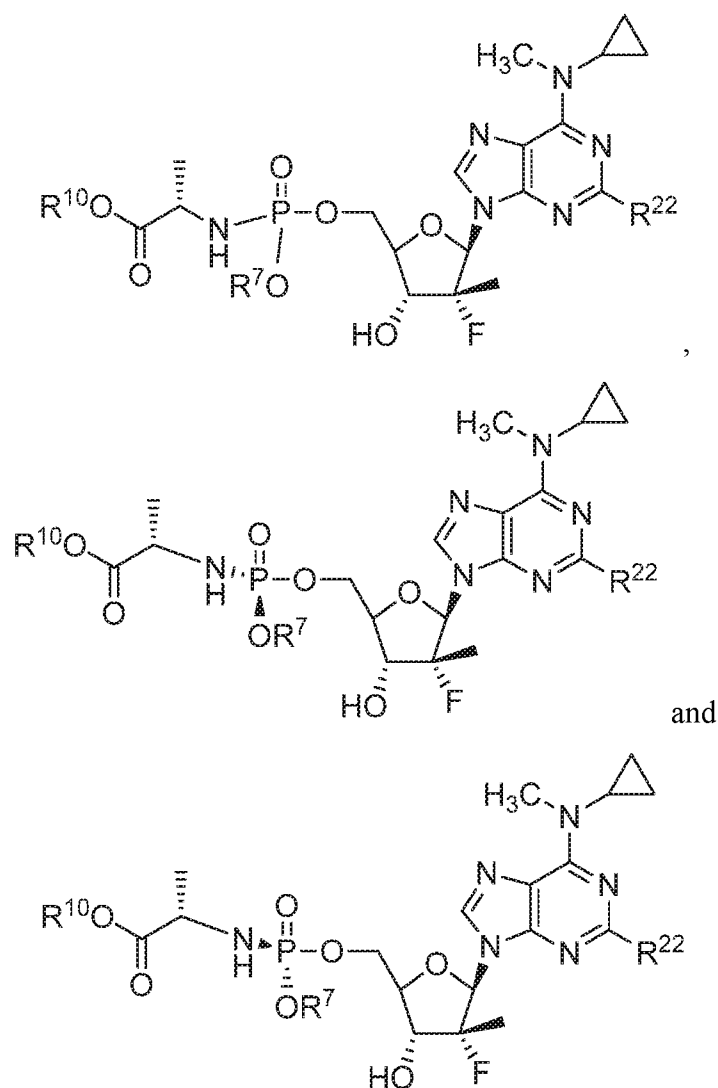
wherein R^{3C}, R⁷ and R¹⁰ are defined in claims 1 and 9;
in the form of an isolated phosphorus S enantiomer.

86. A pharmaceutical composition comprising the compound:



wherein R^{3C}, R⁷ and R¹⁰ are defined in claims 1 and 9;
or a pharmaceutically acceptable salt thereof.

87. A compound of the structure selected from the group consisting of:



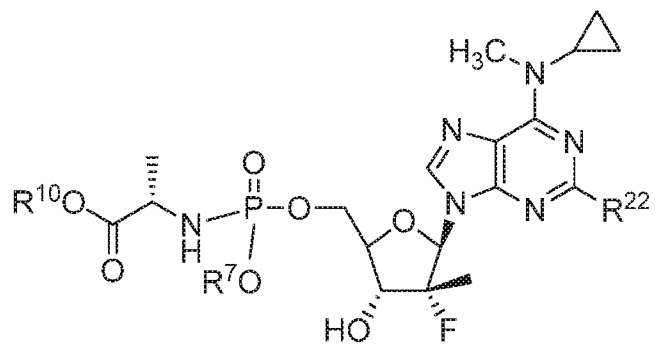
wherein

R^{22} is selected from F, OR^{25} , N_3 or CN;

R^7 and R^{10} are defined in claim 9;

in the form of an isolated phosphorus S enantiomer or a pharmaceutically acceptable salt thereof.

88. A pharmaceutical composition comprising the structure:



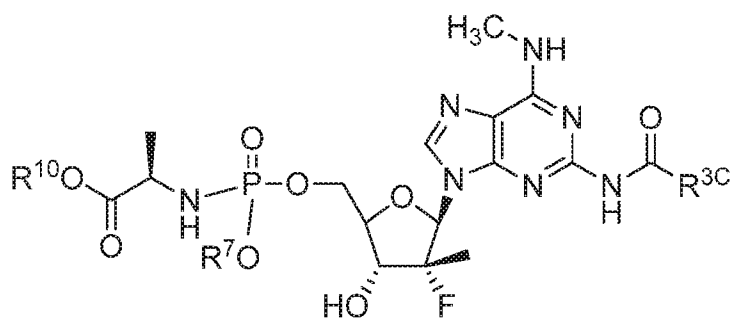
wherein

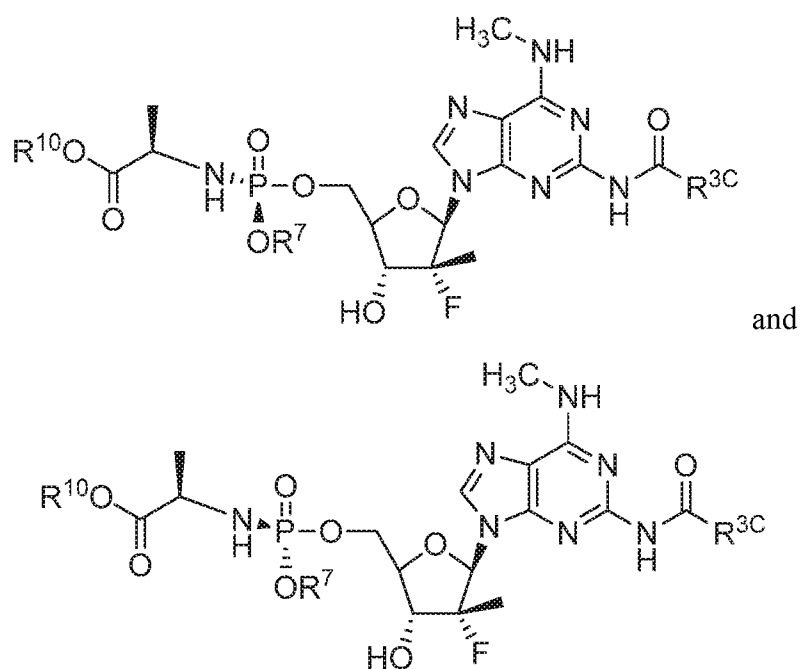
R^{22} is selected from F, OR^{25} , N_3 or CN;

R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

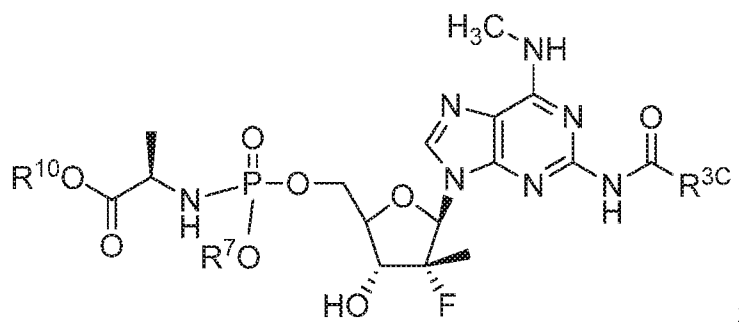
89. A compound of the structure selected from the group consisting of:





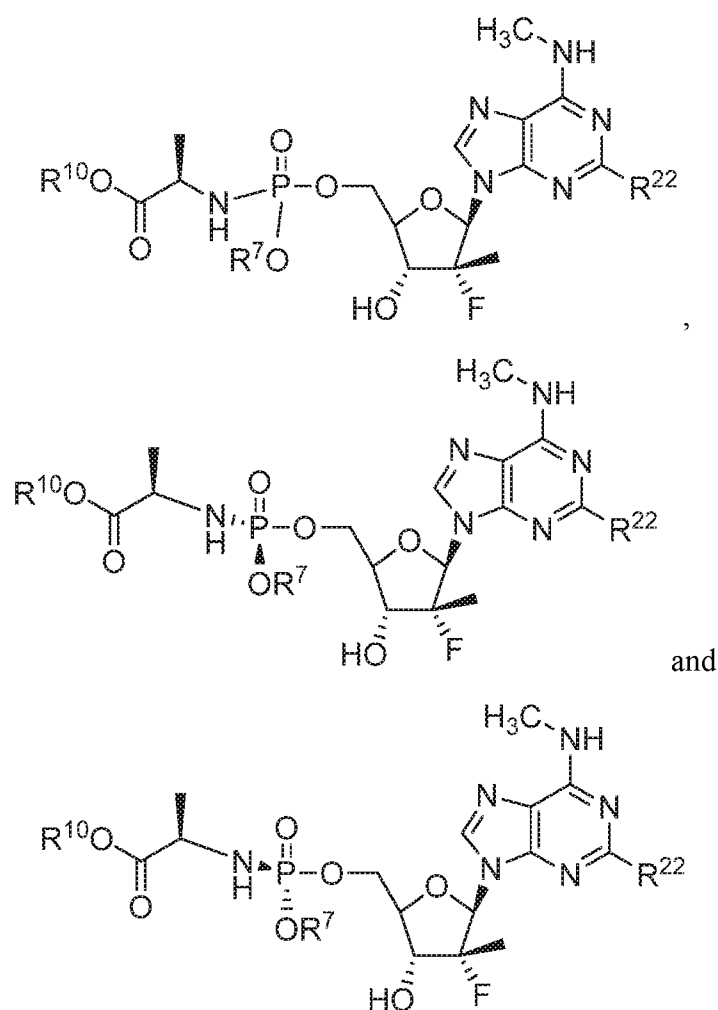
wherein R^7 and R^{10} are defined in claims 1 and 9 and R^{3C} is selected from alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$ and $-O$ -alkyl in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

90. A pharmaceutical composition comprising the structure:



wherein R^7 and R^{10} are defined in claims 1 and 9 and R^{3C} is
or a pharmaceutically acceptable salt thereof.

91. A compound of the structure selected from the group consisting of:



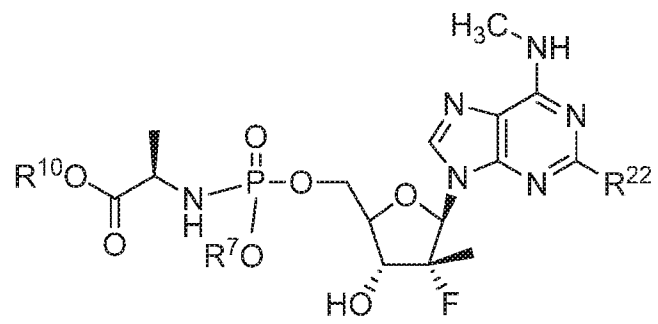
wherein

R²² is selected from F and OR²⁵;

R⁷ and R¹⁰ are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

92. A pharmaceutical composition comprising the structure:



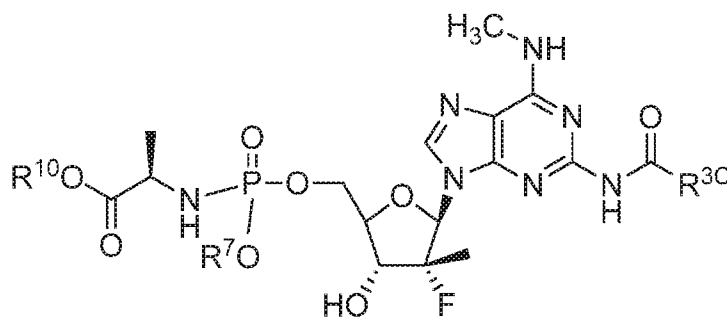
wherein

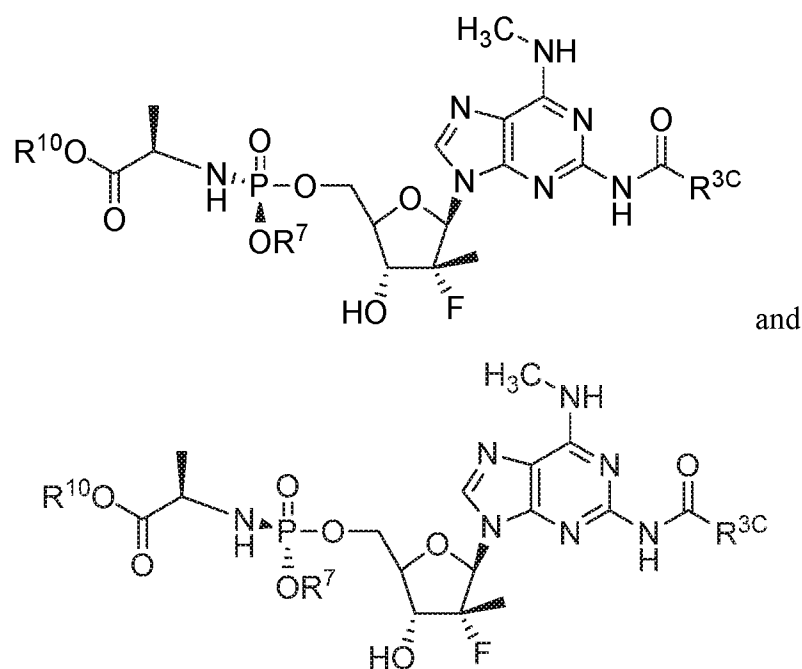
R^{22} is selected from F, OR^{25} , N_3 or CN;

R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

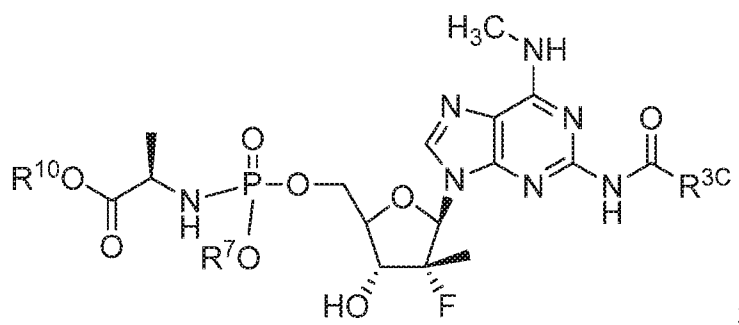
93. A compound of the structure selected from the group consisting of:





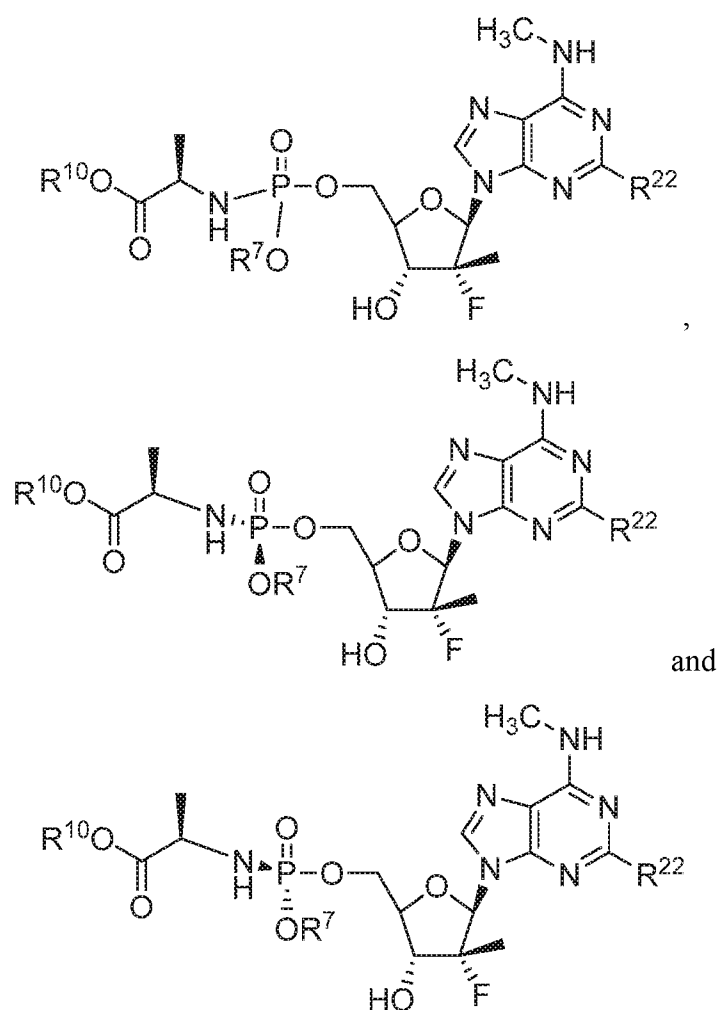
wherein R⁷ and R¹⁰ are defined in claims 1 and 9 and R^{3C} in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

94. A pharmaceutical composition comprising the compound:



wherein R⁷ and R¹⁰ are defined in claims 1 and 9 and R^{3C} is alkyl, alkenyl, alkynyl, -(C₀-C₂)(cycloalkyl), -(C₀-C₂)(heterocyclo), -(C₀-C₂)(aryl), -(C₀-C₂)(heteroaryl) and -O-alkyl or a pharmaceutically acceptable salt thereof.

95. A compound of the structure selected from the group consisting of:



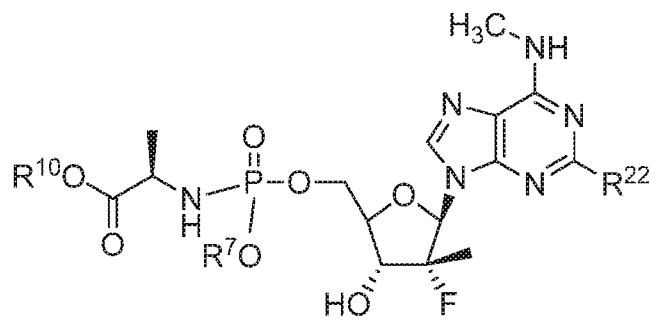
wherein

R²² is selected from F, OR²⁵, N₃ or CN;

R⁷ and R¹⁰ are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

96. A pharmaceutical composition comprising the compound:



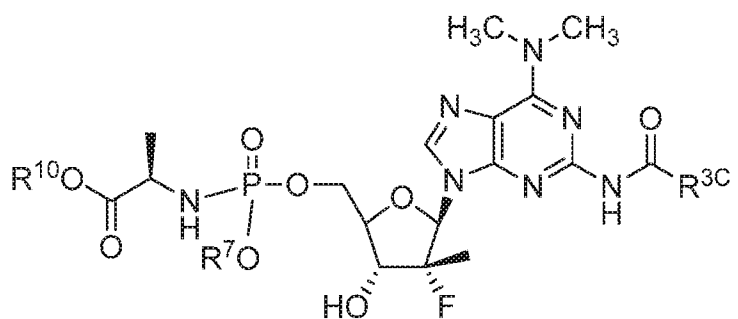
wherein

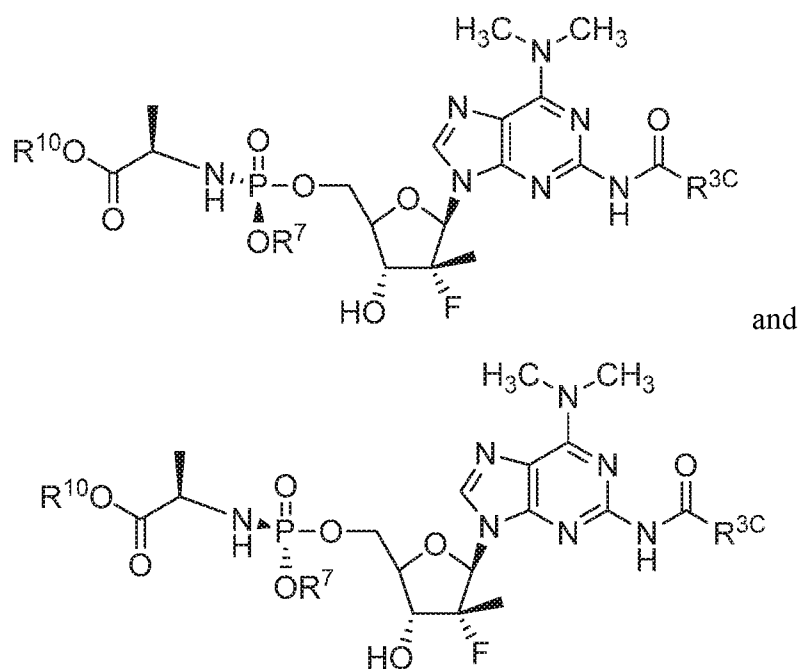
R^{22} is selected from F and OR^{25} ;

R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

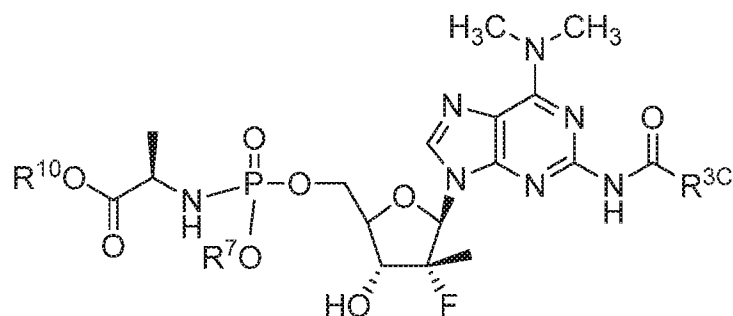
97. A compound of the structure selected from the group consisting of:





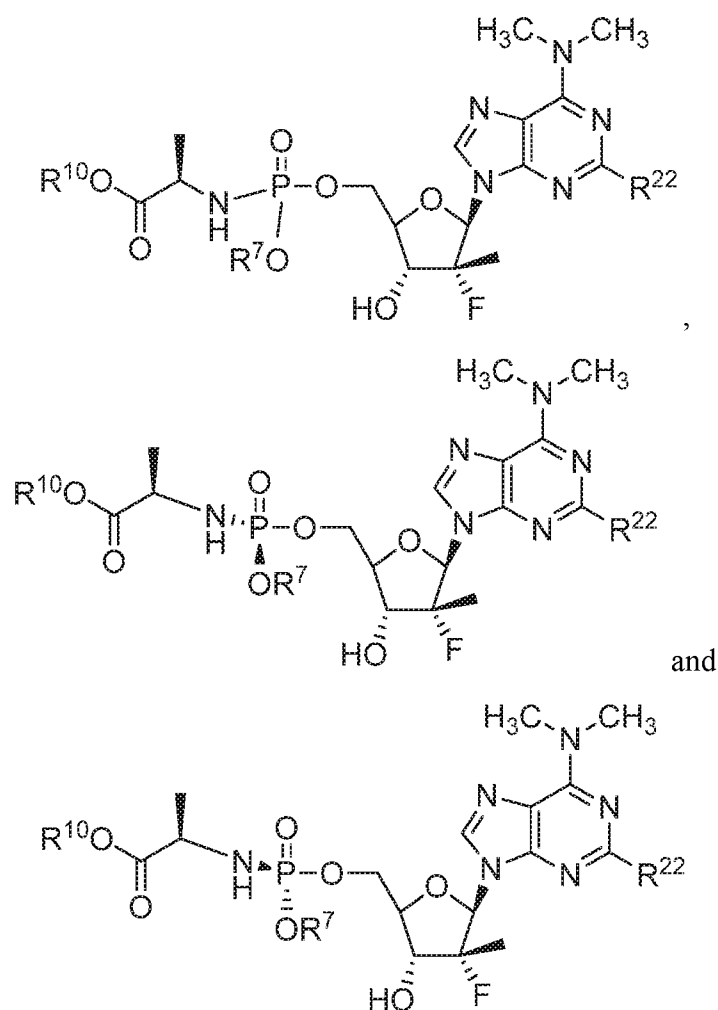
wherein R^7 and R^{10} are defined in claims 1 and 9 and R^{3C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$ and $-O\text{-alkyl}$ in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

98. A pharmaceutical composition comprising the structure:



wherein R^7 and R^{10} are defined in claims 1 and 9; R^{3C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$ and $-O\text{-alkyl}$ or a pharmaceutically acceptable salt thereof.

99. A compound of the structure selected from the group consisting of:



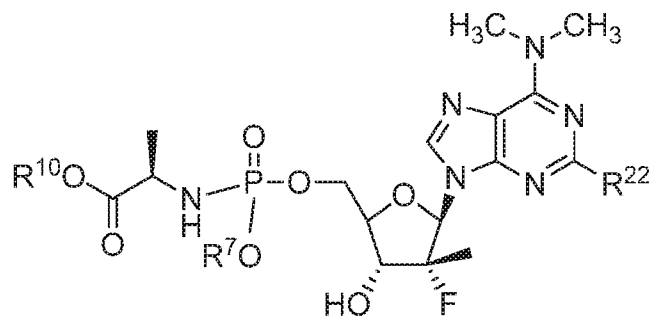
wherein

R²² is selected from F and OR²⁵;

R⁷ and R¹⁰ are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

100. A pharmaceutical composition comprising the structure:



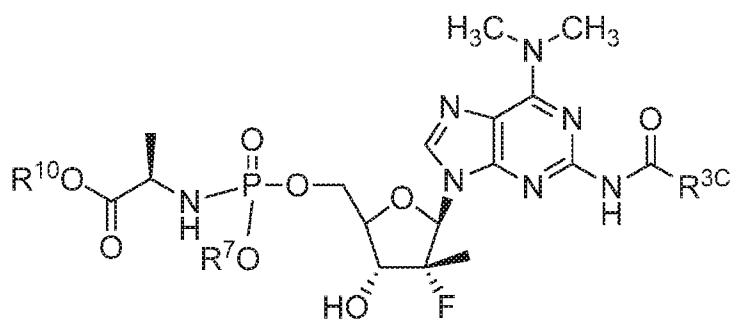
wherein

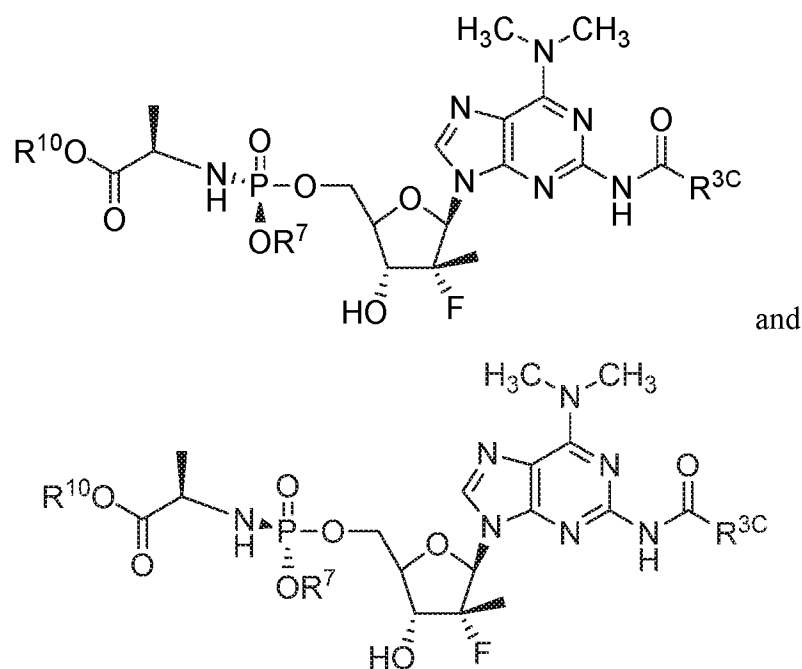
R^{22} is selected from F and OR^{25} ;

R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

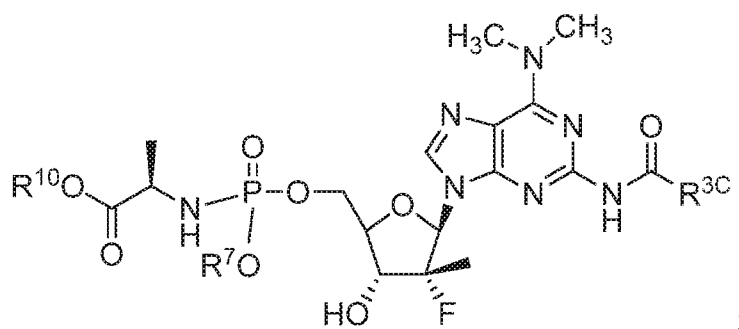
101. A compound of the structure selected from the group consisting of:





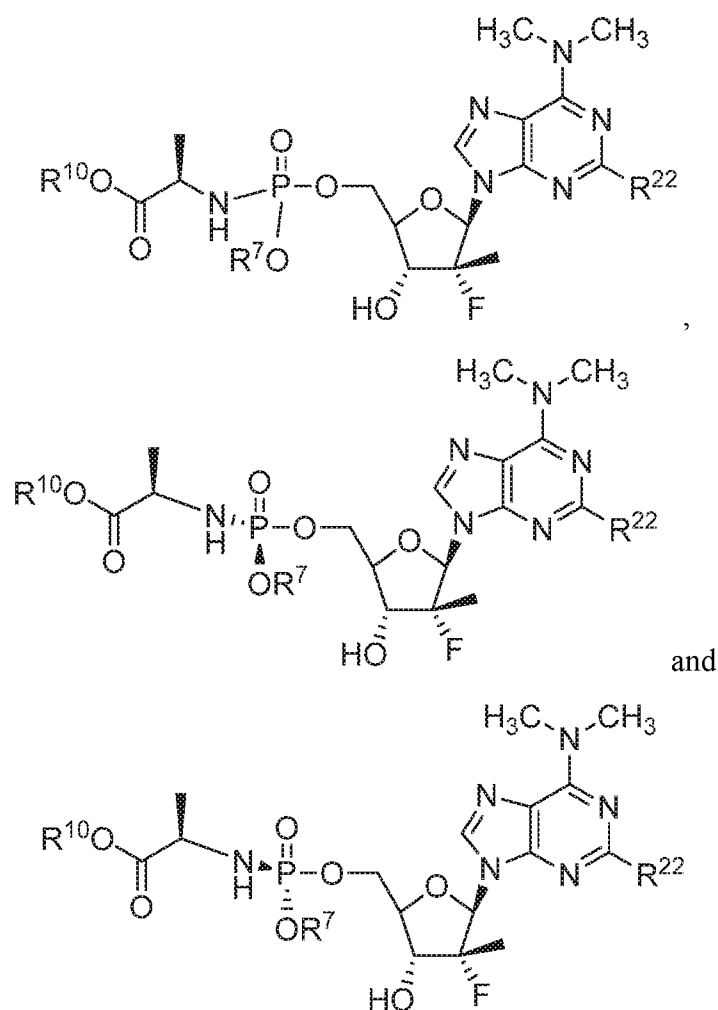
wherein R^7 and R^{10} are defined in claims 1 and 9; and R^{3C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$ and $-O\text{-alkyl}$ in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

102. A pharmaceutical composition comprising the structure:



wherein R^{3C} , R^7 and R^{10} are defined in claims 1 and 9;
or a pharmaceutically acceptable salt thereof.

103. A compound of the structure selected from the group consisting of:



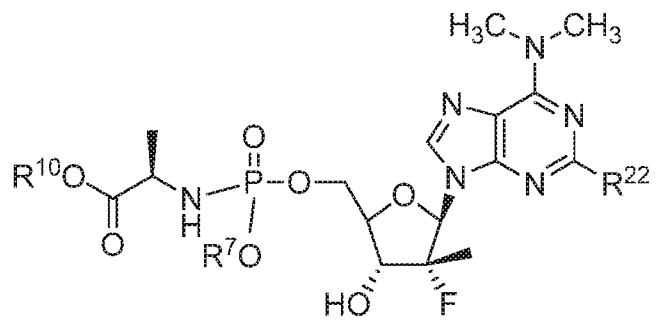
wherein

R²² is selected from F, OR²⁵, N₃ or CN;

R⁷ and R¹⁰ are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

104. A pharmaceutical composition comprising the structure:



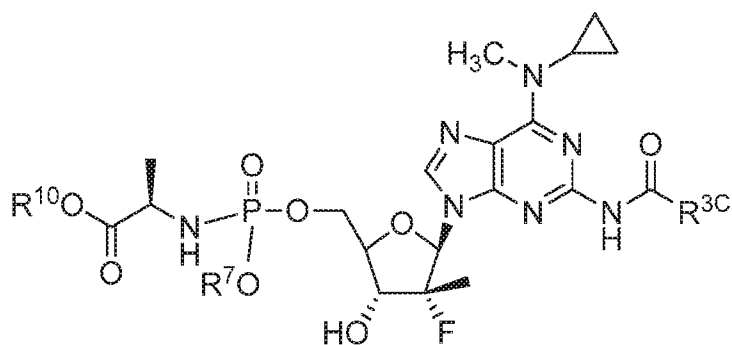
wherein

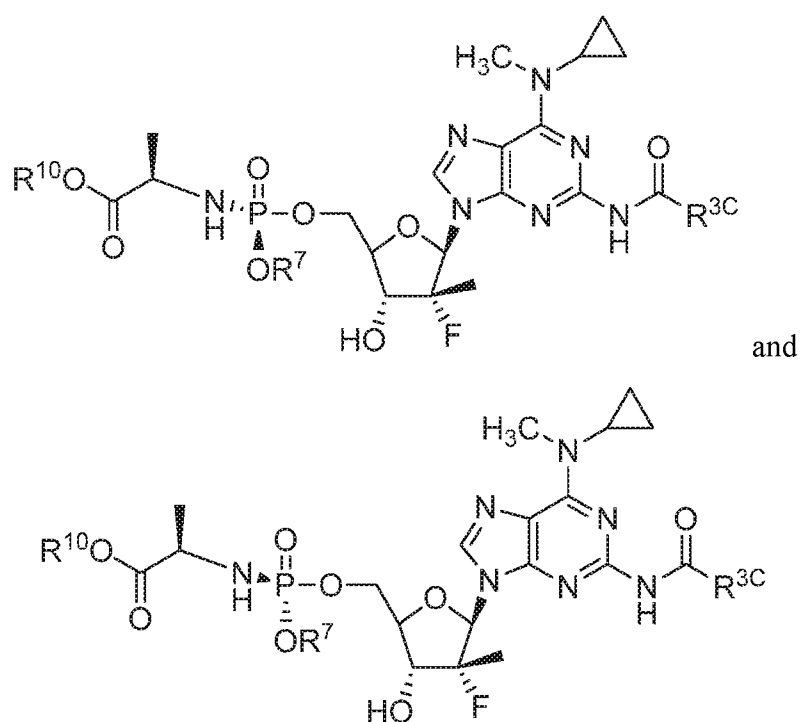
R^{22} is selected from F, OR^{25} , N_3 or CN;

R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

105. A compound of the structure selected from the group consisting of:

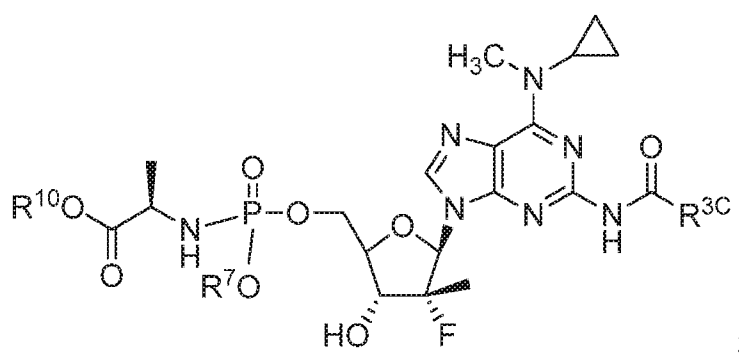




wherein R^{3C} , R^7 and R^{10} are defined in claims 1 and 9;

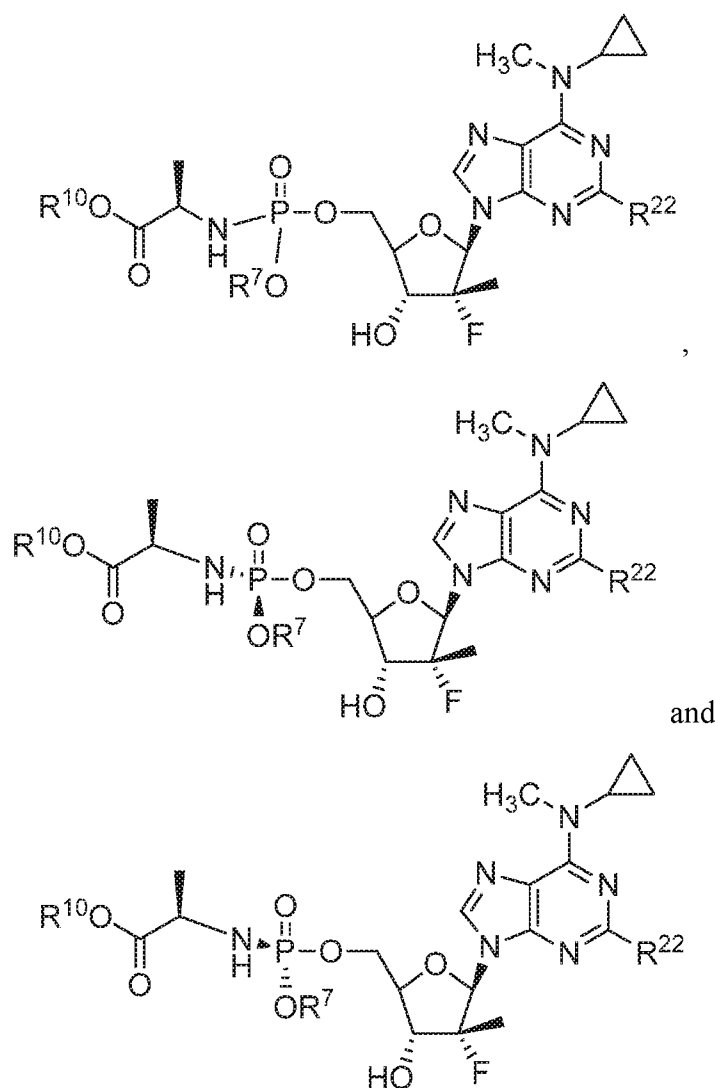
in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

106. A pharmaceutical composition comprising the structure:



wherein R^7 and R^{10} are defined in claims 1 and 9; R^{3C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2)(\text{cycloalkyl})$, $-(C_0-C_2)(\text{heterocyclo})$, $-(C_0-C_2)(\text{aryl})$, $-(C_0-C_2)(\text{heteroaryl})$ and $-O\text{-alkyl}$ or a pharmaceutically acceptable salt thereof.

107. A compound of the structure selected from the group consisting of:



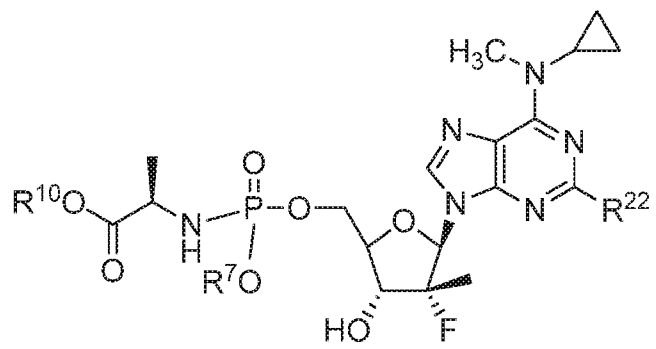
wherein

R^{22} is selected from F and OR^{25} ;

R^7 and R^{10} are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof, or a pharmaceutically acceptable salt thereof.

108. A pharmaceutical composition comprising the structure:



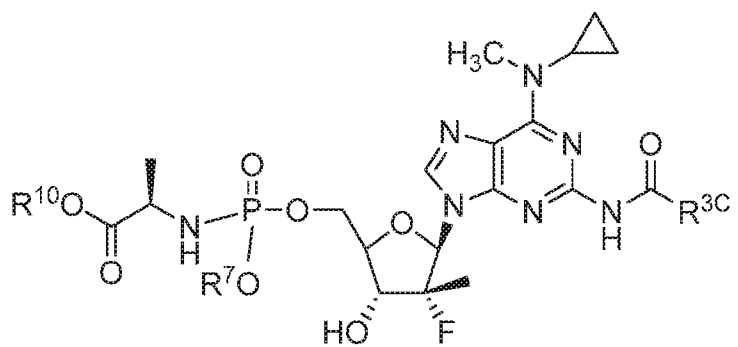
wherein

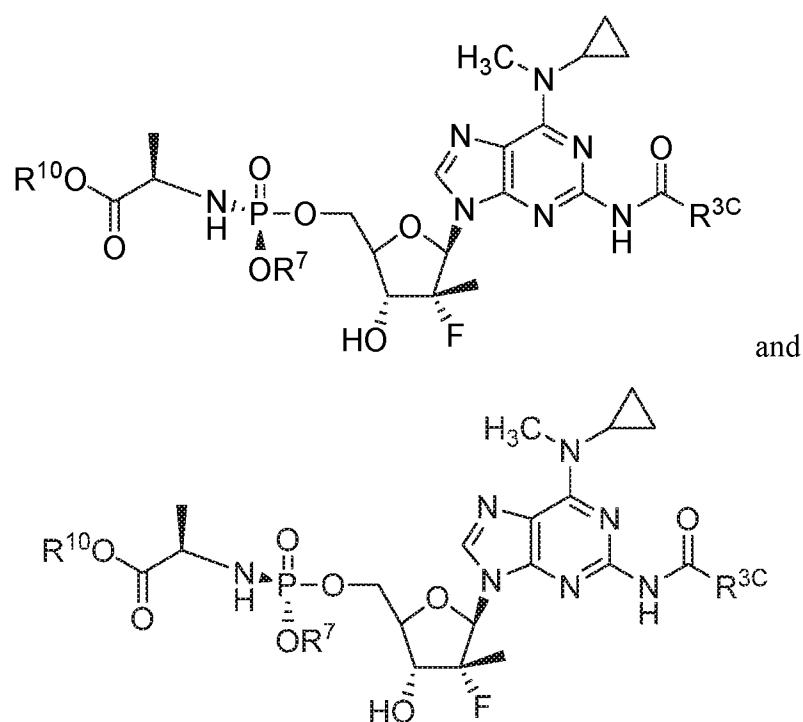
R^{22} is selected from F and OR^{25} ;

R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

109. A compound of the structure selected from the group consisting of:

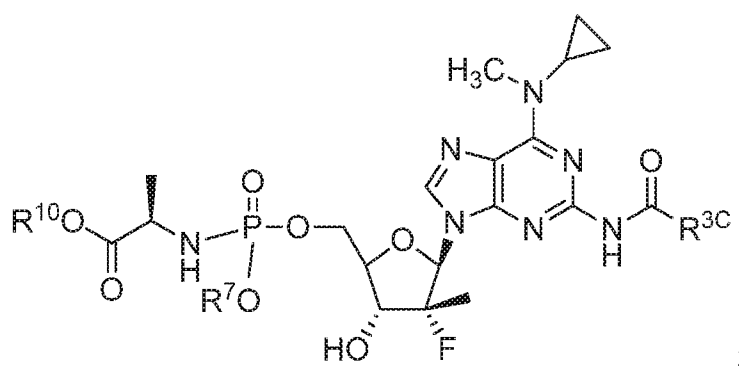




wherein R^{3C} , R^7 and R^{10} are defined in claims 1 and 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof; or a pharmaceutically acceptable salt thereof.

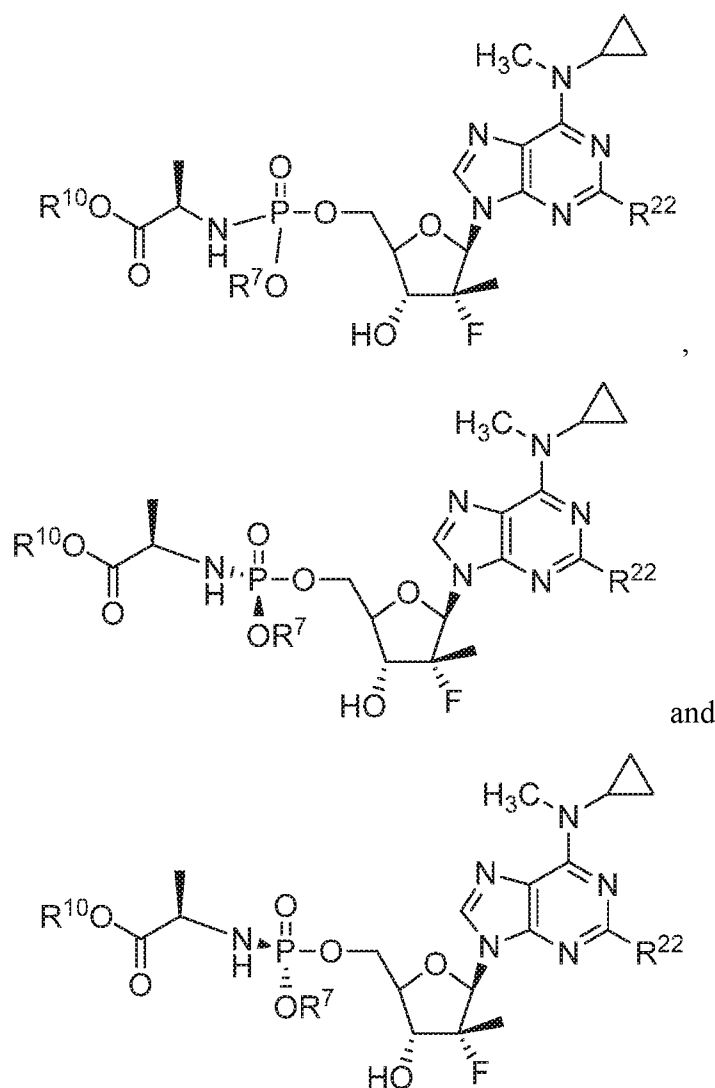
110. A pharmaceutical composition comprising the structure:



wherein R^{3C} , R^7 and R^{10} are defined in claims 1 and 9;

or a pharmaceutically acceptable salt thereof.

111. A compound of the structure selected from the group consisting of:



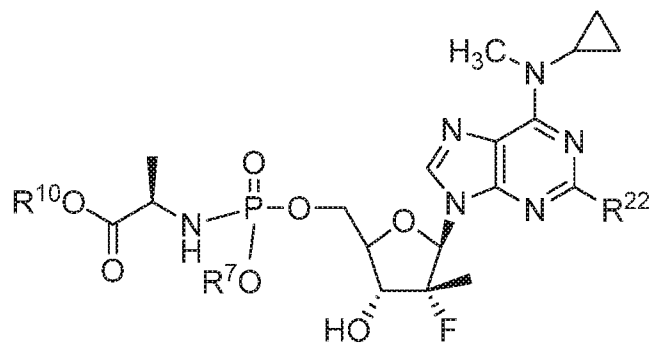
wherein

R^{22} is selected from F, OR^{25} , N_3 or CN;

R^7 and R^{10} are defined in claim 9;

in the form of an isolated phosphorus R or S enantiomer with at least 90% of the designated enantiomer, or a mixture thereof, or a pharmaceutically acceptable salt thereof.

112. A pharmaceutical composition comprising the structure:



wherein

R^{22} is selected from F, OR^{25} , N_3 or CN;

R^7 and R^{10} are defined in claim 9;

or a pharmaceutically acceptable salt thereof.

113. A compound of any of claims 1, 9, 14, 24, or 29-32 wherein R^3 is H.

114. A compound of any of claims 1, 9, 14, 24, or 29-32 wherein R^4 is H.

115. A compound of any of claims 1, 9, 14, 24, or 29-32 wherein R^4 is a mono, di or triphosphate.

116. A compound of any of claims 1, 9, 14, 24, or 29-32 wherein the phosphorus is of the S-configuration.

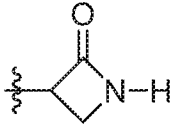
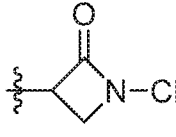
117. A compound of any of claims 1, 9, 14, 24, or 29-32 wherein the phosphorus is of the R-configuration.

118. A compound of any of claims 1, 9, 14, 24, or 29-32 wherein the aminoacid has a D-configuration.

119. A compound of any of claims 1, 9, 14, 24, or 29-32 wherein the aminoacid has a L-configuration.

120. A compound of any of claims 14, 24, 29, 30 or 32 wherein R^{22} is selected from chloro, bromo, fluoro, cyano, azido, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl and n-pentyl, 1,1-dimethylpropyl, 2,2-dimethylpropyl, 3-methylbutyl, 1-methylbutyl, 1-ethylpropyl, vinyl, allyl, 1-butyryl, 2-butyryl, acetylenyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, $-(CH_2)$ -cyclopropyl, $-(CH_2)$ -cyclobutyl, $-(CH_2)$ -cyclopentyl, $-(CH_2)$ -cyclohexyl, aziridine, oxirane, thiirane, azetidine, oxetane, thietane, pyrrolidine, tetrahydrofuran, thiolane,

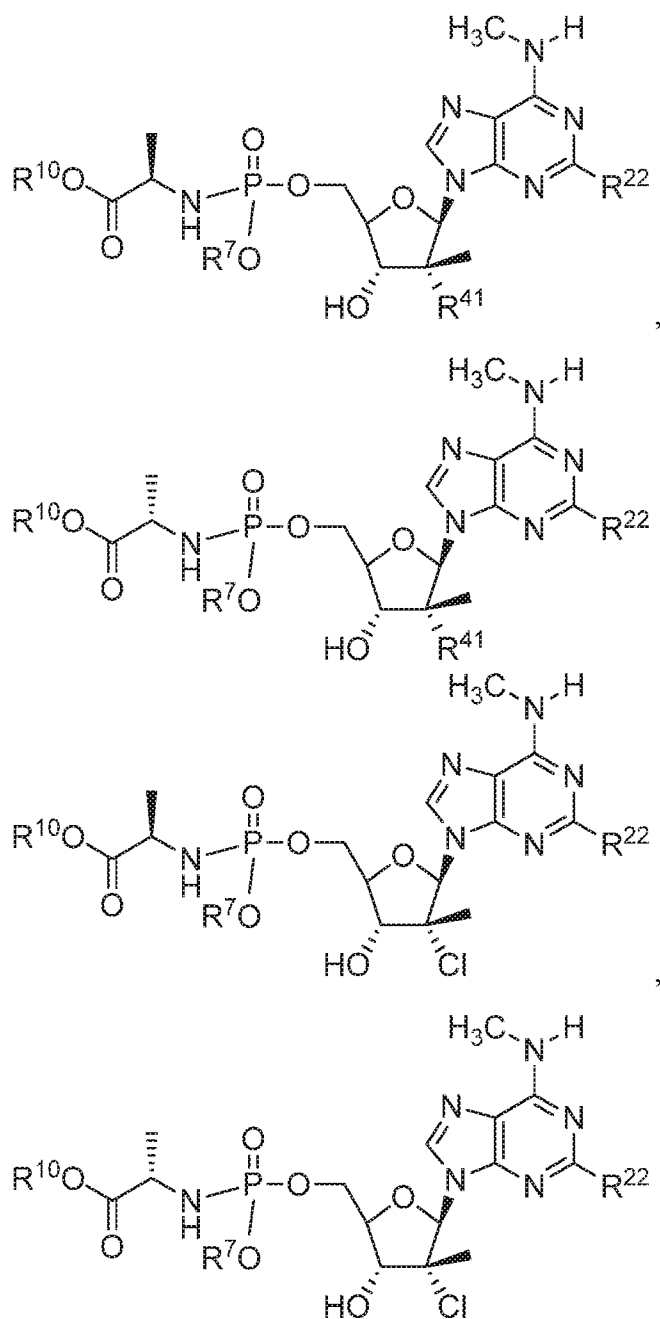
pyrazolidine, piperidine, oxane, thiane, $-(CH_2)$ -aziridine, $-(CH_2)$ -oxirane, $-(CH_2)$ -thiirane, $-(CH_2)$ -azetidine, $-(CH_2)$ -oxetane, $-(CH_2)$ -thietane, $-(CH_2)$ -pyrrolidine, $-(CH_2)$ -tetrahydrofuran, $-(CH_2)$ -thiolane, $-(CH_2)$ -pyrazolidine, $-(CH_2)$ -piperidine, $-(CH_2)$ -oxane, $-(CH_2)$ -thiane, phenyl, pyridyl, $-ONHC(=O)OCH_3$, $-ONHC(=O)OCH_2CH_3$, $-NHOH$, $NHOCH_3$, $-OCH_3$, OC_2H_5 , $-OPh$, OCH_2Ph , $-SCH_3$, $-SC_2H_5$, $-SPh$, SCH_2Ph , $-NH(CH_2)_2NH_2$, $-NH(CH_2)_2N(CH_3)_2$, $-NHNH_2$, $-NHNHCH_3$, $-N=NH$, $-N=NCH_3$, $-N=NCH_2CH_3$, $-NHC(O)NHNH_2$, $-NHC(S)NHNH_2$, $-C(O)NHNH_2$, $-NHSo_2CH_3$, $-NHSo_2CH_2CH_3$, $-So_2NHCH_3$, $-So_2N(CH_3)_2$, $-C(O)NH_2$, $-C(O)NHCH_3$, $-C(O)N(CH_3)_2$, $-CO_2CH_3$, $-CO_2CH_2CH_3$, $-CO_2Ph$, $-CO_2CH_2Ph$, $-So_2CH_3$,

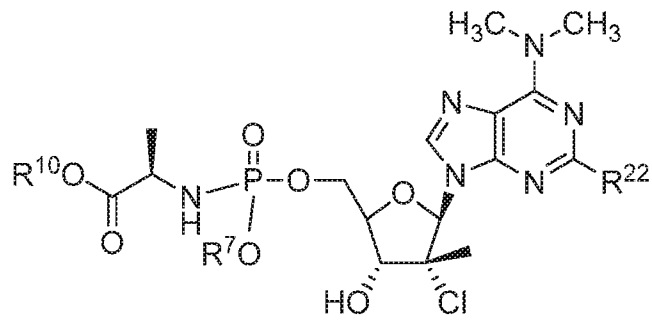
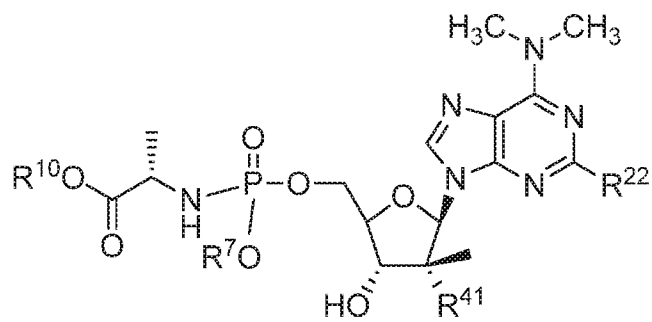
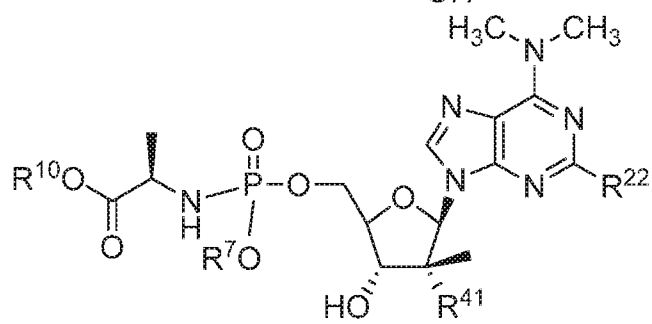
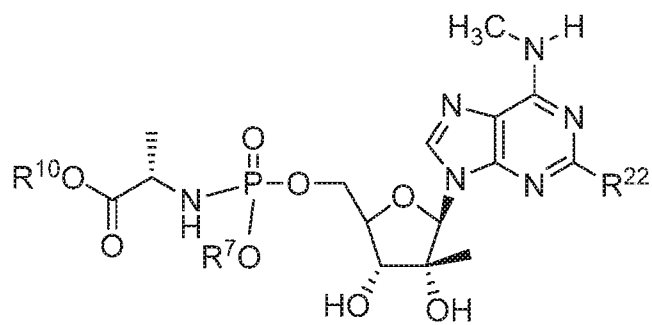
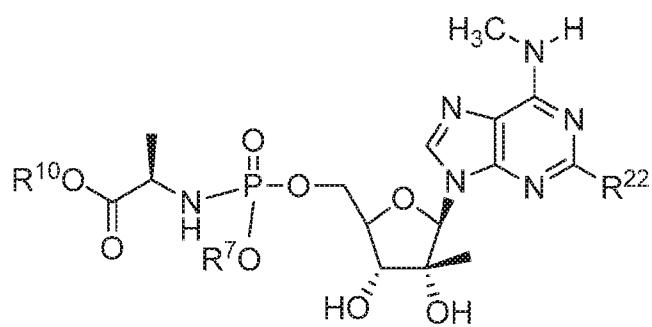
$-So_2CH_2CH_3$, $-So_2Ph$, $-So_2CH_2Ph$, , , $-P(O)H(OH)$, $-P(O)H(OCH_3)$, $-P(O)(OH)(OH)$, $-P(O)(OH)(OCH_3)$, $-P(O)(OCH_3)(OCH_3)$, $-P(O)(OH)(NH_2)$, $-P(O)(OH)(NHCH_3)$, $-P(O)(OH)N(CH_3)_2$, $-NHC(O)CH_3$, $-NHC(O)CH_2CH_3$, $-NHC(O)CH(CH_3)_2$, $-NHC(O)OCH_3$, $-NHC(O)OCH_2CH_3$, $-NHC(O)OCH(CH_3)_2$, $-NHC(O)OCH_2CH_2CH_3$, $-NHC(O)OCH_2CH_2CH_2CH_3$ or $-NHC(O)OCH_2CH_2CH_2CH_2CH_3$.

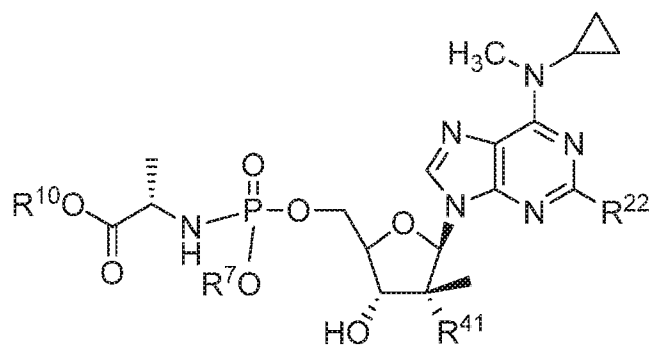
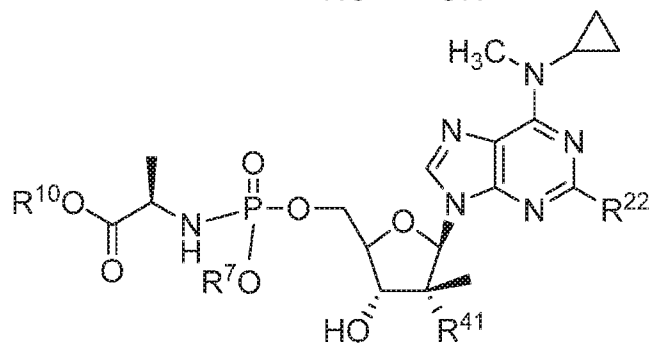
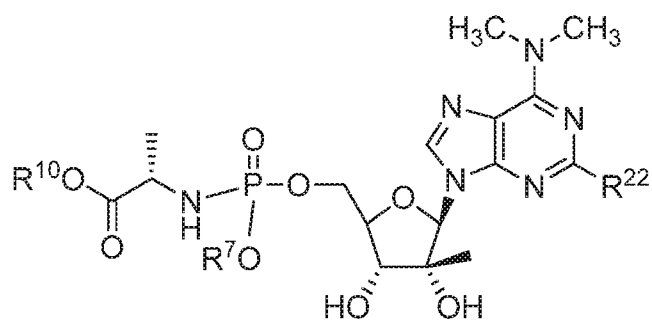
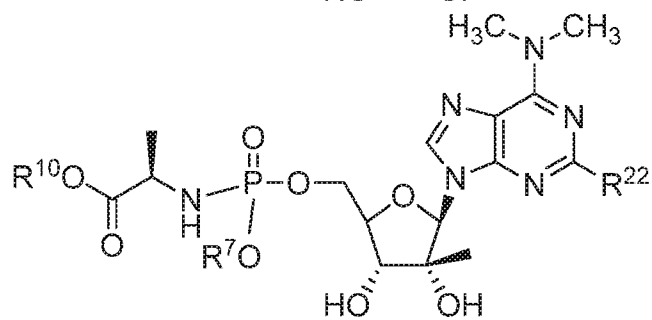
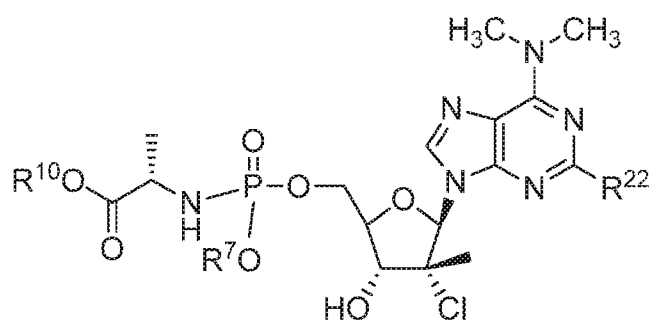
121. A compound of claim 31, wherein R^{41} is fluoro.
122. A compound of claim 31, wherein R^{41} is chloro.
123. A compound of claim 31, wherein R^{41} is hydroxyl.
124. A compound of claim 32, wherein R^{41} is fluoro.
125. A compound of claim 32, wherein R^{41} is chloro.
126. A compound of claim 32, wherein R^{41} is hydroxyl.
127. The compound of any of claims 1, 9, 14, 24 or 29-32 and pharmaceutically acceptable salts and prodrugs thereof for use in the treatment or prophylaxis of a hepatitis C virus infection.
128. The use of a compound of any of claims 1, 9, 14, 24 or 29-32 and pharmaceutically acceptable salts and prodrugs thereof in the manufacture of a medicament for treatment of a hepatitis C virus infection.
129. A method for manufacturing a medicament intended for the therapeutic use for treating a hepatitis C virus infection, characterized in that a compound of any of claims 1, 9, 14, 24 or 29-32 is used in the manufacture.

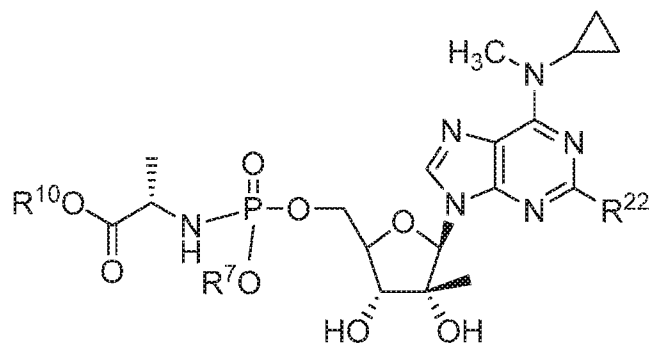
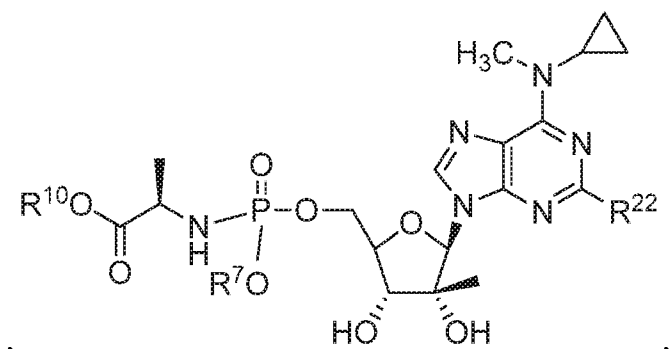
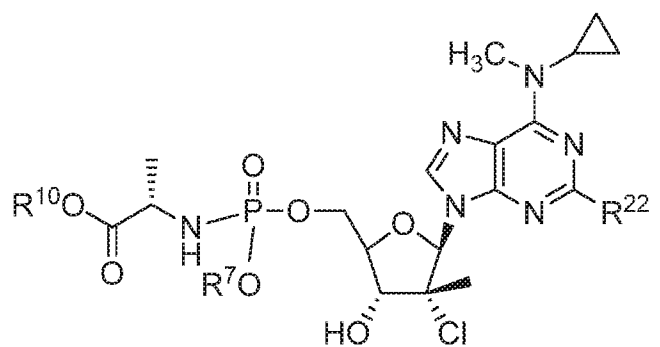
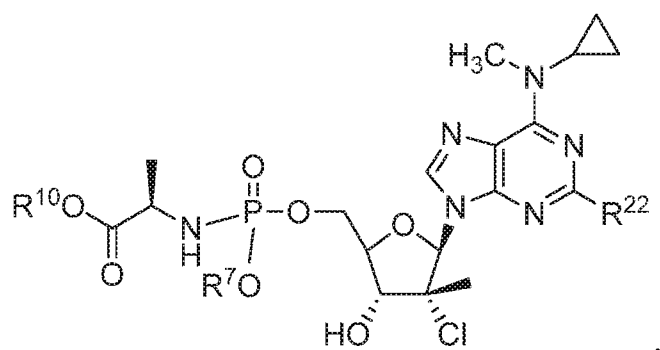
130. A pharmaceutical formulation comprising an effective host-treating amount of the compound of any of claims 1, 9, 14, 24 or 29-32 or a pharmaceutically acceptable salt or prodrug thereof together with a pharmaceutically acceptable carrier or diluent.

131. A compound selected from the group consisting of



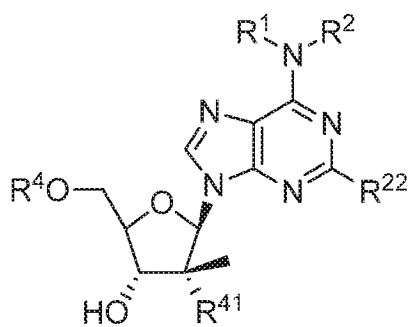






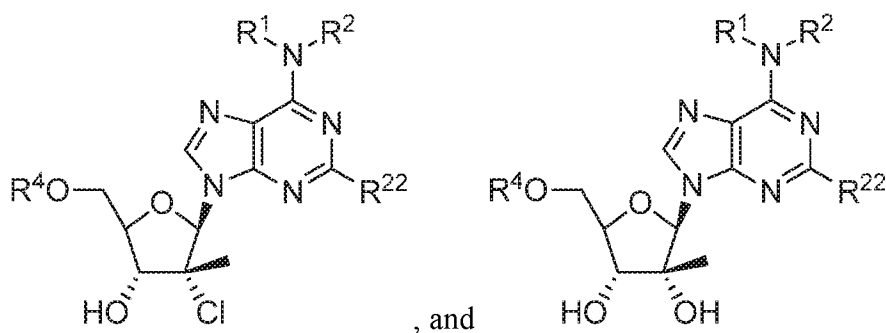
wherein R^7 , R^{10} , R^{22} and R^{41} are as defined in claims 9, 14 and 31.

132. A compound of the formula:



wherein R⁴ is a stabilized phosphate prodrug; and
 R¹, R², R²² and R⁴¹ are as defined in claims 1, 9, 14 and 31;
 or a pharmaceutically acceptable salt thereof.

133. A compound selected from:



wherein R⁴ is a stabilized phosphate prodrug; and
 R¹, R², R²² and R⁴¹ are as defined in claims 1, 9, 14 and 31;
 or a pharmaceutically acceptable salt thereof.

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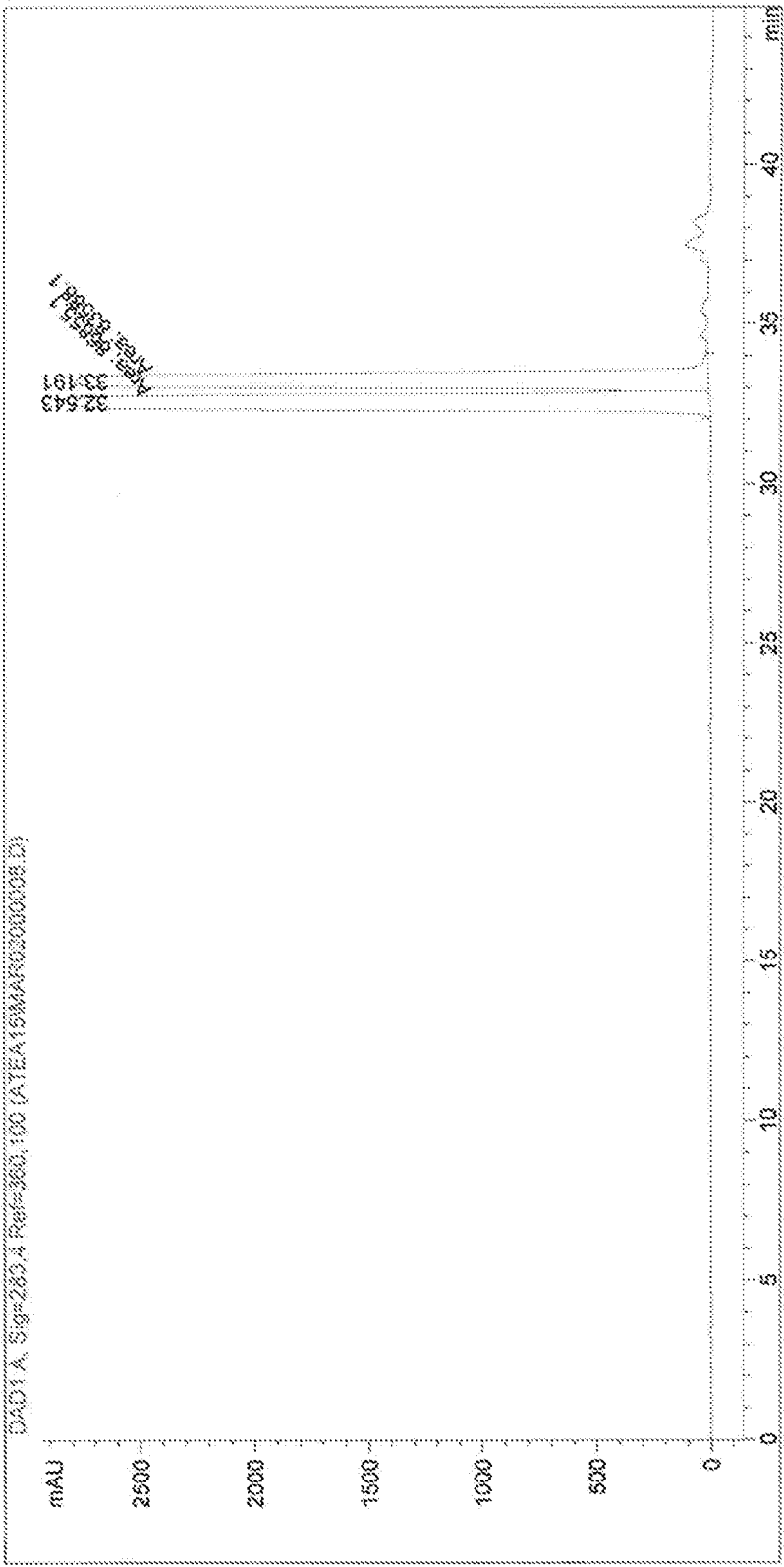


FIG. 1

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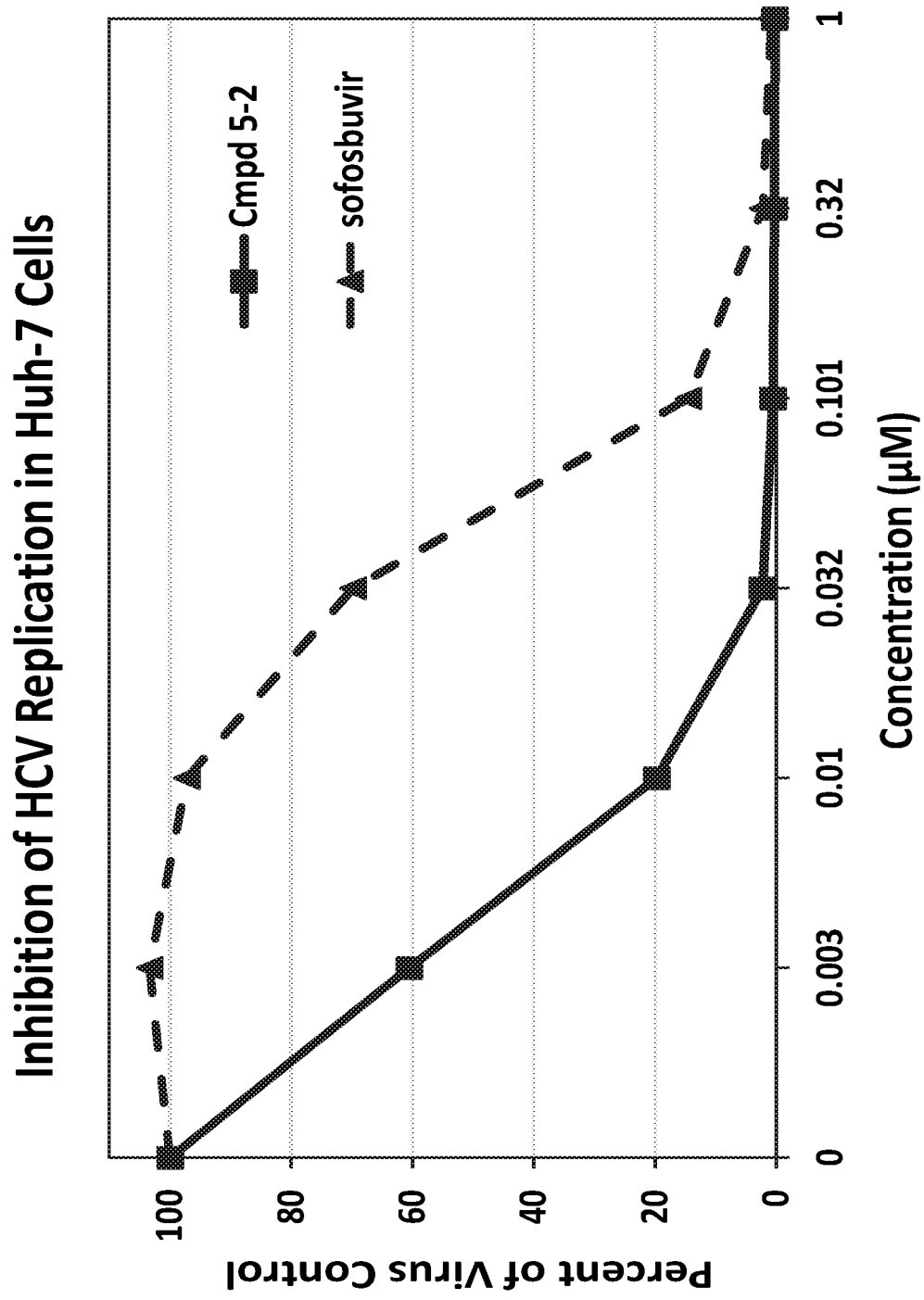


FIG. 2

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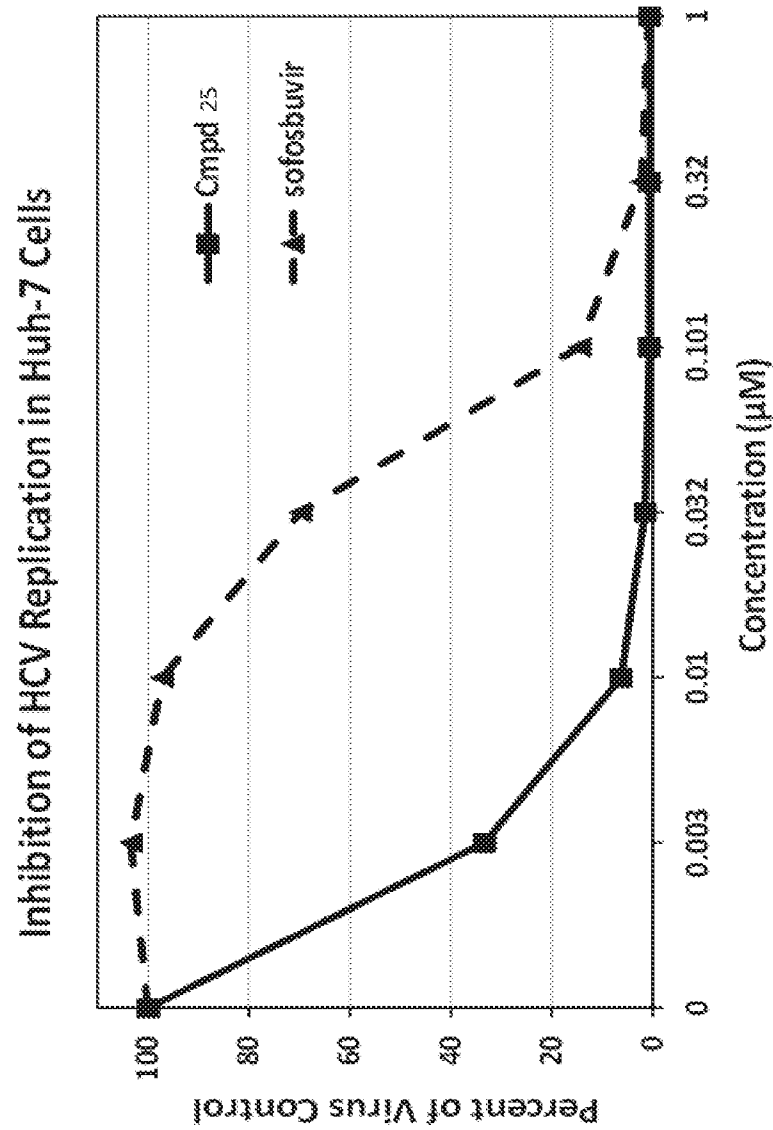


FIG. 3

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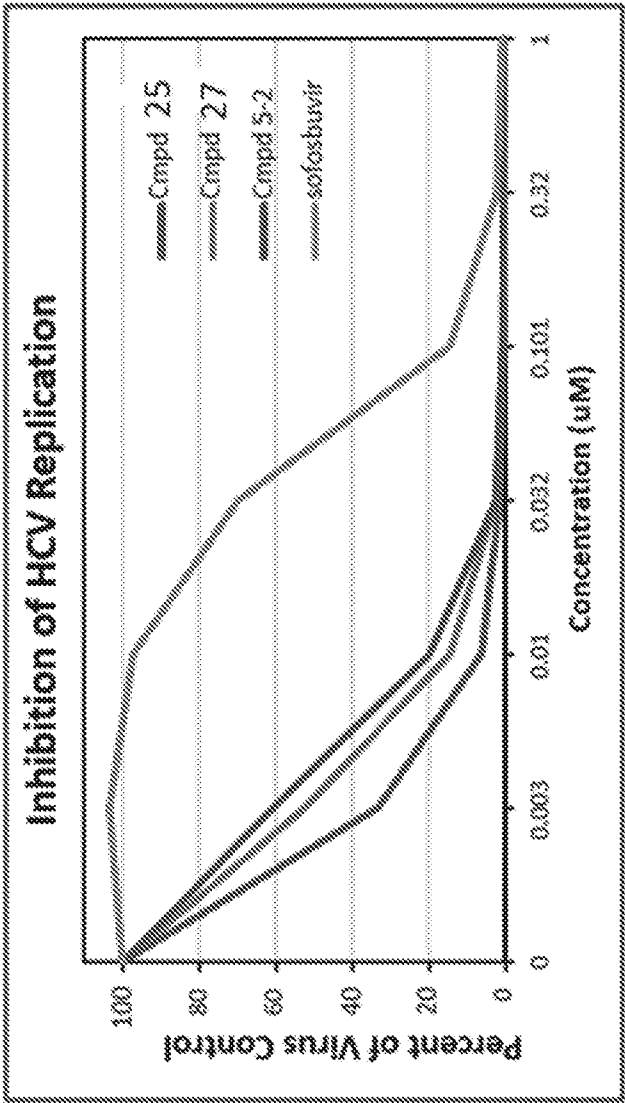


FIG. 4

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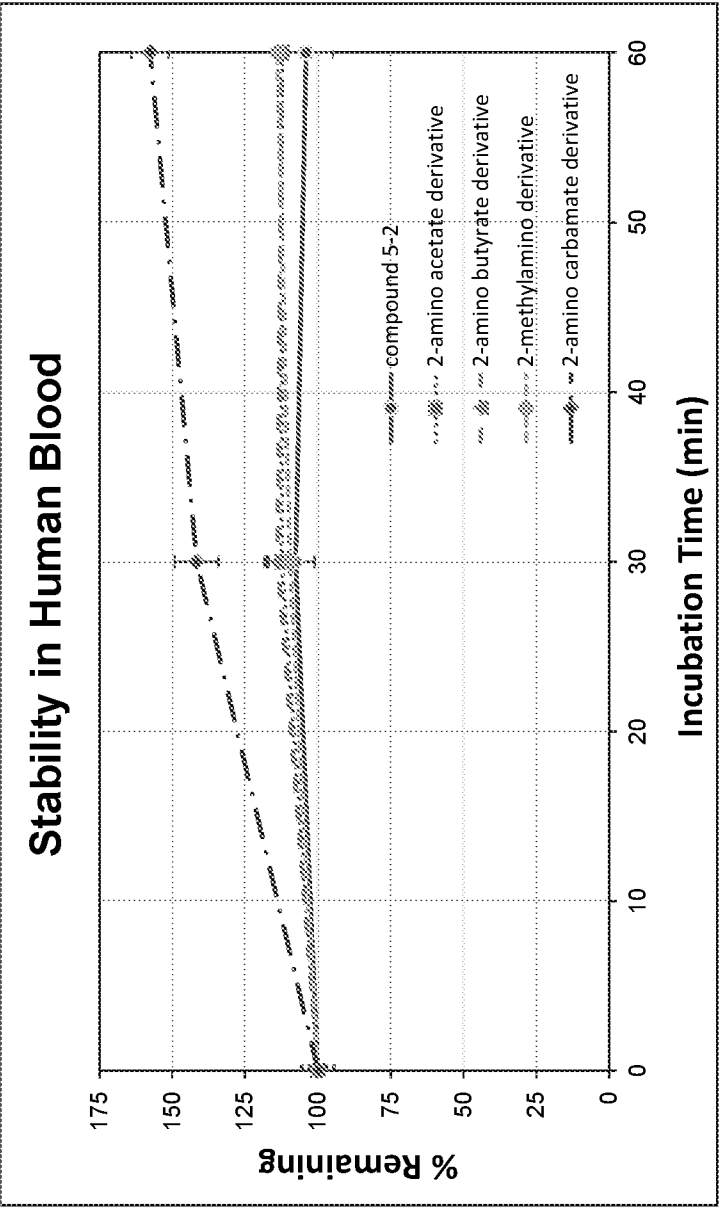


FIG. 5

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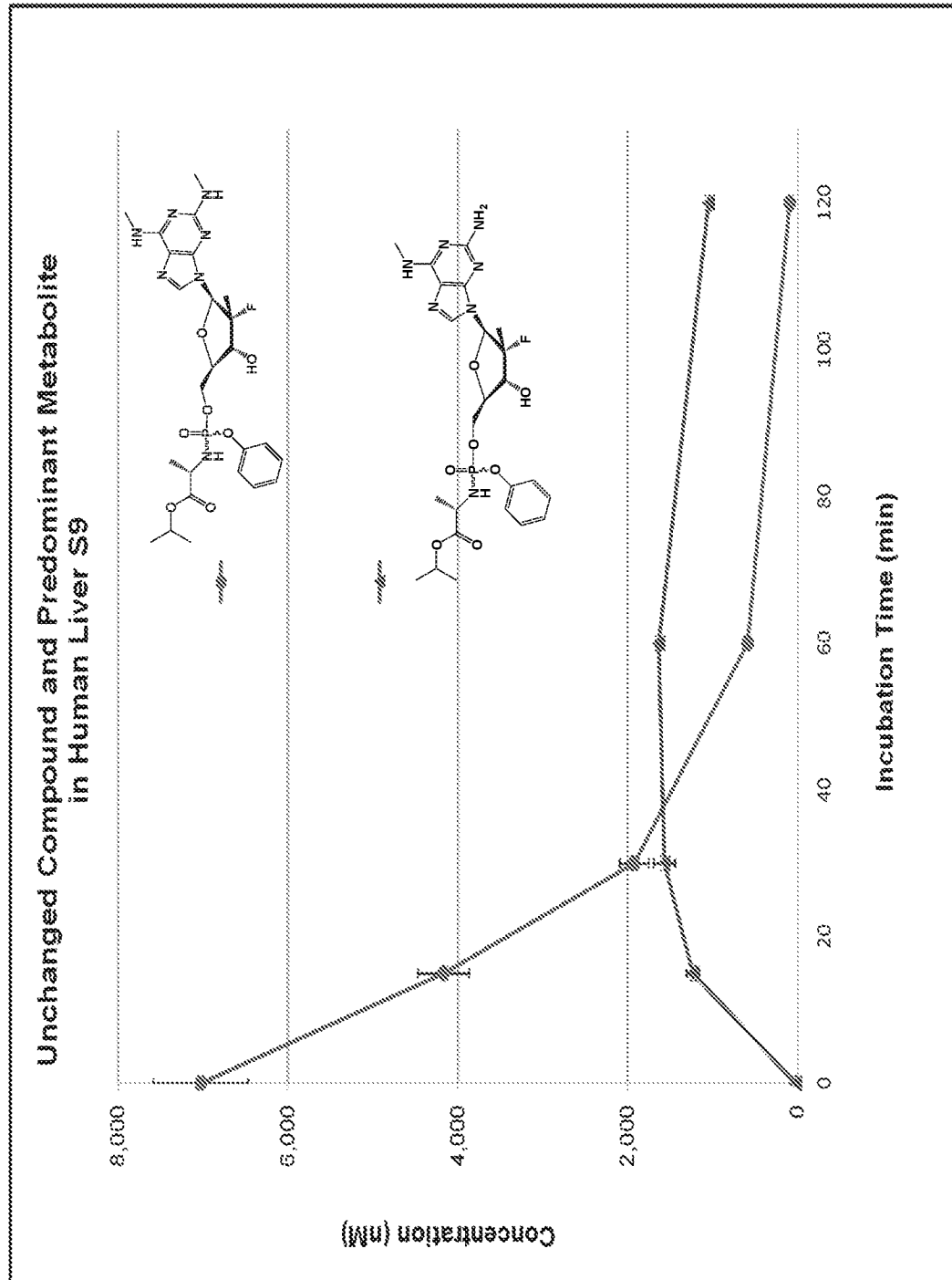


FIG. 6

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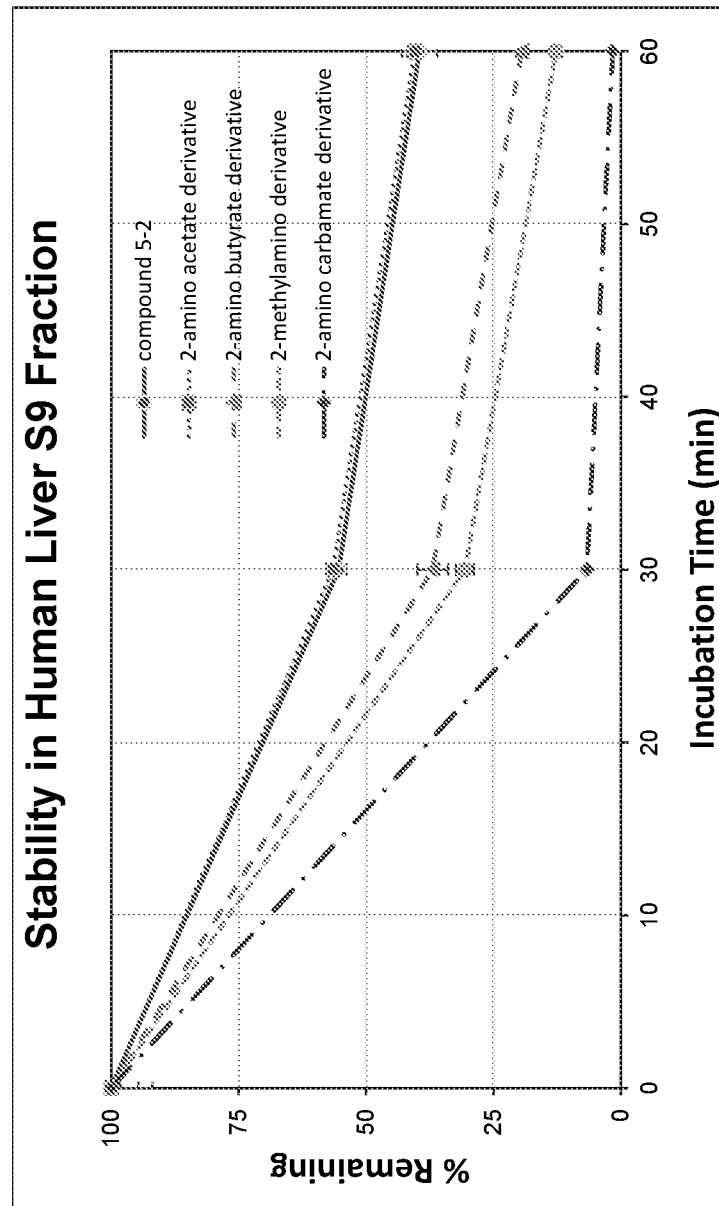


FIG. 7

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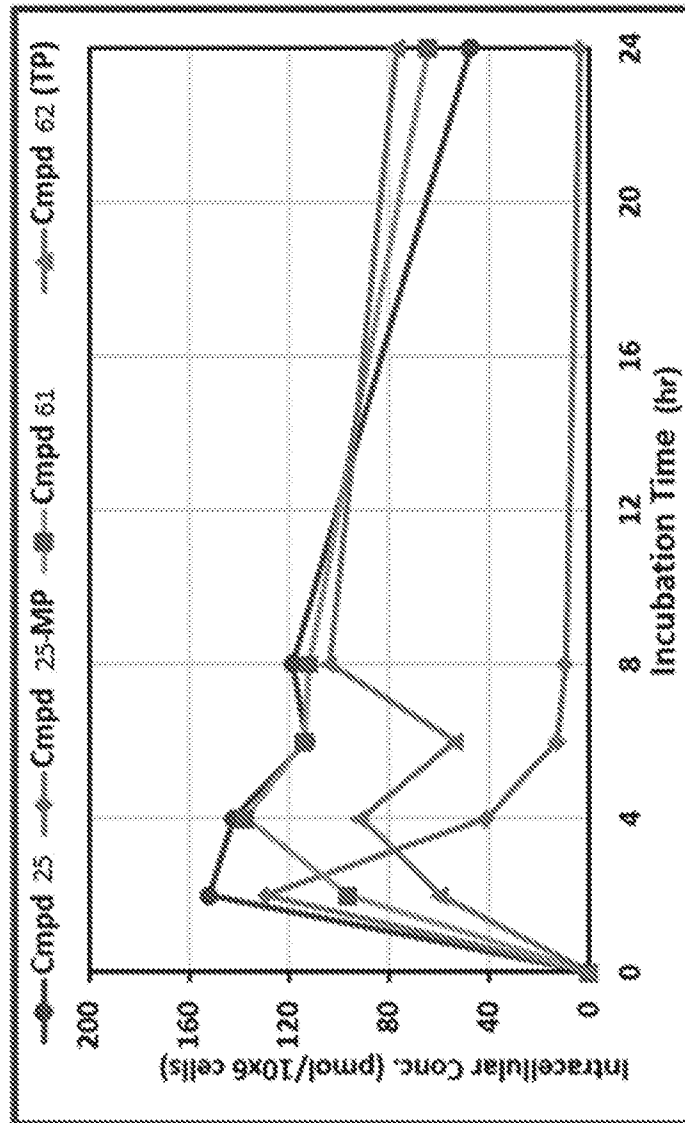


FIG. 8

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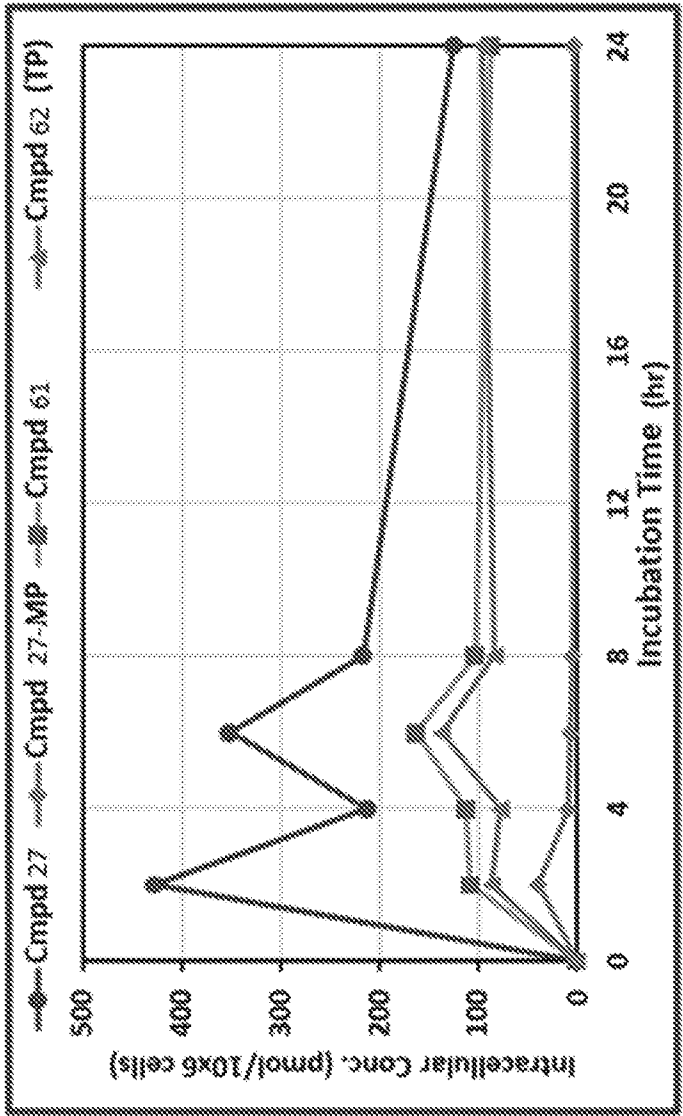


FIG. 9

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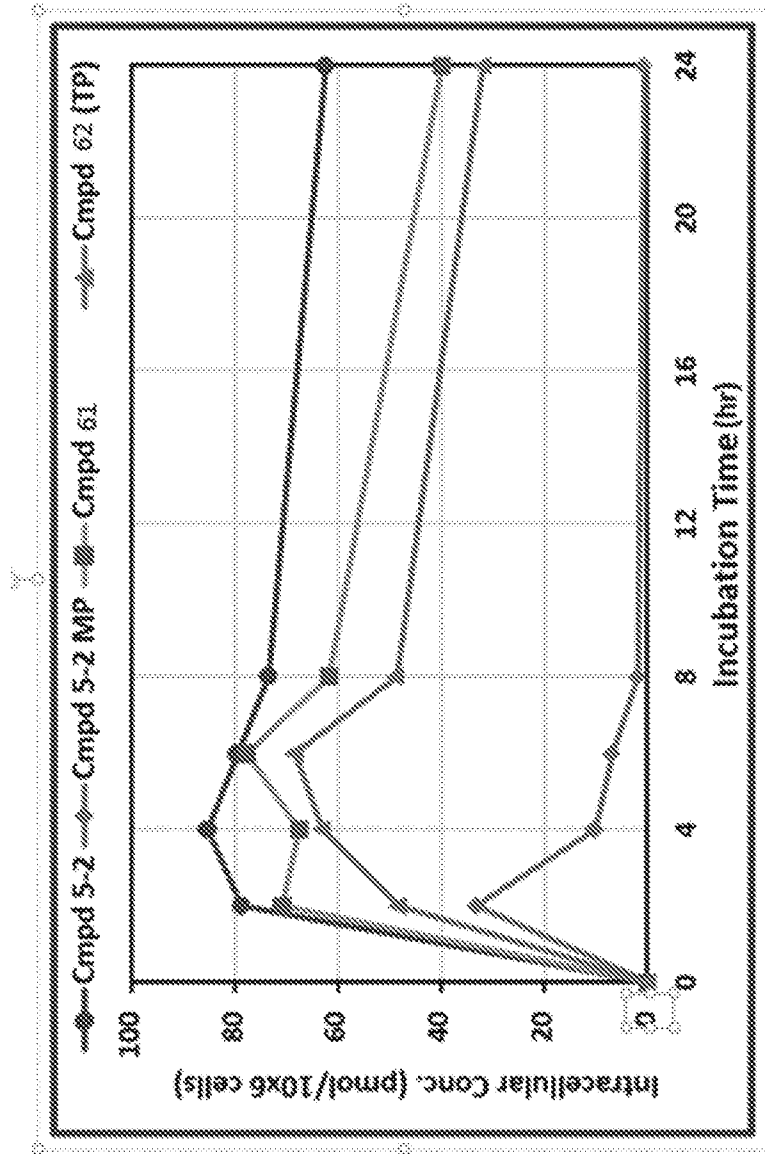


FIG. 10

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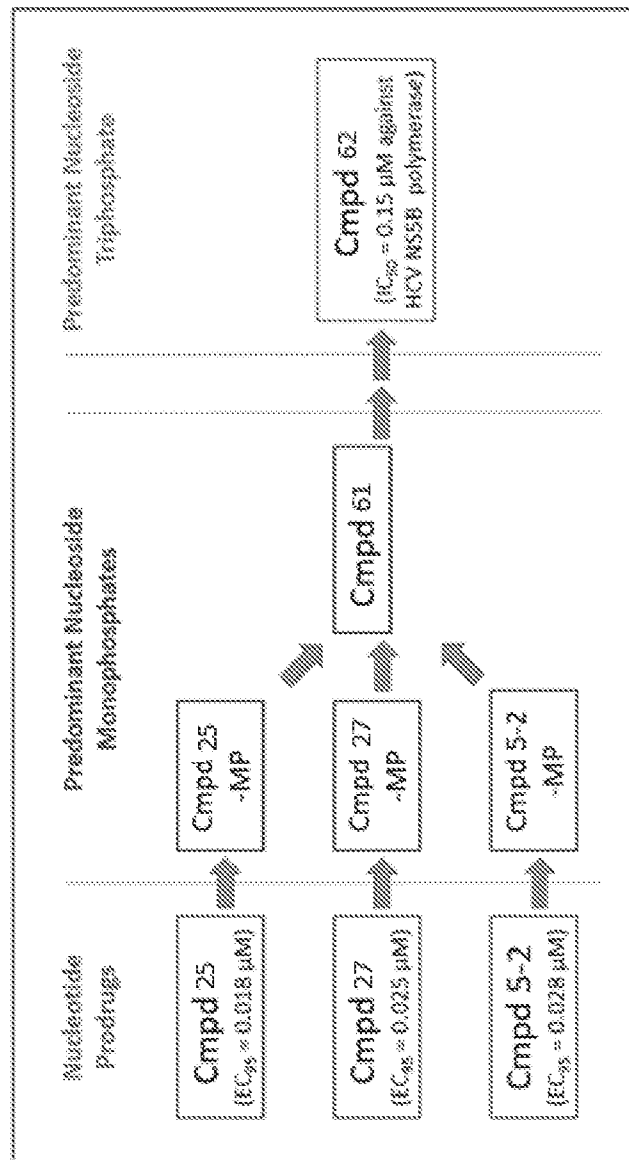


FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/21276

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/7076; C07H 19/16 (2016.01)

CPC - A61K31/7076; C07H19/167; C07H19/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

CPC: A61K31/7076; C07H19/167; C07H19/20

IPC(8): A61K 31/7076; C07H 19/16 (2016.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/45-48; 536/26.7 (See Search Words Below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PATBASE: Full-text = AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO

Google: Scholar/Patents: nucleoside purine HCV phosphoramidate fluorine dimethylamine cyclopropyl methyl amine phenoxy enantiomer fluoro chloro phosphoramidite protease inhibitor ribavirin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/0257121 A1 (CHANG et al.) 20 October 2011 (20.10.2011) para [0003];[0018];[0046];[0063];[0064];[0066];[0069];[0084];[0104];[0110].	1-31, 33-35, 37-39, 41-43, 45-51, 53-61, 63, 64, 67, 68, 71, 72, 75, 76, 79, 80, 83, 84, 87, 88, 91, 92, 95, 96, 99, 100, 103, 104, 107, 108, 111, 112, 121-123
Y	FREEMAN et al. '2-Amino-9-(3-Azido-2,3-Dideoxy- -D-Erythro -Pentofuranosyl) -6-Substituted -9H-Purines: Synthesis and Anti-HIV Activity', Bioorganic and Medicinal Chemistry, 1995, Vol 3, pp 447-458. pg 447, Col 1, para 1; pg 450, Table 1	1-31, 33-35, 37-39, 41-43, 45-51, 53-61, 63, 64, 67, 68, 71, 72, 75, 76, 79, 80, 83, 84, 87, 88, 91, 92, 95, 96, 99, 100, 103, 104, 107, 108, 111, 112, 121-123
Y	US 2004/0063658 A1 (ROBERTS et al.) 01 April 2004 (01.04.2004) para [0059];[0091];[0111];[0113];[0114];[0443]	8, 14-31, 35, 39, 43, 45, 47, 59, 61, 67, 68, 71, 72, 75, 76, 79, 80, 83, 84, 87, 88, 91, 92, 95, 96, 99, 100, 103, 104, 107, 108, 111, 112, 121-123
Y	US 2012/0135951 A1 (SCHINAZI et al.) 31 May 2012 (31.05.2012) para [0100]-[0102]	13

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 May 2016 (18.05.2016)

Date of mailing of the international search report

17 JUN 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/21276

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

32, 36, 40, 44, 52, 62, 65-66, 69-70, 73-74, 77-78, 81-82, 85-86, 89-90, 93-94, 97-98, 101-102, 105-106, 109-110, 113-120, 124-133

3. ☒ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Trends in Active Pharmaceutical Ingredient Salt Selection based on Analysis of the Orange Book Database

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Received August 20, 2007

The Orange Book database published by the U.S. Drug and Food Administration (FDA) was analyzed for the frequency of occurrence of different counterions used for the formation of pharmaceutical salts. The data obtained from the present analysis of the Orange Book are compared to reviews of the Cambridge Structural Database (CSD) and of the Martindale “The Extra Pharmacopoeia”. As well as showing overall distributions of counterion usage, results are broken down into 5-year increments to identify trends in counterion selection. Chloride ions continue to be the most frequently utilized anionic counterions for the formation of salts as active pharmaceutical ingredients (APIs), while sodium ions are most widely utilized for the formation of salts starting from acidic molecules. A strong trend toward a wider variety of counterions over the past decade is observed. This trend can be explained by a stronger need to improve physical chemical properties of research and development compounds.

Introduction

Salt formation is a well-known technique to modify and optimize the physical chemical properties of an ionizable research or development compound. Properties such as solubility, dissolution rate, hygroscopicity, stability, impurity profiles, and crystal habit can be influenced by using a variety of pharmaceutically acceptable counterions.^{1–8} Even polymorphism issues can be resolved in many cases by formation of salts. The crystal structure of a salt is usually completely different from the crystal structure of the conjugate base or acid and also differs from one salt to another. The modification of physical chemical properties, mainly solubility and dissolution rate, may also lead to changes in biological effects such as pharmacodynamics and pharmacokinetics, including bioavailability and toxicity profile.^{1,9,10}

Owing to dramatic changes in the techniques applied in pharmaceutical discovery programs over the past 20 years, the physical chemical properties of development candidates have changed substantially.¹¹ Drug design based on high-throughput screening has in general led to more lipophilic compounds exhibiting low aqueous solubility.

There are many well-known formulation techniques to increase aqueous solubility,^{12–14} e.g., micronization, nanosizing, or complexation with cyclodextrins. The use of solid solutions and solid dispersions is another way to improve bioavailability for development candidates with low solubility. Nevertheless, formation of salts is almost the only chemical technique available to change aqueous solubility and dissolution rate without changing the API molecule. Further options for modifying these properties comprise the choice of the polymorphic form including solvates and formation of cocrystals. Although cocrystals in particular are an innovative way of designing APIs, this method is beyond the scope of this publication. An overview of this topic can be found in ref 15. Salt selection remains an important step at the interface between pharmaceutical research and development. A large number of publications covering

physical chemical properties of pharmaceutical salts and methods for salt screening exist, e.g., refs 4, 16–19 and references included therein. On the other hand, publications giving an overview of approved salt forms are very few.^{1–3} All publications known to the authors dealing with occurrence of counterions for formation of pharmaceutical salts list the counterions and their distribution in the respective data set only at a given point in time. Neither the distribution trends over time nor the causes for these have been analyzed to date.

The present contribution examines the selection of counterions for the formation of salts by analyzing the Orange Book Database²⁰ published by the U.S. Drug and Food Administration (FDA). The Orange Book lists all drug products approved in the U.S. Drug products approved after 1981 are listed including their date of approval. This enables an analysis of the changes in frequency of usage of the different counterions with time. Trends in salt selection over the past 25 years can thus be identified and the outcome of the overall analysis of the Orange Book compared to results based on other sources.

Study Design

The data were compiled from the FDA Orange Book Database as of the end of 2006. At this date, 21 187 drug products were listed, including 1356 chemically “well-defined” APIs. “Well defined” for the purpose of our analysis means that the API molecules are small chemical entities with a defined molar mass, typically below 1000 Da and that their chemical structure is completely known. Dosage forms containing multiple APIs, peptide hormones, biological APIs like antibodies, enzymes, extracts, and proteins, metal complexes, polymeric salt forms, inorganic APIs, and markers were excluded from our analysis. The APIs were classified into three categories: Category I consists of salts formed from basic molecules containing at least one atom suitable for protonation. Category II comprises salts formed from acidic species. Finally, category III is represented by APIs that are used as nonsalt forms. This class also includes zwitterions. Counterions are reported according to their type of charge as cations and anions. The stoichiometry of the salts is not discussed separately: for

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[†] Merck KGaA.

[‡] Johann Wolfgang Goethe University.

Table 1. Distribution of FDA Approved APIs among Categories I–III

overall	pre-1982 (%)	1982–1986 (%)	1987–1991 (%)	1992–1996 (%)	1997–2001 (%)	2002–2006 (%)
Category I: API Salts Formed of Basic Entities						
38.6	38.4	42.0	40.2	38.0	40.3	32.7
Category II: API Salts Formed of Acidic Entities						
12.8	13.6	10.1	11.1	13.3	11.1	14.6
Category III: Nonsalt APIs						
48.6	48.0	47.9	48.7	48.7	48.6	52.7

example, the occurrence of bromides includes bromides and dibromides. Furthermore, the APIs were arranged by year of approval to analyze how trends in the choice of salt forms have changed in recent decades. Prior to 1981, no date of approval is given in the Orange Book. Therefore, the drug products approved before 1982 are summarized under “pre-1982”. The period from 1982 to 2006 has been divided into five intervals, each comprising 5 years. After completion of the analysis of all chemically well-defined APIs, a separate assessment of the subset of APIs of oral (844 APIs) and injectable (482 APIs) dosage forms was made. Our analysis shows how the route of administration influences the choice of a specific salt form. This observation can be assigned to the different requirements of the two routes of administration. For example, for the two basic compounds biperiden and pentazocine, the chloride salts are used for oral dosage forms, whereas the lactate salts are used for injectable dosage forms.

Results and Discussion

Distribution of API Salts Formed of Basic and Acidic Molecules and APIs in Nonsalt Forms. The 1356 chemically well-defined APIs listed in the Orange Book comprise 659 (48.6%) APIs in nonsalt forms, 523 (38.6%) salts formed from basic compounds, and 174 (12.8%) salts formed from acidic molecules. Thirty-eight different anions and 15 cations are used as counterions for the formation of salts. Thereof, 16 anions and 8 cations were only used once. During the past 25 years, 25 anions and 7 cations have been used to form salts. The ratios of APIs obtained by salt formation of molecules exhibiting basic properties, API salts obtained from acidic species, and APIs in nonsalt forms have remained virtually constant. This is shown in Table 1. During 2002–2006, there has been some decrease in the percentage of APIs obtained as salts of basic compounds. This leads to a small increase in both of the other categories. Figure 1 shows the corresponding distribution of APIs among the three categories used in oral and injectable dosage forms. Together, oral and injectable formulations represent the majority of FDA-approved formulations. However, the requirements placed on an API for oral and injectable dosage forms are quite different. For oral dosage forms, a key prerequisite of the API is a certain minimum solubility in the pH range of the gastrointestinal tract. An adequate dissolution rate and a sufficient permeability are also important. If these requirements are not fulfilled, bioavailability will be insufficient to achieve the desired therapeutic effect. In the case of solutions for injection, considerations such as pH of the solution, osmolarity, and solubility in a small volume are important for efficient and pain-free administration. In many cases, this can lead to situations where a considerably higher solubility is required for injectables than for oral formulations.

Distribution of Anionic Counterions Used To Form Pharmaceutical Salts. A summary of all anions used along with their distribution during different time periods is given in

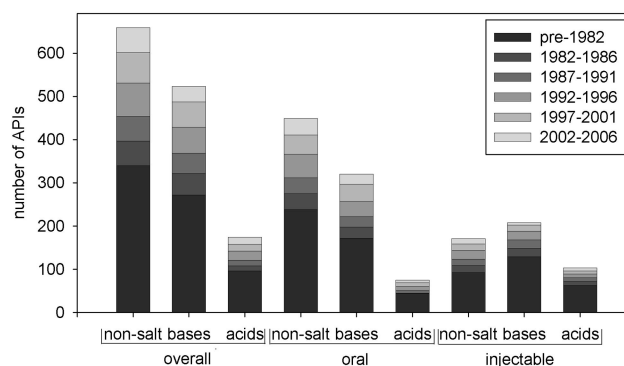
**Figure 1.** Classification and distribution of species in the Orange Book according to their type of charge and administration route.

Table 2. Figure 2 displays the overall distribution of anions, whereas Figure 3 depicts the most recent period, 2002–2006. The anion encountered most frequently in FDA-approved pharmaceutical salts is the chloride ion. The fraction of chlorides increased from 52.9% (pre-1982) to 63.8% (1987–1991), remained almost constant at 63.3% over the next 5 years (1992–1996) and decreased significantly to 38.9% (2002–2006) over the past 10 years. The anion encountered with highest frequency after chloride is sulfate. However, it accounts for only 7.5% of APIs formed from basic molecules. Its peak incidence was 12.0% during the period 1982–1986. Further acidic counterions frequently encountered include bromides, with a total incidence of 4.6%, as well as maleates and mesylates, both with incidences of 4.2%.

There appears to be some tendency for “fashions” in anionic counterion selection, with certain counterions showing a noticeably higher occurrence during one period compared to their overall usage. For example, nitrates represented 8.0% of anionic counterions during the 1982–1986 period. The average usage of nitrates is only 1.7%. Further examples include acetate with a maximum incidence of 12.7% during 1987–1991 and an overall usage of 3.3%. Tartrates exhibited a higher incidence of 6.7% in 1992–1996 than the average of 3.8%. Fumarates showed most frequent utilization during 1997–2001, contributing 8.6% of FDA-approved salts formed of basic molecules during this period. They yielded an average fraction of 1.7%. For mesylates, the same is true with a peak occurrence of 13.8% during the same period and an average incidence of 4.2%. The number of anions used to form salts has varied during the past 25 years between 11 and 15 per 5-year period. In total, there are only two anions with an average incidence of more than 5% over the whole period. These are the chlorides and sulfates. Nevertheless, during the individual 5-year intervals, there are several anions reaching fractions of more than 5%. For example, in the pre-1982 period these are bromides and maleates. From 1982 to 1986, acetates and nitrates are encountered in more than 5% of the APIs of category I. From 1987 to 1991, acetate and from 1992 to 1996 tartrate are the only anions other than chloride that were used to form more than 5% of the FDA-approved salts of basic molecules. After 1996, a broader variety of anions has reached an incidence of more than 5% usage. During 1997–2001 five anions exhibit an occurrence of more than 5%: bromides, chlorides, citrates, fumarates, and mesylates. From 2002 to 2006, seven different anions including bromides, chlorides, maleates, mesylates, phosphates, sulfates, and tartrates had an incidence of 5% or more. These figures indicate a strong, recent trend toward increased diversity of anions applied for the formation of salts in category I. The trend can be explained as a consequence of the changes in research techniques

Table 2. Distribution of Anions Used in APIs of Category I

	overall (%)	pre-1982 (%)	1982–1986 (%)	1987–1991 (%)	1992–1996 (%)	1997–2001 (%)	2002–2006 (%)
acetate	3.3	1.5	8.0	12.7		3.5	2.8
benzoate	0.2					1.7	
besylate	0.8	0.4	2.0		3.3		
bromide	4.6	5.2	4.0	2.1	1.7	5.2	8.3
camphorsulfonate	0.2	0.4					
chloride	53.4	52.9	52.0	63.8	63.3	46.6	38.9
chlorotheophyllinate	0.2	0.4					
citrate	2.7	2.6	2.0		3.3	5.2	2.8
ethandisulfonate	0.2	0.4					
fumarate	1.7	0.4		2.1	3.3	8.6	
gluceptate	0.2	0.4					
gluconate	0.4	0.7					
glucuronate	0.2				1.7		
hippurate	0.2	0.4					
iodide	1.0	1.5	2.0				
isethionate	0.4	0.4	2.0				
lactate	1.3	1.5	4.0	2.1			
lactobionate	0.2	0.4					
laurylsulfate	0.2	0.4					
malate	0.4	0.4					2.8
maleate	4.2	5.5	2.0		3.3	3.5	5.6
mesylate	4.2	2.6	2.0	4.3	1.7	13.8	8.3
methysulfate	0.4	0.7					
naphthoate	0.2				1.7		
napsylate	0.4	0.7					
nitrate	1.7	0.7	8.0	2.1	1.7		2.8
octadecanoate	0.2	0.4					
oleate	0.2			2.1			
oxalate	0.2						2.8
pamoate	0.8	1.1				1.7	
phosphate	2.7	3.3		2.1	1.7	1.7	5.6
polygalacturonate	0.2	0.4					
succinate	1.2	0.7			3.3	1.7	2.8
sulfate	7.5	9.6	12.0	4.3	1.7	3.5	5.6
sulfosalicylate	0.2	0.4					
tartrate	3.8	3.7		2.1	6.7	3.5	8.3
tosylate	0.4	0.4					2.8
trifluoroacetate	0.2				1.7		
number of salts	523	272	50	47	60	58	36

employed by the pharmaceutical industry. The extensive use of combinatorial chemistry and high-throughput screening in drug discovery has led to higher lipophilicity and commensurate lower solubility and dissolution rate of new drug candidates over the past 20 years. This in turn has necessitated a more intensive search for appropriate salts as a tool to improve physical chemical properties, a search typically conducted at the end of lead optimization or during exploratory development.

Distribution of Cationic Counterions Used To Form Pharmaceutical Salts. All cationic counterions together with their respective incidences are listed in Table 3. Figure 4 shows the overall distribution of cations in salts formed from chemical entities exhibiting acidic properties. In Figure 5, the relative occurrence during the last period from 2002 to 2006 is depicted. Among the cations used to form API salts of acidic molecules, the sodium ion strongly dominates with an incidence of 75.3% over the entire period. From 1982 to 1991, the fraction of sodium salts was more than 90%. This decreased to 62.5% during the

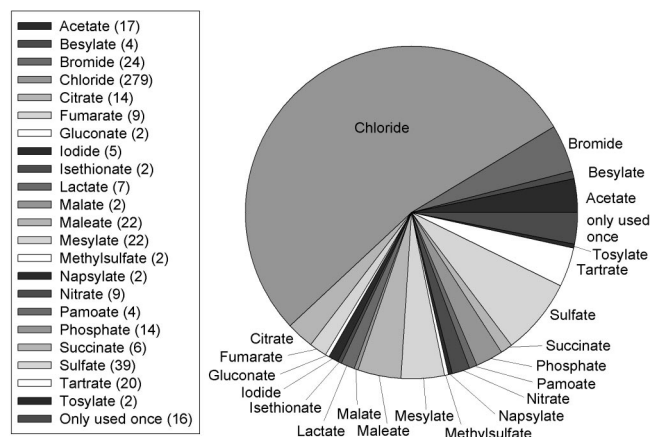
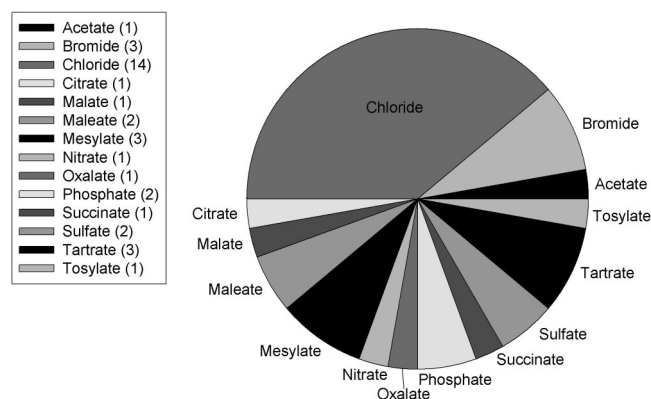
**Figure 2.** Overall distribution of anions used in APIs of category I in the Orange Book.**Figure 3.** Distribution of anions used in APIs of category I from 2002 to 2006.

Table 3. Distribution of Cations Used in APIs of Category I

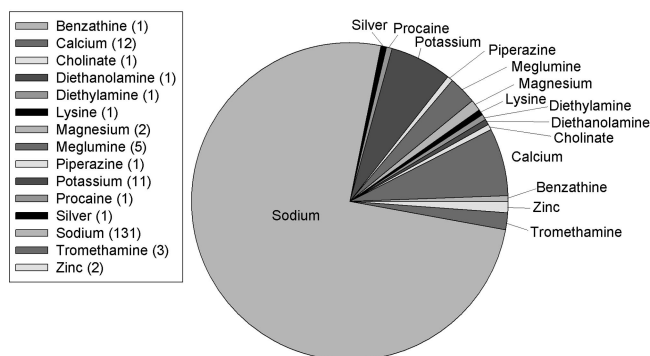
	overall (%)	pre-1982 (%)	1982–1986 (%)	1987–1991 (%)	1992–1996 (%)	1997–2001 (%)	2002–2006 (%)
benzathine	0.6	1.0					
calcium	6.9	7.3			9.5		18.8
choline	0.6	1.0					
diethanolamine	0.6	1.0					
diethylamine	0.6	1.0					
lysine	0.6						6.3
magnesium	1.2					6.3	6.3
mequmine	2.9	5.2					
piperazine	0.6	1.0					
potassium	6.3	6.3			14.3	6.3	6.3
procaine	0.6	1.0					
silver	0.6	1.0					
sodium	75.3	72.9	91.7	92.3	66.7	87.5	62.5
tromethamine	1.7			7.7	9.5		
zinc	1.2	1.0	8.3				
number of salts	174	96	12	13	21	16	16

2002–2006 period. The second most common cation is calcium with an average incidence of 6.9%. Its peak frequency of 18.8% was reached during 2002–2006. Another cation with frequent usage is potassium. On average, 6.3% of the FDA-approved drugs of category II are potassium salts. Potassium salts show their highest relative occurrence during 1992–1996, yielding 14.3% of API salts obtained from acidic entities. Benzathine, choline, diethanolamine, diethylamine, mequmine, piperazine, procaine, and silver have not been used over the past 25 years. They were only used once each during the time frame before end of 1981. Lysine and magnesium were both introduced as counterions during the past 10 years.

Only two basic counterions were utilized in each of the two 5-year periods 1982–1986 (sodium, zinc) and 1987–1991 (sodium, tromethamine). This number increased from three in the period 1992–1996 to five in the period 1997–2001 to six in the period 2002–2006. This analysis indicates that the trend toward a wider diversity of counterions observed for usage of anions is also occurring with cations.

Salts Used in Oral Formulations. Of the 1356 chemically well-defined APIs listed in the Orange Book, 844 are used for oral delivery. A total of 449 (53.2%) of them are nonsalt forms, 320 (37.9%) salts are formed from molecules exhibiting basic properties, and 75 (8.9%) are salts formed from entities with acidic behavior. A total of 30 different anions have been used, 17 of them during the past 25 years. Only eight cations have been employed for formation of salts from acidic moieties, five of which were employed over the past 25 years. The analysis shows that 15 anions and 3 cations were only used once.

Distribution of Anionic Counterions Used in Oral Formulations. Relative incidences of all anions used in FDA-approved oral formulations are presented in Table 4. The anion

**Figure 4.** Overall distribution of cations used in APIs of category II in the Orange Book.

applied most frequently in APIs utilized in oral formulations is chloride. Its fraction increased from 55.8% (pre-1982) through 65.4% (1982–1986) to 79.2% (1987–1991). After this period, there was a continuous decrease from 65.7% (1992–1996) through 45.0% (1997–2001) to 34.8% (2002–2006). Other important anions for oral delivery comprise sulfate with an incidence of 7.5%, maleate with 6.9%, and mesylate with 4.4% over the whole period. Mesylate salts exhibited a peak incidence of 15.0% during 1997–2001. Citrate salts were also frequently encountered during the same period, with 7.5% compared to an average fraction of 3.4% over the whole time period. The fifth anion according to frequency of usage ranking is bromide with an average value of 4.1% and a peak occurrence of 8.7% in 2002–2006.

During each of the periods from 1982 to 1986 and 1987–1991, salts containing five different anions were approved in oral formulations. Between 1992 and 1996, 10 different anions were used in API salts in newly approved drug products intended for oral use. During the two last periods of 1997–2001 and 2002–2006, 11 anions were applied per period. Thus, the overall trend toward a higher variety of acids and bases used for formation of salts is reflected in APIs for oral application.

Distribution of Cationic Counterions Used in Oral Formulations. All cations encountered as counterions for formation of API salts used in products for oral delivery are summarized in Table 5. Sodium represents the most common cation of this category. Its average frequency of occurrence during the whole time period analyzed is 65.3%. It strongly fluctuates during the different 5-year time periods with a relative

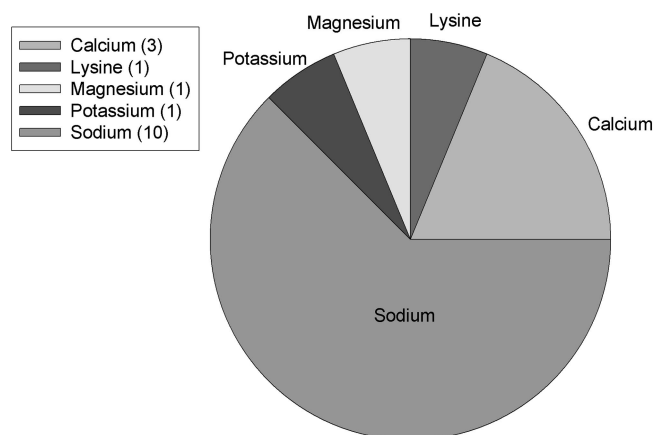
**Figure 5.** Distribution of cations used in APIs of category II from 2002 to 2006.

Table 4. Distribution of Anions for API Used in Oral Dosage Forms

	overall (%)	pre-1982 (%)	1982–1986 (%)	1987–1991 (%)	1992–1996 (%)	1997–2001 (%)	2001–2006 (%)
acetate	0.9	0.6	7.7				
benzoate	0.3					2.5	
besylate	0.6	0.6			2.9		
bromide	4.1	5.2				5.0	8.7
chloride	56.6	55.8	65.4	79.2	65.7	45.0	34.8
chlorthephyllinate	0.3	0.6					
citrate	3.4	4.1			2.9	7.5	
ethandisulfonate	0.3	0.6					
fumarate	1.6	0.6		4.2	2.9	5.0	
gluconate	0.3	0.6					
hippurate	0.3	0.6					
iodide	0.3	0.6					
lactate	0.3	0.6					
laurylsulfate	0.3	0.6					
malate	0.3						4.4
maleate	6.9	8.7	3.9		5.7	5.0	8.7
mesylate	4.4	1.7		8.3	2.9	15.0	8.7
methysulfate	0.6	1.2					
napsylate	0.6	1.2					
nitrate	0.6		3.9		2.9		
octadecanoate	0.3	0.6					
oxalate	0.3						4.4
pamoate	0.9	1.7					
phosphate	2.5	2.9				2.5	8.7
polygalacturonate	0.3	0.6					
succinate	1.9	1.2			5.7	2.5	4.4
sulfate	7.5	7.6	19.2	4.2	2.9	5.0	8.7
tartrate	2.8	1.7		4.2	5.7	5.0	4.4
tosylate	0.3						4.4
number of salts	320	172	26	24	35	40	23

Table 5. Distribution of Cations for API Used in Oral Dosage Forms

	overall (%)	pre-1982 (%)	1982–1986 (%)	1987–1991 (%)	1992–1996 (%)	1997–2001 (%)	2002–2006 (%)
benzathine	1.3	2.3					
calcium	12.0	11.4			11.1		50.0
choline	1.3	2.3					
magnesium	2.7					11.1	16.7
piperazine	1.3	2.3					
potassium	13.3	13.6			33.3		16.7
sodium	65.3	68.2	100.0	83.3	44.4	88.9	16.7
tromethamine	2.7			16.7	11.1		
number of salts	75	44	1	6	9	9	6

fraction of at least 68.2% until 1991. This value decreased to 44.4% during 1992–1996. During the following period, 1997–2001, there was an increase to 88.9% followed by a huge drop to just 16.7% during 2002–2006. The strong fluctuations are caused by the small absolute numbers of approved drug products containing salts formed from acidic entities. There were a maximum of nine drugs approved in this category for oral usage during each of the 5-year periods. The second common cation is potassium with an average fraction of 13.3% over the whole period and a peak of 33.3% in 1992–1996. The third important cation for oral dosage forms, which accounted for a total frequency of 12.0% and a peak of 50.0% during the last period from 2002 to 2006, is calcium. Thus, calcium and potassium have changed positions in usage ranking for oral dosage forms in recent times.

A good example of how the counterion affects the physical chemical properties of an API in oral formulations is diclofenac and its salts. There are both sodium and potassium salts of diclofenac applied in drug products for oral delivery. The free acid is not used in FDA-approved drug products. Only the diclofenac sodium salt is utilized for extended and delayed release tablet dosage forms. In contrast, the diclofenac potassium salt is used for immediate release tablets. This suggests that

the different salt forms may influence dissolution rates. Fini et al.²¹ have discussed the difference in dissolution behavior between these salt forms.

Salts Used in Injectable Formulations. The 482 APIs used for injectable formulations consist of 171 (35.5%) nonsalt forms, 208 (43.2%) API salts of basic molecules, and 103 (21.4%) salts of acidic entities, whereas in APIs utilized in oral formulations about half of the APIs were used as nonsalt forms; in injectable formulations only about one-third were employed as noncharged forms. This shows that formation of salts is even more important for injectable dosage forms than for oral formulations. The more frequent usage of salt forms in injectable formulations can be explained by the need for even higher solubility compared to oral formulations. An oral dosage form needs to completely dissolve in 250 mL of aqueous media in the physiological relevant pH range of 1–8 to be classified as highly soluble with reference to the Biopharmaceutical Classification System.²² Typically, the preferred injectable dosage form comprises a volume of a few milliliters. If the solubility of the API is too low for this application, an infusion formulation becomes necessary. In many cases, there is a difference of at least one order of magnitude with respect to the solubility required for the formulation of an API as an injectable versus

Table 6. Distribution of Anions for API Used in Injectable Dosage Forms

	overall (%)	pre-1982 (%)	1982–1986 (%)	1987–1991 (%)	1992–1996 (%)	1997–2001 (%)	2002–2006 (%)
acetate	5.8	2.3	5.0	26.3		14.3	16.7
besylate	1.4	0.8	5.0		5.0		
bromide	4.3	3.9	5.0	5.3	5.0	7.1	
camphorsulfonate	0.5	0.8					
chloride	53.4	54.3	60.0	42.1	55.0	50.0	50.0
chlorthephyllinate	0.5	0.8					
citrate	2.4	1.6	5.0		5.0		16.7
ethandisulfonate	0.5	0.8					
fumarate	0.5				5.0		
gluceptate	0.5	0.8					
gluconate	0.5	0.8					
glucuronate	0.5	-			5.0		
iodide	1.0	1.6					
isethionate	1.0	0.8	5.0				
lactate	2.9	3.1	5.0	5.3			
lactobionate	0.5	0.8					
malate	0.5	0.8					
maleate	1.4	2.3					
mesylate	3.9	3.1				21.4	16.7
nitrate	0.5	0.8					
oleate	0.5			5.3			
pamoate	0.5					7.1	
phosphate	3.4	3.9		5.3	5.0		
succinate	0.5				5.0		
sulfate	8.2	10.9	10.0	5.3			
tartrate	3.9	4.7		5.3	5.0		
tosylate	0.5	0.8					
trifluoroacetate	0.5				5.0		
number of salts	208	129	20	19	20	14	6

Table 7. Distribution of Cations for API Used in Injectable Dosage Forms

	overall (%)	pre-1982 (%)	1982–1986 (%)	1987–1991 (%)	1992–1996 (%)	1997–2001 (%)	2002–2006 (%)
benzathine	1.0	1.6					
calcium	2.9	4.8					
diethanolamin	1.0	1.6					
diethylamin	1.0	1.6					
lysine	1.0						14.3
mequmine	4.9	7.9					
potassium	1.0	1.6					
procaine	1.0	1.6					
sodium	85.4	79.4	100.0	88.9	100.0	100.0	85.7
tromethamine	1.0			11.1			
number of salts	103	63	9	9	8	7	7

an oral dosage form, with higher solubility generally required for APIs used in injectable dosage forms. The increased percentage of APIs employed as salt forms in injectable dosage forms shows that formation of salts is a practical way to achieve this objective. A total of 28 different anions and 10 different cations were used as counterions for formation of salts utilized in FDA-approved injectable formulations. Seventeen anions and only three cations were used over the past 25 years.

Distribution of Anionic Counterions Used in Injectable Formulations. A summary of the frequency of occurrence of all anions used for the formation of salts of basic molecules in injectable formulations is presented in Table 6. As for oral dosage forms, the most important anion is chloride with an average fraction of 53.4%. This incidence has remained quite stable, exhibiting a minimum of 42.1% and a maximum of 60.0%. During the last two periods (1997–2001 and 2002–2006) the fraction was 50.0% each. The second widely used anion is sulfate with a total fraction of 8.2%. However, after 1991 no further sulfate salts have been approved for injectable dosage forms. The third anion in frequency of occurrence ranking is acetate with an average fraction of 5.8% and a peak value of 26.3% during the 1987–1991 period. During the following

period, from 1992 to 1996, there were no further FDA-approved acetate salts. On the other hand, during the last two periods 1997–2001 and 2002–2006 the relative fraction of acetates increased to 14.3% and 16.7%. Frequent usage of mesylates over the past 10 years, with a relative frequency of occurrence of 21.4% during the 1997–2001 period and 16.7% during the 2002–2006 period, is apparent from Table 6. This is in strong contrast to the period from 1982 to 1996 in which no mesylate salts were approved for injectable dosage forms. In contrast to API salts containing anionic counterions intended for oral formulations, a trend toward a broader variety of anions cannot be observed for injectable formulations.

Distribution of Cationic Counterions Used in Injectable Formulations. In category II, 38 of the 40 APIs used in injectable formulations and approved over the past 25 years are sodium salts. Beyond the sodium salts, there is only one tromethamine salt approved in 1989 and one lysine salt approved in 2006. A summary together with the 63 salt forms approved before 1982 is given in Table 7.

Comparison with Analysis of Data from the Cambridge Structural Database. Haynes, Jones, and Motherwell searched the Cambridge Structural Database (CSD) for the

occurrence of salts with pharmaceutically acceptable counterions.²³ It is mentioned that the CSD is a database that is not limited to pharmaceuticals. Rather, it contains many substances used in other industries, such as pigments. The analysis of Haynes et al. was published in 2005, covering a time span of more than 80 years. Haynes et al. received 6021 hits for anions and 587 hits for cations. A hit represents one structure of an organic salt found in the CSD. Because of the fact that the CSD is not a database exclusively comprising APIs, it is difficult to obtain pharmaceutically relevant trends in salt selection from this database.

Haynes et al. searched the CSD for salt forms containing pharmaceutically acceptable counterions. For this search they used 69 different anions and 21 different cations. However, since the authors faced difficulties in determining charges and the bonding type of metal atoms, they were unable to differentiate appropriately between ionic and covalent compounds. This problem forced the authors to omit all compounds containing metal atoms. Because metal cations are the most frequently used cationic counterions in the Orange Book, a comparison of the data between the Orange Book and CSD for cations is not meaningful.

As a consequence, only the results for anionic counterions are compared with the Orange Book data. The comparison of the relative occurrence of anions used as counterions for the formation of salts shows large differences between the CSD and the Orange Book analysis. As one example, bromides used for formation of salts account for a much higher share in the CSD (23.3%) than in the Orange Book (4.6%). In contrast to this observation, the results for chlorides agree quite well: 47.7% in the CSD and 53.4% in the Orange Book. The maleate, mesylate, and sulfate fractions in the CSD are distinctly lower than in the Orange Book: 1.3% (CSD) versus 4.2% (Orange Book) for maleates, 1.1% (CSD) versus 4.2% (Orange Book) for mesylates, and 2.7% (CSD) versus 7.5% (Orange Book) for sulfates.

The ratio of salts formed with anionic counterions to salts formed with cationic counterions in the CSD analysis is about 10 to 1. The respective ratio obtained from the Orange Book is roughly 3 to 1. This reflects the large fraction of compounds left out by neglecting substances containing metal cations in the CSD analysis. Nonsalt forms of API were not considered in the CSD analysis.

The CSD analysis for cationic counterions loses pharmaceutical relevance by using a database that includes non-API substances and leaves out metal cations as counterions. Surprisingly, the analysis for anionic counterions gives the right order of magnitude for most anions. Nevertheless, examples such as the bromide salts show that the CSD results are not sufficiently reliable. In conclusion, analysis of a very general database like the CSD cannot be expected to and does not yield results relevant in a pharmaceutical environment.

Comparison with Analysis of Data from Martindale. Berge, Bighley, and Monkhouse published a review article about pharmaceutical salts in 1977.¹ In this article, the distribution of counterions at that time was presented. Their list was based on Martindale's "The Extra Pharmacopoeia", 26th edition, from 1974. The authors listed 80 different anions and 21 different cations used as counterions for formation of pharmaceutical salts. At that time, 53 anions and 14 cations were classified as FDA-approved. The distribution of counterions obtained in this analysis is comparable to the average values from the Orange Book compilation obtained 30 years later. This can be derived from the data summarized in Table 8. The good agreement is

Table 8. Comparison of Orange Book (2006) Data with Data from Berge, Monkhouse, and Bighley (1993 and 1974)

counterion	Martindale, 1974 (%)	Martindale, 1993 (%)	Orange Book, 2006 (%)
bromide	7.6	5.7	4.6
chloride	47.7	48.9	53.4
maleate	3.0	3.1	4.2
mesylate	2.0	3.2	4.2
sulfate	7.8	6.1	7.5
calcium	10.5	12.2	6.9
potassium	10.8	9.8	6.3
sodium	62.0	57.7	75.3

not surprising because the trend toward a broader variety of counterions first started to have a notable impact on distributions around the mid-1990s. Because of the large number of APIs approved before that point in time, the average distribution is still dominated by drug products approved earlier.

There is a second publication by the same authors on this topic.³ This analysis is based on Martindale's "The Extra Pharmacopoeia", 30th edition, from 1993. It lists 112 different anions and 38 cations. Some of the counterions have not been newly introduced for formation of API salts but simply listed with the respective trivial names. This leads to multiple references of the same counterion. Another circumstance leading to the increased variety of anionic and cationic counterions at this time is the fact that quite a lot of counterions were used in only one case. Although the database changed considerably from 1974 to 1993, results are still in quite good agreement for the most important counterions and compare well with the data from our Orange Book analysis. Some examples of important counterions are given in Table 8.

One must keep in mind that the Orange Book only contains drug products approved in the U.S. In contrast, the "Martindale Extra Pharmacopoeia" contains drug products from all over the world. A further reason for differences between both databases is the way salt forms and formulations are counted, e.g., if salt forms used in drug products containing more than one API are considered as separate use of the counterion.

Conclusions

This contribution proves that there is a trend away from using a small selection of counterions for formation of pharmaceutical salts toward a much broader variety of ions. This trend started in the 1990s and has accelerated significantly during recent years. The separate analysis for APIs used in oral and injectable dosage forms confirms that trends in the choice of counterions depend on the route of administration.

The comparison with data from other databases indicates the importance of the choice of the data source. Only pharmaceutical databases will give pharmaceutically relevant results reflecting the specific needs for development of new drugs. The data from the Orange Book agrees well with data from older pharmaceutical sources; this is exemplified by comparison with data from Martindale's "The Extra Pharmacopoeia". Finally, it is speculated that the trend toward more diversity in pharmaceutical salts will be even more pronounced in the near future, as increasingly challenging molecules are selected for predevelopment and clinical development.

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References

- (1) Berge, S. M.; Bighley, L. D.; Monkhouse, D. C. Pharmaceutical salts. *J. Pharm. Sci.* **1977**, *66* (1), 1–19.

- (2) Gould, P. L. Salt selection for basic drugs. *Int. J. Pharm.* **1986**, *33*, 201–217.
- (3) Bighley, L. D.; Berge, S. M.; Monkhouse, D. C. Salt Forms of Drugs and Absorption. In *Encyclopaedia of Pharmaceutical Technology*; Swarbrick, J., Boylan, J. C., Eds.; Marcel Dekker, Inc.: New York, 1996; Vol. 13, pp 453–499.
- (4) Stahl, P. H.; Wermuth, C. G., Eds. *Handbook of Pharmaceutical Salts: Properties, Selection and Use*; VCH: Zurich, Switzerland, and Weinheim, Germany, 2002.
- (5) Badawy, S. I. F. Effect of salt form on chemical stability of an ester prodrug of a glycoprotein IIb/IIIa receptor antagonist in solid dosage forms. *Int. J. Pharm.* **2001**, *223*, 81–87.
- (6) Agharkar, S.; Lindenbaum, S.; Higuchi, T. Enhancement of solubility of drug salts by hydrophilic counterions: properties of organic salts of an antimalarial drug. *J. Pharm. Sci.* **1976**, *65* (5), 747–749.
- (7) Li, S.; Wong, S. M.; Sethia, S.; Almoazen, H.; Joshi, Y. M.; Serajuddin, A. T. M. Investigation of solubility and dissolution of a free base and two different salt forms as a function of pH. *Pharm. Res.* **2005**, *22* (4), 628–635.
- (8) O'Connor, K. M.; Corrigan, O. I. Comparison of the physicochemical properties of the *N*-(2-hydroxyethyl)pyrrolidine, diethylamine and sodium salt forms of diclofenac. *Int. J. Pharm.* **2001**, *222*, 281–293.
- (9) Lin, S.-L.; Swartz, C. J.; Huebner, C. F. Preformulation investigation I: relation of salt forms and biological activity of an experimental antihypertensive. *J. Pharm. Sci.* **1972**, *61* (9), 1418–1422.
- (10) Gwak, H.-S.; Choi, J.-S.; Choi, H.-K. Enhanced bioavailability of piroxicam via salt formation with ethanolamides. *Int. J. Pharm.* **2005**, *297*, 156–161.
- (11) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235–249.
- (12) Rasenack, N.; Mueller, B. W. Poorly water-soluble drugs for oral delivery: a challenge for pharmaceutical development. Part I. *Pharm. Ind.* **2005**, *67* (3), 323–326.
- (13) Rasenack, N.; Mueller, B. W. Poorly water-soluble drugs for oral delivery: a challenge for pharmaceutical development. Part II. *Pharm. Ind.* **2005**, *67* (4), 447–451.
- (14) Rasenack, N.; Mueller, B. W. Poorly water-soluble drugs for oral delivery: a challenge for pharmaceutical development. Part III. *Pharm. Ind.* **2005**, *67* (5), 583–591.
- (15) Vishweshwar, P.; McMahon, J. A.; Bis, J. A.; Zaworotko, M. J. Pharmaceutical co-crystals. *J. Pharm. Sci.* **2006**, *95* (3), 499–516.
- (16) Morris, K. R.; Fakes, M. G.; Thakur, A. B.; Newman, A. W.; Singh, A. K.; Venit, J. J.; Spagnuolo, C. J.; Serajuddin, A. T. M. An integrated approach to the selection of optimal salt form for a new drug candidate. *Int. J. Pharm.* **1994**, *105*, 209–217.
- (17) Bastin, R. J.; Bowker, M. J.; Slater, B. J. Salt selection and optimisation procedures for pharmaceutical new chemical entities. *Org. Process Res. Dev.* **2000**, *4* (5), 435–427.
- (18) Engel, G. L.; Farid, N. A.; Faul, M. M.; Richardson, L. A.; Winneroski, L. L. Salt form selection and characterization of LY333531 mesylate monohydrate. *Int. J. Pharm.* **2000**, *198*, 239–247.
- (19) Balbach, S.; Korn, C. Pharmaceutical evaluation of early development candidates “the 100mg-approach”. *Int. J. Pharm.* **2004**, *275*, 1–12.
- (20) U.S. Food and Drug Administration Orange Book. <http://www.fda.gov/cder/ob/>.
- (21) Fini, A.; Fazio, G.; Hervás, M.-J. F.; Holgado, M. A.; Rabasco, A. M. Factors governing the dissolution of diclofenac salts. *Eur. J. Pharm. Sci.* **1996**, *4*, 231–238.
- (22) Amidon, G. L.; Lennernäs, H.; Shah, V. P.; Crison, J. R. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.* **1995**, *12* (3), 413–420.
- (23) Haynes, D. A.; Jones, W.; Motherwell, W. D. S. Occurrence of pharmaceutically acceptable anions and cations in the Cambridge Structural Database. *J. Pharm. Sci.* **2005**, *94* (10), 2111–2120.

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Determination of 19 antiretroviral agents in pharmaceuticals or suspected products with two methods using high-performance liquid chromatography

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Abstract

Three classes of antiretroviral agents are usually available for the treatment of HIV infection: nucleoside reverse transcriptase inhibitors (IN), non-nucleoside reverse transcriptase inhibitors (INN) and protease inhibitors (IP). Two methods by reversed-phase liquid chromatography were developed for the analysis of 19 antiretroviral molecules belonging to these three therapeutic classes and used in medicinal products. Both of these HPLC techniques use a C18 column and UV detection. The first method is for IN family analysis and allows eight molecules to be separated: zalcitabine, lamivudine, amdoxovir, emtricitabine, didanosine, stavudine, zidovudine and abacavir. The second method is for INN and IP family analysis and allows 11 molecules to be separated: fosamprenavir, nevirapine, indinavir, amprenavir, saquinavir, atazanavir, ritonavir, lopinavir, efavirenz, nelfinavir and tipranavir. The combination of these two methods makes possible the quality control of mono-, bi- or tri-therapy pharmaceutical products and the detection of illegal products sold particularly in developing countries.

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Keywords: HIV; Antiretroviral agent; Nucleoside reverse transcriptase inhibitors; Non-nucleoside reverse transcriptase inhibitors; Protease inhibitors; HPLC–UV; Illegal products

1. Introduction

Availability of good quality medicines is of major importance both in developed and developing countries. Counterfeit of essential medicines, such as antibiotics, antimalarials and antiretrovirals, is a public health danger that can lead to life threatening situations [1,3]. In such a context and in order to make available screening methods for suspected products, a methodology has been developed to assess the quality of most antiretroviral medicines available in 2006 for HIV treatment.

In order to check medicine quality and, in particular, generic specialities distributed in developing countries, the aim of our laboratory was to develop a protocol for the identification and quantification of the most antiretroviral molecules available in the international market. These molecules belong mainly to three therapeutic families [2]: nucleoside/nucleotide reverse transcriptase inhibitors (IN), non-nucleoside reverse transcrip-

tase inhibitors (INN) and protease inhibitors (IP). Researching in scientific literature [4–15] and compiling a library of antiviral molecules led us to develop and validate two complementary HPLC methods (on C18 column with solvent gradient and UV detection). The first method allowed eight IN family molecules to be separated: zalcitabine, lamivudine, amdoxovir, emtricitabine, didanosine, stavudine, zidovudine and abacavir. The second method allowed 11 molecules belonging to the INN and IP families to be separated: fosamprenavir, nevirapine, indinavir, amprenavir, saquinavir, atazanavir, ritonavir, lopinavir, efavirenz, nelfinavir and tipranavir. For active ingredient verification and quantification, these methods were designed to be as easy as possible to work.

2. Experimental

2.1. Reagents

Standards and compounds were kindly obtained from the respective pharmaceutical companies: atazanavir sulfate, didanosine, efavirenz and stavudine from Bristol Myers Squibb

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(Epernon, France); zidovudine, lamivudine, abacavir sulfate, amprenavir and fosamprenavir calcic from GlaxoSmithKline (Greenford, UK); amdoxovir, emtricitabine and tenofovir disoproxil fumarate from Gilead (Paris, France); enfuvirtide, nelfinavir mesylate, saquinavir mesylate and zalcitabine from Roche (Neuilly-sur-seine and Fontenay-sous-bois, France); indinavir sulfate from Merck Charp & Dohme–Chibret (Clermont-Ferrand, France), lopinavir and ritonavir from Abbott (Saint-Rémy-sur-Avre, France); nevirapine anhydride, nevirapine hemihydrate and tipranavir from Boeringer Ingelheim (Paris, France).

Acetonitrile (Carlo Erba, France) and methanol (BDH, France) were HPLC grade. Ammonium acetate, potassium dihydrogenphosphate (KH_2PO_4), acetic acid 100% (Prolabo, France), potassium hydroxide 37% (Carlo Erba, France) were analytical-reagent grade. Water was ultra pure HPLC grade (Milli-Q, Millipore).

2.2. Instrumentation and chromatographic conditions

The HPLC system consisted of a Waters model composed of a 600 quaternary pump, a 996 diode array detector and a Wisp 717+ autosampler injector operated with the Millenium 32 software.

The first HPLC method enabled IN molecules identification and assay. The separation was performed using an YMC-pack ODS-AM, 250×4.6 mm, $5 \mu\text{m}$ analytical column (Interchim, France). The mobile phase was composed of an ammonium acetate buffer (25 mM adjusted to pH 4.0 with acetic acid 100%) filtered through a $0.45 \mu\text{m}$ polypropylene membrane (GHP, Pall Gelman) and ultrasonically degassed for 15 min. A gradient with ammonium acetate buffer and methanol was programmed. The proportion of methanol stayed at 5% for 5 min, rose to 30% in 25 min and stayed at 30% for 15 min before falling back down to 5% in 5 min. The system was then equilibrated for 10 min under initial conditions. The mobile phase was delivered

at a flow rate of 1 ml/min. The sample injection volume was $10 \mu\text{l}$.

The second HPLC method enabled INN and IP molecules identification and assay. The separation was performed using a Symmetry C18 250×4.6 mm, $5 \mu\text{m}$ column (Waters, France). The mobile phase was composed of potassium phosphate buffer (50 mM adjusted to pH 5.65 with potassium hydroxide 100%) filtered through a $0.45 \mu\text{m}$ polypropylene membrane (GHP, Pall Gelman) and ultrasonically degassed for 15 min. A gradient with the potassium phosphate buffer and acetonitrile was programmed. The proportion of acetonitrile stayed at 40% for 5 min, rose to 60% in 35 min and stayed at 60% for 5 min before falling back down to 40% in 1 min. The system was then equilibrated for 4 min under initial conditions. The mobile phase was delivered at a flow rate of 1.5 ml/min. The sample injection volume was $20 \mu\text{l}$. A summary of the two methods is reported in Table 1.

2.3. Calibration curves preparation

Substance solubilities were determined. Standards for IN family molecules were dissolved in water. Standards for INN and IP family molecules were dissolved in methanol, except for atazanavir sulphate which is water soluble and fosamprenavir calcic which is soluble in acidified water. Enfuvirtide, belonging to a fourth family (entry inhibitors), was used to study possible interferences. This molecule is soluble in dimethylformamide.

Stock solutions of IN family molecules were prepared in water at a 0.5 mg/ml concentration. A calibration range was carried out in a dilution solvent (methanol 5%–water 95%) in order to obtain concentrations of 10, 25, 50, 75 and $100 \mu\text{g/ml}$ for each compound. Stock solutions of INN and IP family molecules were prepared in methanol at the concentration of 0.5 mg/ml. A calibration range was carried out in order to obtain concentrations

Table 1
Summary of HPLC methods

	Method 1			Method 2		
Column	YMC pack ODS-AM, 250×4.6 mm, $5 \mu\text{m}$			Symmetry C18 250×4.6 , $5 \mu\text{m}$		
Mobile phase A	Ammonium acetate buffer 0.025 M pH 4.0			Potassium phosphate buffer 50 mM pH 5.65		
Mobile phase B	Methanol			Acetonitrile		
Gradient	Time (min)	%A	%B	Time (min)	%A	%B
	0	95	5	0	60	40
	5	95	5	5	60	40
	30	70	30	40	40	60
	45	70	30	45	40	60
	50	95	5	46	60	40
	60	95	5	50	60	40
Flow rate	1.0 ml/min			1.5 ml/min		
UV detection	$\lambda = 270$ nm			$\lambda = 260$ nm		
Injection volume	$10 \mu\text{l}$			$20 \mu\text{l}$		
Column temperature	Ambient			30°C		
Run time	60 min			50 min		
Dissolve solvent	Milli-Q water			Methanol		
Dilutents	Milli-Q water 95%–methanol 5%			Initial mobile phase		

of 10, 25, 50, 75 and 100 µg/ml for each molecule. The initial mobile phase (acetonitrile 40%–ammonium acetate buffer 60%) was used as dilution solvent.

2.4. Sample preparation

The sample weight (equivalent to two or four tablets or capsules accurately weighed) was placed directly into a graduated flask with a little water, and it underwent magnetic stirring for 30 min. The preparation was then diluted to volume with water for medicine containing IN family molecules or diluted with methanol for medicine containing INN and IP family molecules. The solution was centrifuged for 15 min at 4000 rotation/min. Supernatant dilutions were then performed with the methanol 5%–water 95% mixture for the first family and with the mobile phase (acetonitrile 40%–ammonium acetate buffer 60%) for the two other families in order to obtain approximately 50 µg/ml sample solutions. Solutions were filtered by acrodisk GHP 0.45 µm (Pall Gelman) before injection. It should be noted that a test has shown that acrodisk filtered solutions did not modify studied peak areas.

3. Results

3.1. Specificity and selectivity

UV spectra of IN family molecules differ from each other and are compound specific, except for zalcitabine and lamivudine, and stavudine and zidovudine for which spectra are similar. Maximum absorption ranges between 250 and 290 nm. A 270 nm wavelength was chosen for the analysis, being appropriate for whole molecules determination in the selected concentration range. UV spectra of INN and IP family molecules also differ from each other and are compound specific, except for fosamprenavir and amprenavir for which spectra are similar. Maximum absorption ranges between 240 and 280 nm. A 260 nm wavelength was chosen for the analysis, being appropriate for whole molecules determination in the selected concentrations range. The use of a photodiode array detector allowed, on one hand, antiretroviral molecule identification by comparison with the reference spectrum and on the other hand, the detection of other non-antiretroviral molecules added to the speciality in case of falsification.

There is no interference of IN family molecules during the analysis of INN and IP family molecules, and vice versa. The study was supplemented by injecting three additional compounds which did not cause any interference: enfuvirtide (entry inhibitor antiretroviral), hypoxanthine (degradation product of didanosine) and aciclovir (antiviral). Analysing over 110 medicinal products enabled the verification of the absence of interference of excipients commonly used in tablet and capsule formulation. Symmetry factor and resolution were calculated for each peak in both chromatographic systems studied, in accordance to the recommendations of the European Pharmacopeia 5th edition. Results are reported in Tables 2 and 3. The symmetry factor always complied with the recommendations of the European Pharmacopeia (between 0.8 and 1.5) and the

Table 2

Chromatographic parameters of method 1 for IN family

Method 1 for IN			
Name	t _R (min)	Symmetry	Resolution
Zalcitabine	14.1	1.0	–
Lamivudine	18.2	1.0	12.7
Amdoxovir	20.1	1.1	6.9
Emtricitabine	23.4	1.1	12.3
Didanosine	24.0	1.1	2.3
Stavudine	24.4	1.0	1.5
Zidovudine	37.3	1.0	44.9
Abacavir	44.2	1.1	18.4

resolution was always higher than 1.5 (minimal resolution for a complete separation).

3.2. Concentration range

The study of method linearity was carried out on 10–25–50–75 and 100 µg/ml standard solutions, and the study of reliability was carried out on a 50 µg/ml standard solution (Figs. 1–4).

3.3. Linearity and precision

Validation was done according to recommendations [16,17] and using AVA software (3rd version). The statistical study applied to the eight IN compounds (Table 4) led to the following conclusions on method n°1:

- variances in linearity and precision were homogeneous for the three series of injections (carried out on three different days),
- the correlation coefficient of the linear calibration curve was always greater than 0.996,
- the linearity of the calibration curve was shown (statistical tests of variance homogeneity, comparison of y-intercept with 0, existence of a significant slope and validity of the linear curve are in compliance),
- the relative standard deviation of injections, repeatability and intermediate precision were always less than 1.2% (except for Didanosine, a fragile molecule in solution which presented an intermediate precision RSD of 5.7%).

Table 3

Chromatographic parameters of method 2 for INN and IP families

Method 2 for INN and IP			
Name	t _R (min)	Symmetry	Resolution
Fosamprenavir	2.3	1.2	–
Nevirapine	2.7	1.3	2.8
Indinavir	6.7	1.2	18.1
Amprenavir	11.5	1.2	14.0
Saquinavir	19.8	1.1	20.6
Atazanavir	20.7	1.1	2.2
Ritonavir	23.0	1.1	5.3
Lopinavir	25.6	1.1	5.9
Efavirenz	27.2	1.1	3.5
Nelfinavir	34.2	1.0	13.6
Tipranavir	39.5	1.1	8.2

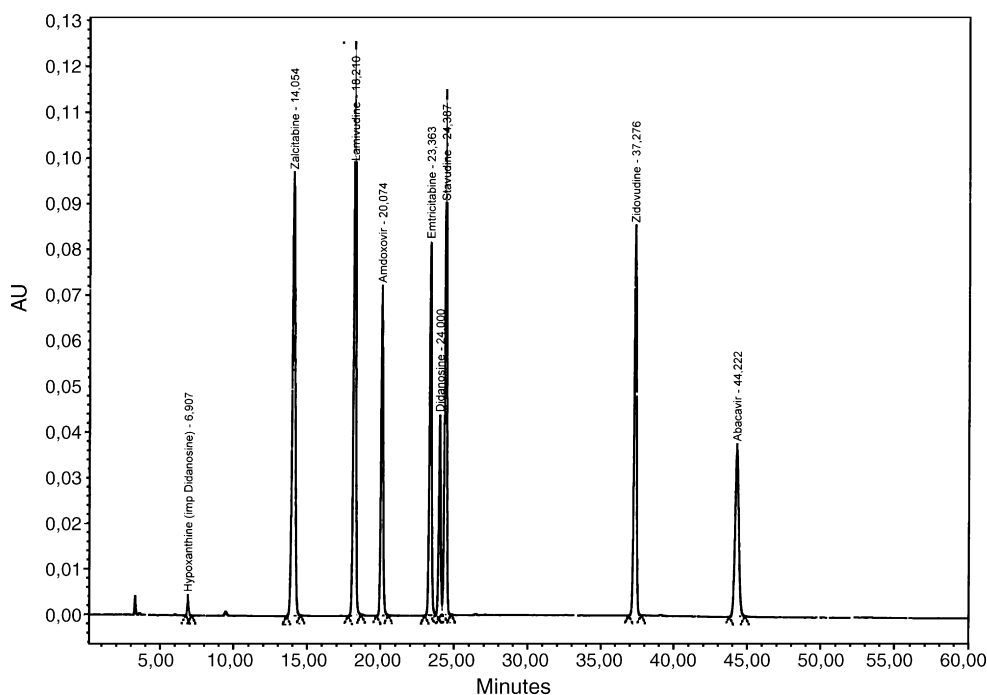


Fig. 1. Method 1 chromatogram (IN family).

The statistical study applied to the 11 INN and IP compounds (Table 5) allowed the following conclusions to be drawn on method n°2:

- variances in linearity and precision were homogeneous for the three series of injections (carried out on three different days),
- the correlation coefficient of the linear calibration curve was always greater than 0.999,
- the linearity of the calibration curve was shown (statistical tests of variance homogeneity, comparison of y-intercept with 0, existence of a significant slope and validity of the linear curve are in compliance),
- the relative standard deviation of injections, repeatability and intermediate precision were always less than 1.0% (except for Lopinavir which presented a RSD of 2% because of low absorbance at maximum absorption $\lambda = 260$ nm).

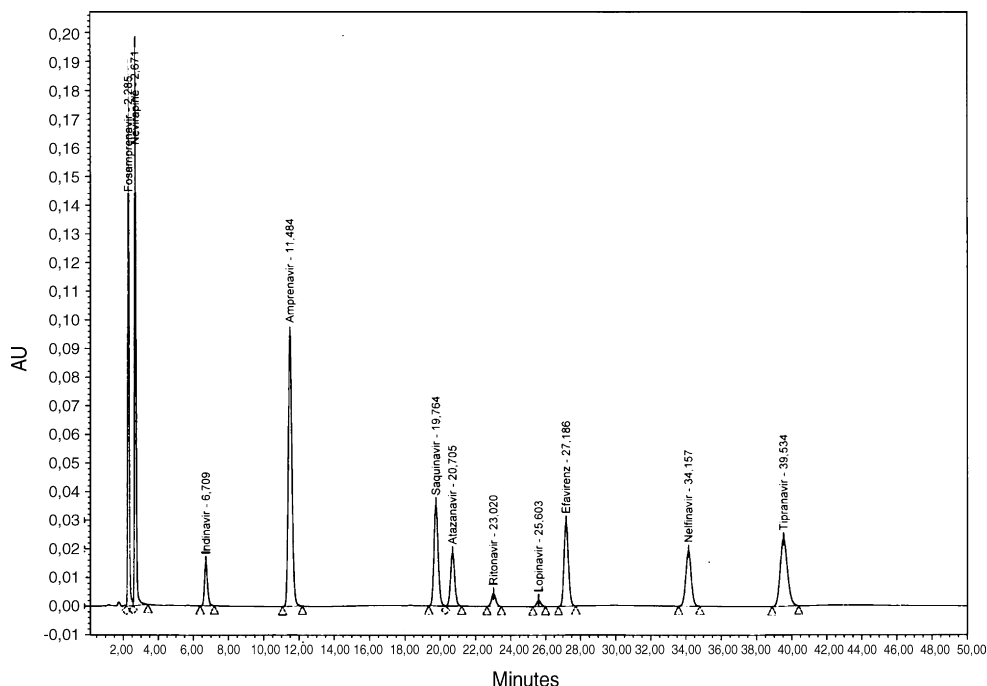


Fig. 2. Method 2 chromatogram (INN and IP families).

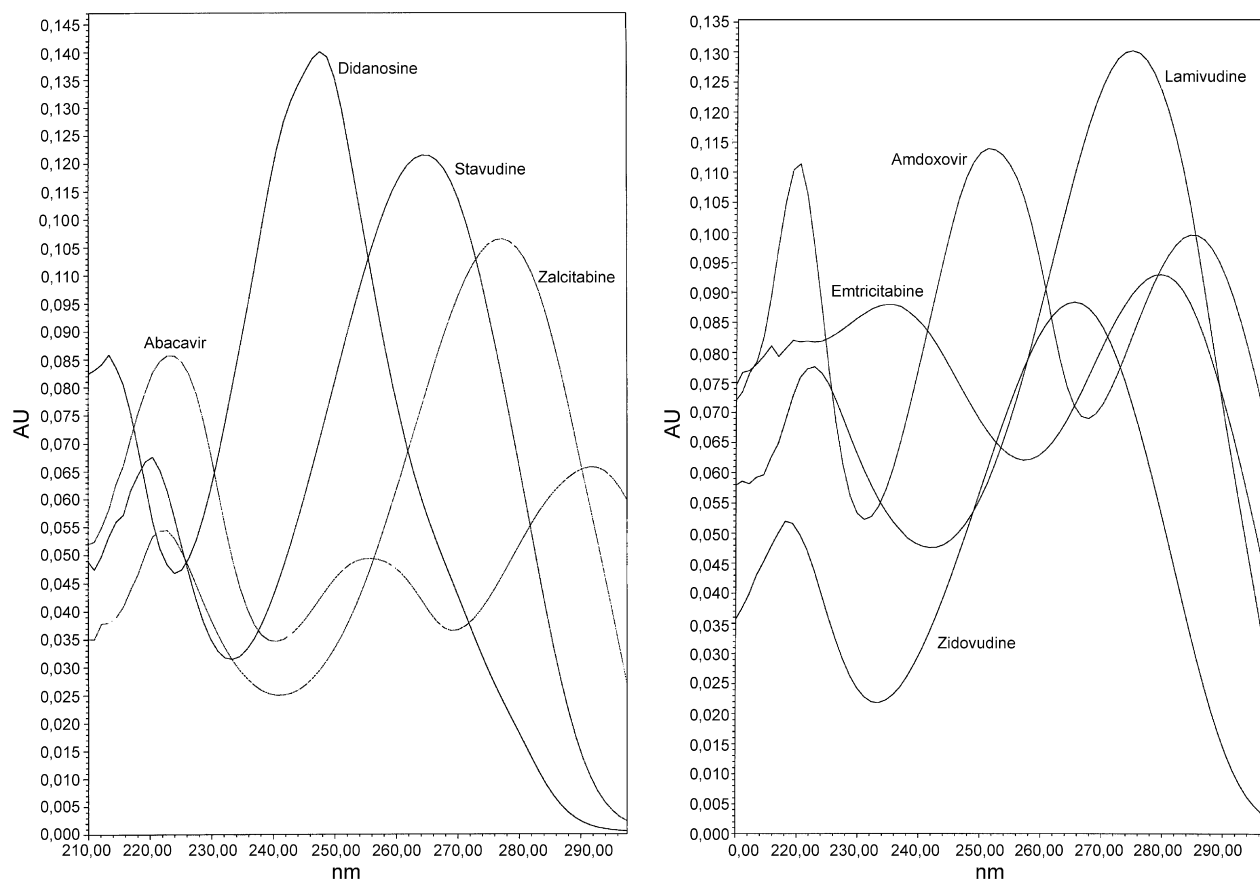


Fig. 3. UV spectra of IN family molecules.

3.4. Sample analysis

Several medicinal products (110) taken from the African and Asian markets were tested. Among these products, different forms were available, such as tablets, hard and soft capsules and oral suspensions. These were presented as mono-therapy (61), bi-therapy (24) and tri-therapy (25) products. The analysis involved determining the declared active ingredient content. Both HPLC methods made possible the verification of the absence of interference by excipients commonly used in tablet and capsule formulation. The developed

methods were thus specific for ARV and excipients of tested products.

3.5. Solution stability

A stability study was specifically performed on didanosine. The molecule degrades by 30% in 6 h in acidic solutions (mobile phase) giving hypoxanthine as impurity. However, didanosine remains stable in aqueous solutions and in the solvent mixture (water 95%–methanol 5%). For this reason, all method 1 standard solutions were prepared in solvent

Table 4
Statistical study of method 1 for IN family

	Theoretical value	Results	Conclusion
LINEARITY			
Slope	/	6824 to 26806	
Y-intercept	/	–8936 to 8210	
Correlation coefficient	/	0.996 to 1.000	
Variances homogeneity (Cochran)	$C(0.05; 6; 2) = 0.6161$	0.5664 to 0.6085	Non significant
Y-intercept comparison with 0 (Student)	$t(0.05; 16) = 2.1199$	0.4515 to 1.1239	Non significant
Significant slope existence (Fisher)	$F(0.001; 1; 16) = 16.1202$	1848.9 to 16455.3	Highly significant
Validity of calibration curve (Fisher)	$F(0.05; 4; 12) = 3.2592$	0.1299 to 0.4214	Non significant
PRECISION			
Variances homogeneity (Cochran)	$C(0.05; 3; 4) = 0.7457$	0.4373 to 0.6114	Non significant
Repeatability RSD	/	0.41% to 0.84%	
Intermediate precision RSD	/	0.72% to 1.17% except didanosine = 5.7%	

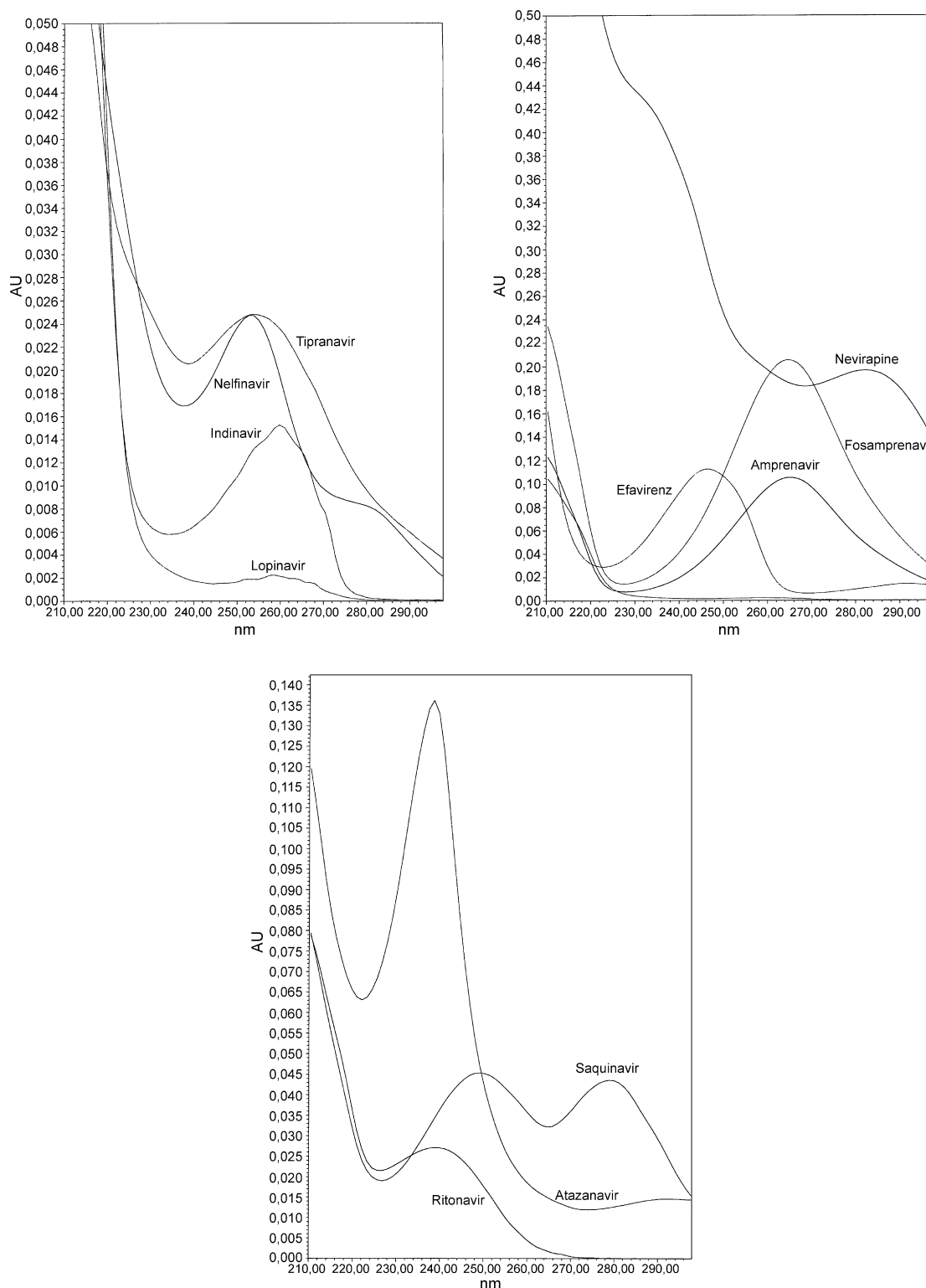


Fig. 4. UV spectra of INN and IP family molecules.

mixture (water 95%–methanol 5%). Didanosine, a fragile molecule in solution and unstable in acidic medium, must be injected within 4 h after being set in the water solution and diluted in the solvent mixture (water 95%–methanol 5%). Stability of others molecules in solution was shown over at least 48 h.

4. Discussion

The two developed HPLC methods allowed separation, identification and quantification of 19 antiretroviral agents. HPLC columns were reversed phase, and detection was done in the ultraviolet region at 270 nm and 260 nm. Mobile phases were

Table 5
Statistical study of method 2 for INN and IP families

	Theoretical value	Results	Conclusion
LINEARITY			
Slope	/	726 to 27576	
Y-intercept	/	−5141 to −518	
Correlation coefficient	/	0.999 to 1.000	
Variances homogeneity (Cochran)	$C(0.05; 6; 2) = 0.6161$	0.4193 to 0.5324	Non significant
Y-intercept comparison with 0 (Student)	$t(0.05; 16) = 2.119905$	0.1829 to 1.8682	Non significant
Significant slope existence (Fisher)	$F(0.001; 1; 16) = 16.1202$	8271.4 to 106203.5	Highly significant
Validity of calibration curve (Fisher)	$F(0.05; 4; 12) = 3.2592$	0.0166 to 1.7110	Non significant
PRECISION			
Variances homogeneity (Cochran)	$C(0.05; 3; 6) = 0.4160$	0.4039 to 0.6706	Non significant
Repeatability RSD	/	0.30 to 0.79% except lopinavir = 1.97%	
Intermediate precision RSD	/	0.32% to 0.85% except lopinavir = 1.97%	

binary with a buffer gradient (pH 4.0 for method 1 and pH 5.65 for method 2) and an organic solvent (methanol for method 1 and acetonitrile for method 2). Several columns were tested (YMC-pack ODS-AQ, Stability RP18, YMC-pack ODS-AM and Symmetry C18) and gave chromatographic profiles similar to those obtained with the columns used for the validations. It was possible to transpose method 1 on a LC/MS system in order to obtain additional structural information [12,13]. Method 2 transposition was not initiated yet.

A UV mono wavelength detector can be used. The choice of detection wavelengths was made after studying absorption spectra of all the molecules. These maxima ranged between 240 nm and 290 nm; 270 nm was chosen for method 1 and 260 nm for method 2. Lopinavir had a maximum absorption at 260 nm, but its low signal brought coefficients of variation of repeatability and intermediate reliability slightly greater than the other compounds but always less than 2%. To confirm wavelength choice, the determination of several selected medicinal products was carried out with the presented method's wavelength and with that of the maximum of absorption of the molecule to be proportioned. These tests showed exactly the same results.

The solubility of all compounds was studied during the preparation of standards and sample solutions. The dead volume (1.5 min) of each method was determined by the injection of a non retained compound. The 19 studied molecules were suitably chromatographed. A compound from the IN family was withdrawn from the study: tenofovir disoproxil fumarate [6]. This compound appeared in dead volume with method 1. However, under our experimental conditions, the peak shape was symmetrical and did not show interference. Its UV spectrum was determined and its purity was calculated by the software. The validation of the method for this molecule's analysis was not scientifically possible, but tenofovir could be identified in a product if no other compound is detected in the dead volume.

The solutions were stable at 48 h minimum in their dilution solvent. Only didanosine presented rapid degradation in an acidic medium. It was, therefore, necessary to dissolve this compound in water, dilute it in the water 95%–methanol 5% mixture and then inject it within 4 h after preparing the sample.

The analysis lasted 60 min for method 1 and 50 min for method 2, but the separation of all the compounds was carried out in 45 min. Compounds were correctly separated and peak resolution was always greater or equal to 1.5. A series of three IN molecules was eluted in less than 1 min (between 23.4 and 24.4 min). The peak resolution was however correct because the resolution of the two nearest compound (didanosine and stavudine) was sufficient (according to the European pharmacopeia 5th edition). Moreover, the association of these two molecules does not exist in any commercial trade.

Method validation was based on a statistical study of linearity and precision. The calibration range selected was rather broad, from 10 µg/ml to 100 µg/ml, making possible in one single injection the analysis of products containing several active ingredients having wide variations in their quantities (example: lamivudine 150 mg, stavudine 30 mg). Method precision was checked with the average concentration of 50 µg/ml.

Analysis of 110 medicinal products issued from the African and Asian market allowed the specificity of the method to be checked. The absence of interference with the excipients of the analyzed formulations was emphasized, and the purity of the chromatographic peaks was shown. The controlled drugs were in the form of hard and soft capsules and tablets. These two methods demonstrated a tri-therapy medicinal product falsification: presence of the first ARV molecule, substitution of the second ARV molecule by another ARV compound and replacement of the third ARV compound by an inactive molecule. An analysis by LC/MS was able to determine the nature of the falsified molecule.

5. Conclusion

A research in scientific literature shows that several studies exist concerning antiretroviral compounds in biological samples. Some publications describe a simultaneous determination of several molecules [4–13]. For drug control, several HPLC techniques exist and allow the determination of one or two molecules. The emergence of antiretroviral generic medicinal products and the search for counterfeits, falsifications or fake

products in this field led to the need to develop new comprehensive methods.

Two complementary HPLC methods were developed for this purpose: The first studied 8 antiretroviral molecules of the IN class and the second studied 11 antiretroviral molecules of INN and IP classes. These two HPLC methods enabled the separation, identification and quantification of 19 molecules among the 26 compounds which are currently indexed. Of the seven remaining molecules, five are under development [2], one is not analyzable with any of the two methods (Tenofovir), three were not obtained (elvucitabine, delavirdine and capravirine) and three were not indexed at the beginning of the study (alovudine, etravirine and calanolide). Nevertheless, analysing 19 molecules made it possible to cover the majority of the antiretroviral substances commonly found in the international market.

Concentrations of these molecules used in medicinal products range from 30 mg to 600 mg per tablet or capsule. Analysing more than 110 products made it possible to check the absence of interference by excipients used for the formulation. The linearity established between 10 µg/ml and 100 µg/ml was checked for all the molecules as well as the analysis precision. The use of a photodiode array detector allowed an identification of the chromatographic peaks by their UV spectrum and the verification of their purity. Creating a library of raw material antiretroviral molecules in the laboratory provided a rapid answer to the whole of these qualitative and quantitative analyses.

References

- [1] P. Aldhous, *Nature* 434 (2005) 132.
- [2] Alphabetical list of drugs for HIV, www.aidsmeds.com/List.htm (consulted the 20/01/2006).
- [3] A.K. Deisingh, *Analyst* 130 (2005) 271.
- [4] K. Titier, F. Lagrange, F. Pehourcq, L. Edno-Mcheik, N. Moore, M. Molinard, *Thérapie* 57 (2002) 169.
- [5] G. Aymard, M. Legrand, N. Trichereau, B. Diquet, *J. Chromatogr. B* 744 (2000) 227.
- [6] V. Julien, J.M. Treluyer, G. Pons, E. Rey, *J. Chromatogr. B* 785 (2003) 377.
- [7] M. Sarasa-Nacenta, Y. Lopez-Pua, J. Mallolas, J.L. Blanco, J.M. Gatell, J. Carné, *J. Chromatogr. B* 757 (2001) 325.
- [8] V. Proust, K. Toth, A. Hulin, A.M. Taburet, F. Gimenez, E. Singlas, *J. Chromatogr. B* 742 (2000) 453.
- [9] C. Marzolini, A. Telenti, T. Buclin, J. Biollaz, L.A. Decosterd, *J. Chromatogr. B* 740 (2000) 43.
- [10] P.W. Hugen, C.P. Verweij van Wissen, D.M. Burger, E.W. Wuis, P.P. Koopmans, Y.A. Hekster, *J. Chromatogr. B* 727 (1999) 139.
- [11] R.P. Van Heeswijk, R.M. Hoetelmans, R. Harms, P.L. Meenhorst, J.W. Mulder, J.M. Lange, J.H. Beijnen, *J. Chromatogr. B* 719 (1998) 159.
- [12] K.M. Rentsh, *J. Chromatogr. B* 788 (2003) 339.
- [13] K.M. Cormmentuyn, H. Rosing, M.J.X. Hillebrand, A.D.R. Huitena, J.H. Beijnen, *J. Chromatogr. B* 804 (2004) 359.
- [14] M.L. Foisy, J.P. Sommadossi, *J. Chromatogr. B* 721 (1999) 239.
- [15] S. Notari, A. Bocedi, G. Ippolito, P. Narciso, L.P. Pucillo, G. Tossini, R.P. Donnorso, F. Gasparrini, P. Ascenzi, *J. Chromatogr. B* 831 (2006) 258.
- [16] Official J. Eur. Commun., L221 (2002) 8.
- [17] SFSTP commission, Validation of quantitative analytical procedure, harmonization of approaches, STP pharma pratiques, 13 (2003) 1.



US 20140187773A1

(19) **United States**(12) **Patent Application Publication**
Liu et al.(10) **Pub. No.: US 2014/0187773 A1**(43) **Pub. Date: Jul. 3, 2014**(54) **TENOFOVIR ALAFENAMIDE**
HEMIFUMARATE

(60) Provisional application No. 61/524,224, filed on Aug. 16, 2011.

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Chiu Yu, San Francisco, CA (US)(51) **Int. Cl.**
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CPC **C07F 9/65616** (2013.01)
USPC **544/244**(73) Assignee: **GILEAD SCIENCES, INC.**, Foster
City, CA (US)(21) Appl. No.: **14/197,873**(57) **ABSTRACT**(22) Filed: **Mar. 5, 2014****Related U.S. Application Data**(63) Continuation of application No. 13/586,358, filed on
Aug. 15, 2012.

A hemifumarate form of 9-[(R)-2-[[[(S)-1-(isopropoxy-carbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine (tenofovir alafenamide), and antiviral therapy using tenofovir alafenamide hemifumarate (e.g., anti-HIV and anti-HBV therapies).

Figure 1

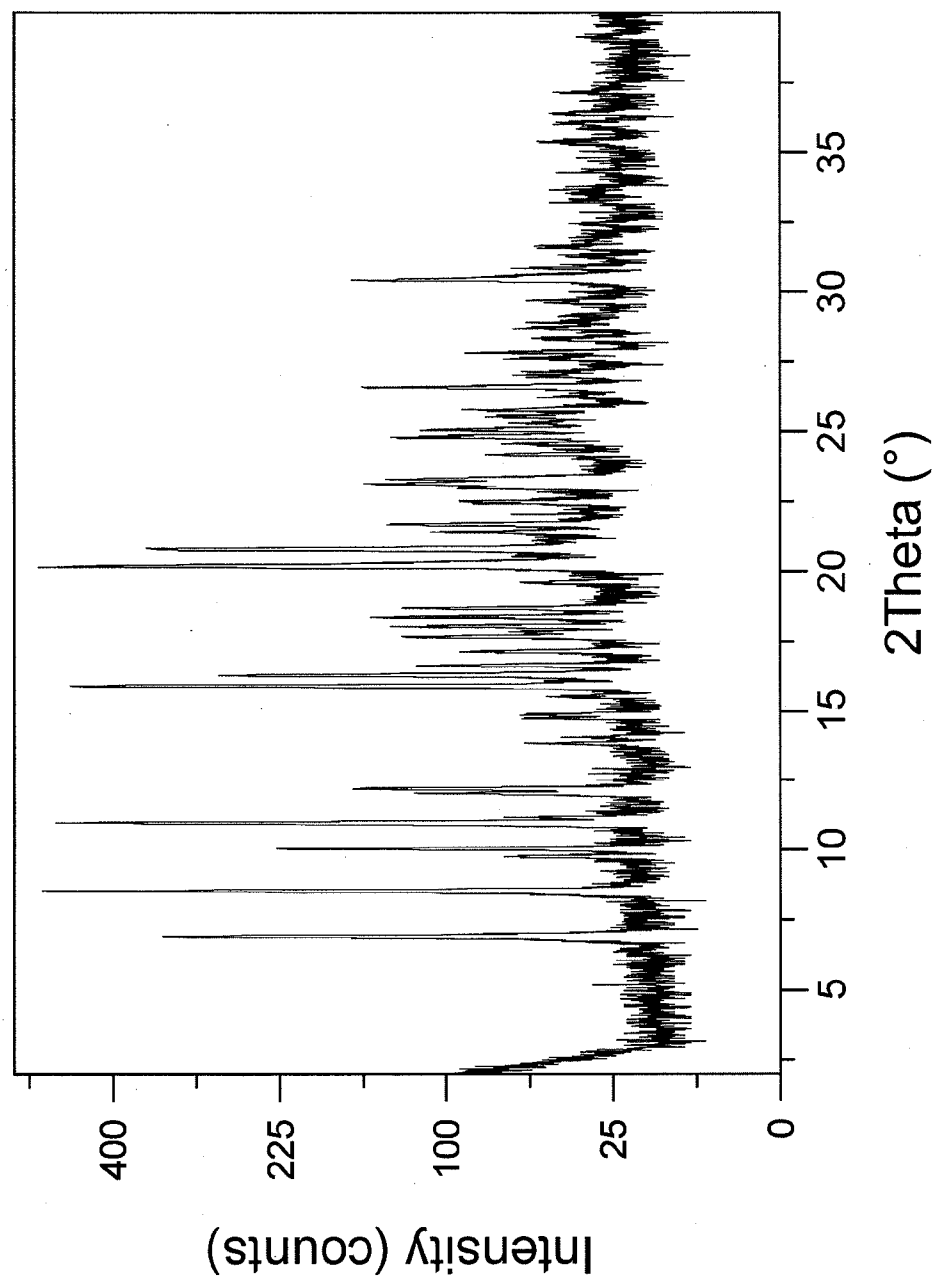


Figure 2

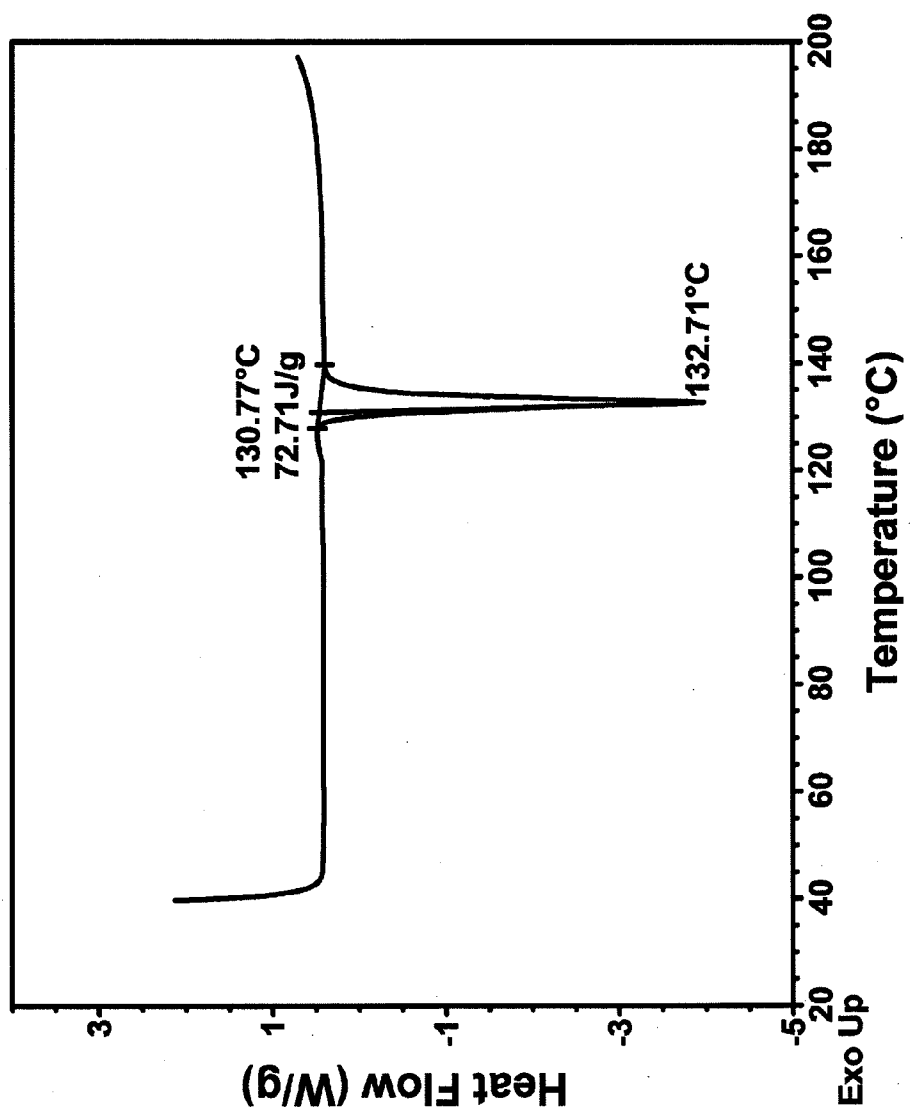


Figure 3

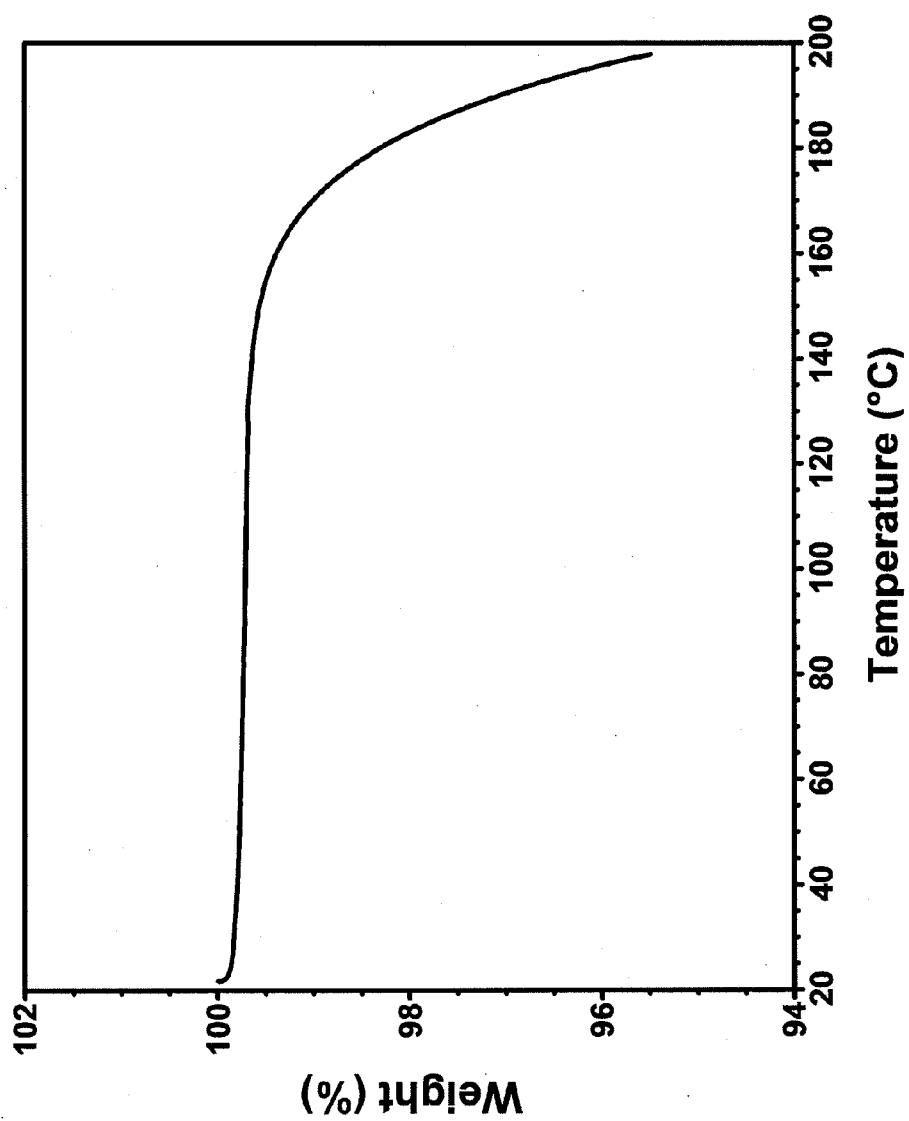
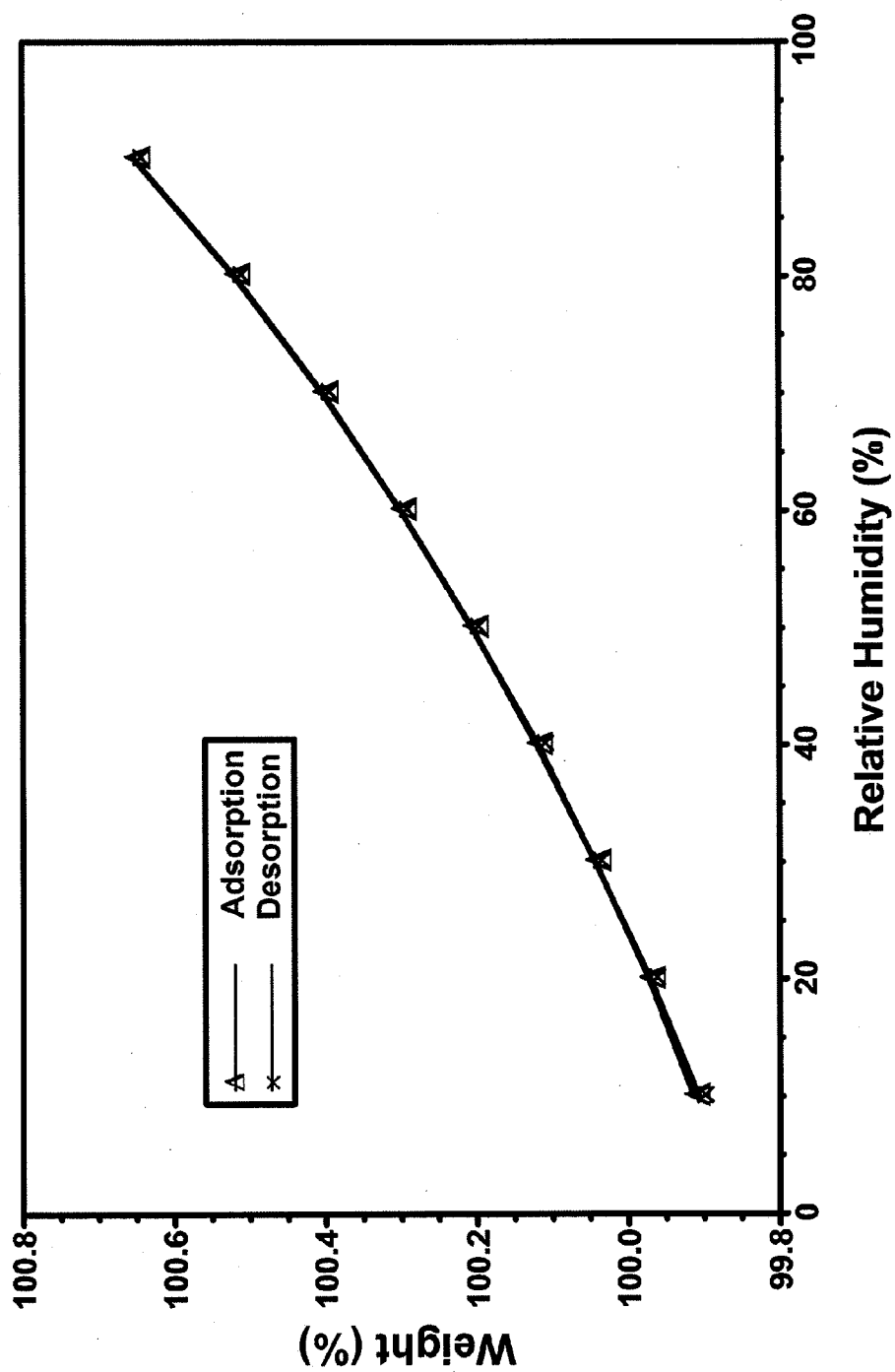


Figure 4



TENOFOVIR ALAFENAMIDE HEMIFUMARATE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority from U.S. Provisional Patent Application No. 61/524,224, filed Aug. 16, 2011, the content of which is hereby incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

Description of Related Art

[0002] U.S. Pat. Nos. 7,390,791 and 7,803,788 (the content of each of which is incorporated by reference herein in its entirety) describe certain prodrugs of phosphonate nucleotide analogs that are useful in therapy. One such prodrug is 9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine. This compound is also known by the Chemical Abstract name L-alanine, N—[(S)-[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]phenoxyphosphinyl-, 1-methylethyl ester. U.S. Pat. Nos. 7,390,791 and 7,803,788 also disclose a monofumarate form of this compound and its preparation method (see, e.g., Example 4).

SUMMARY OF THE INVENTION

[0003] Described is a hemifumarate form of 9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine. The name for 9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine is tenofovir alafenamide. The hemifumarate form of tenofovir alafenamide is also referred to herein as tenofovir alafenamide hemifumarate.

[0004] In one embodiment of the invention is provided tenofovir alafenamide hemifumarate.

[0005] In another embodiment is provided tenofovir alafenamide hemifumarate, wherein the ratio of fumaric acid to tenofovir alafenamide is 0.5 ± 0.1 , or 0.5 ± 0.05 , or 0.5 ± 0.01 , or about 0.5.

[0006] In one embodiment is provided tenofovir alafenamide hemifumarate in a solid form.

[0007] In one embodiment is provided tenofovir alafenamide hemifumarate that has an X-ray powder diffraction (XRPD) pattern having 2theta values of $6.9 \pm 0.2^\circ$ and $8.6 \pm 0.2^\circ$. In another embodiment is provided tenofovir alafenamide hemifumarate wherein the XRPD pattern comprises 2theta values of $6.9 \pm 0.2^\circ$, $8.6 \pm 0.2^\circ$, $11.0 \pm 0.2^\circ$, $15.9 \pm 0.2^\circ$, and $20.2 \pm 0.2^\circ$.

[0008] In one embodiment is provided tenofovir alafenamide hemifumarate that has a differential scanning calorimetry (DSC) onset endotherm of $131 \pm 2^\circ \text{C}$., or $131 \pm 1^\circ \text{C}$.

[0009] In one embodiment is provided a pharmaceutical composition comprising tenofovir alafenamide hemifumarate and a pharmaceutically acceptable excipient. In another embodiment is provided the pharmaceutical composition, further comprising an additional therapeutic agent. In a further embodiment, the additional therapeutic agent is selected from the group consisting of human immunodeficiency virus (HIV) protease inhibiting compounds, HIV nonnucleoside inhibitors of reverse transcriptase, HIV nucleoside inhibitors

of reverse transcriptase, HIV nucleotide inhibitors of reverse transcriptase, HIV integrase inhibitors, and CCR5 inhibitors.

[0010] In one embodiment is provided a method for treating a human immunodeficiency virus (HIV) infection comprising administering to a subject in need thereof a therapeutically effective amount of tenofovir alafenamide hemifumarate. In another embodiment is provided a method for treating an HIV infection comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising tenofovir alafenamide hemifumarate. In a further embodiment, the method comprises administering to the subject one or more additional therapeutic agents selected from the group consisting of HIV protease inhibiting compounds, HIV nonnucleoside inhibitors of reverse transcriptase, HIV nucleoside inhibitors of reverse transcriptase, HIV integrase inhibitors, and CCR5 inhibitors.

[0011] In one embodiment is provided a method for treating a hepatitis B virus (HBV) infection comprising administering to a subject in need thereof a therapeutically effective amount of tenofovir alafenamide hemifumarate. In another embodiment is provided a method for treating an HBV infection comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition comprising tenofovir alafenamide hemifumarate.

[0012] In one embodiment is provided a method for preparing a pharmaceutical composition comprising combining tenofovir alafenamide hemifumarate and a pharmaceutically acceptable excipient to provide the pharmaceutical composition.

[0013] In one embodiment is provided a method for preparing tenofovir alafenamide hemifumarate comprising subjecting a solution comprising a suitable solvent; fumaric acid; tenofovir alafenamide; and, optionally, one or more seeds of tenofovir alafenamide hemifumarate to conditions that provide for the crystallization of the fumaric acid and the tenofovir alafenamide. In one embodiment, the solvent comprises acetonitrile. In another embodiment, the solution is subjected to a temperature in the range of from about 0°C . to about 75°C .

[0014] In one embodiment is provided tenofovir alafenamide hemifumarate for use in medical therapy.

[0015] In one embodiment is provided the use of tenofovir alafenamide hemifumarate for the prophylactic or therapeutic treatment of an HIV infection. In another embodiment is provided the use of tenofovir alafenamide hemifumarate to treat an HIV infection. In a further embodiment is provided the use of tenofovir alafenamide hemifumarate for the preparation or manufacture of a medicament for the treatment of an HIV infection. In another further embodiment is provided tenofovir alafenamide hemifumarate for use in treating an HIV infection.

[0016] In one embodiment is provided the use of tenofovir alafenamide hemifumarate for the prophylactic or therapeutic treatment of an HBV infection. In another embodiment is provided the use of tenofovir alafenamide hemifumarate to treat an HBV infection. In a further embodiment is provided the use of tenofovir alafenamide hemifumarate for the preparation or manufacture of a medicament for the treatment of an HBV infection. In another further embodiment is provided tenofovir alafenamide hemifumarate for use in treating an HBV infection.

[0017] In some embodiments of the invention, the methods of treating and the like comprise administration of multiple

daily doses. In other embodiments, the methods of treating and the like comprise administration of a single daily dose.

[0018] In one embodiment of the invention is provided a composition consisting essentially of tenofovir alafenamide hemifumarate.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0019] FIG. 1 shows the X-ray powder diffraction (XRPD) pattern of tenofovir alafenamide hemifumarate.

[0020] FIG. 2 shows a graph of the DSC analysis of tenofovir alafenamide hemifumarate.

[0021] FIG. 3 shows a graph of the thermogravimetric analysis (TGA) data for tenofovir alafenamide hemifumarate.

[0022] FIG. 4 shows a graph of the dynamic vapor sorption (DVS) analysis of tenofovir alafenamide hemifumarate.

DETAILED DESCRIPTION OF THE INVENTION

[0023] Specific values listed within the present description for radicals, substituents, and ranges are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

[0024] In one embodiment, there is provided a hemifumarate form of tenofovir alafenamide (i.e., tenofovir alafenamide hemifumarate). This form may have a ratio (i.e., a stoichiometric ratio or mole ratio) of fumaric acid to tenofovir alafenamide of 0.5 ± 0.1 , 0.5 ± 0.05 , 0.5 ± 0.01 , or about 0.5, or the like.

[0025] In one embodiment, tenofovir alafenamide hemifumarate consists of fumaric acid and tenofovir alafenamide in a ratio of 0.5 ± 0.1 .

[0026] In one embodiment, tenofovir alafenamide hemifumarate consists essentially of fumaric acid and tenofovir alafenamide in a ratio of 0.5 ± 0.1 .

[0027] In one embodiment, tenofovir alafenamide hemifumarate has an XRPD pattern comprising 2theta values of $6.9 \pm 0.2^\circ$, $8.6 \pm 0.2^\circ$, $10.0 \pm 0.2^\circ$, $11.0 \pm 0.2^\circ$, $12.2 \pm 0.2^\circ$, $15.9 \pm 0.2^\circ$, $16.3 \pm 0.2^\circ$, $20.2 \pm 0.2^\circ$, and $20.8 \pm 0.2^\circ$.

[0028] In one embodiment, tenofovir alafenamide hemifumarate has an XRPD pattern comprising at least four 2theta values selected from $6.9 \pm 0.2^\circ$, $8.6 \pm 0.2^\circ$, $10.0 \pm 0.2^\circ$, $11.0 \pm 0.2^\circ$, $12.2 \pm 0.2^\circ$, $15.9 \pm 0.2^\circ$, $16.3 \pm 0.2^\circ$, $20.2 \pm 0.2^\circ$, and $20.8 \pm 0.2^\circ$.

[0029] In one embodiment, tenofovir alafenamide hemifumarate has a DSC onset endotherm of $131 \pm 2^\circ \text{C}$., or $131 \pm 1^\circ \text{C}$.

[0030] In one embodiment, a tenofovir alafenamide hemifumarate composition comprises less than about 5% by weight of tenofovir alafenamide monofumarate.

[0031] In one embodiment, a tenofovir alafenamide hemifumarate composition comprises less than about 1% by weight of tenofovir alafenamide monofumarate.

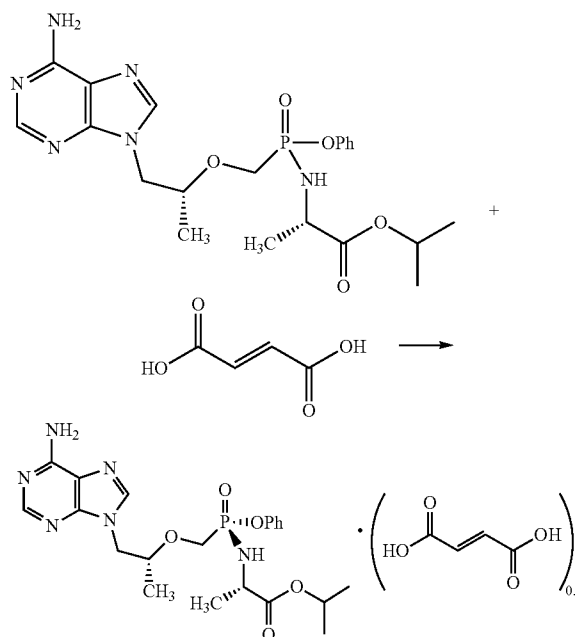
[0032] In one embodiment, a tenofovir alafenamide hemifumarate composition comprises less than about 0.5% by weight of tenofovir alafenamide monofumarate.

[0033] In one embodiment, a tenofovir alafenamide hemifumarate composition comprises no detectable tenofovir alafenamide monofumarate.

[0034] Tenofovir alafenamide (i.e., the compound 9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine) can be prepared as described in U.S. Pat. No. 7,390,791.

Selective Crystallization

[0035] In one embodiment, tenofovir alafenamide hemifumarate can be prepared using selective crystallization. An example of a scheme for this preparation method is as follows.



[0036] The method can be carried out by subjecting a solution comprising: a) a suitable solvent; b) fumaric acid; c) tenofovir alafenamide; and, optionally, d) one or more seeds comprising tenofovir alafenamide hemifumarate, to conditions that provide for the crystallization of fumaric acid and tenofovir alafenamide. The starting solution can contain the single diastereomer of tenofovir alafenamide or a mixture of tenofovir alafenamide and one or more of its other diastereomers (e.g., GS-7339, as described in U.S. Pat. No. 7,390,791).

[0037] The selective crystallization can be carried out in any suitable solvent. For example, it can be carried out in a protic solvent or in an aprotic organic solvent, or in a mixture thereof. In one embodiment, the solvent comprises a protic solvent (e.g., water or isopropyl alcohol). In another embodiment, the solvent comprises an aprotic organic solvent (e.g., acetone, acetonitrile (ACN), toluene, ethyl acetate, isopropyl acetate, heptane, tetrahydrofuran (THF), 2-methyl THF, methyl ethyl ketone, or methyl isobutyl ketone, or a mixture thereof). In one embodiment, the solvent comprises ACN or a mixture of ACN and up to about 50% methylene chloride (by volume). The selective crystallization also can be carried out at any suitable temperature, for example, a temperature in the range of from about 0°C . to about 70°C . In one specific embodiment, the resolution is carried out at a temperature of about 0°C .

[0038] One major advantage of the hemifumarate form of tenofovir alafenamide over the monofumarate form is its exceptional capability to purge GS-7339 (i.e., 9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine; described in, e.g., U.S. Pat. No.

7,390,791), which is the major diastereomeric impurity in the active pharmaceutical ingredient. Thus, the hemifumarate form of tenofovir alafenamide can be more readily and easily separated from impurities than the monofumarate form. Other major advantages of tenofovir alafenamide hemifumarate over the monofumarate form include improved thermodynamic and chemical stability (including long-term storage stability), superior process reproducibility, superior drug product content uniformity, and a higher melting point.

[0039] Tenofovir alafenamide hemifumarate is useful in the treatment and/or prophylaxis of one or more viral infections in man or animals, including infections caused by DNA viruses, RNA viruses, herpesviruses (e.g., CMV, HSV 1, HSV 2, VZV), retroviruses, hepadnaviruses (e.g., HBV), papillomavirus, hantavirus, adenoviruses and HIV. U.S. Pat. No. 6,043,230 (incorporated by reference herein in its entirety) and other publications describe the antiviral specificity of nucleotide analogs, such as tenofovir disoproxil. Like tenofovir disoproxil, tenofovir alafenamide is another prodrug form of tenofovir, and can be used in the treatment and/or prophylaxis of the same conditions.

[0040] Tenofovir alafenamide hemifumarate can be administered by any route appropriate to the condition to be treated. Suitable routes include oral, rectal, nasal, topical (including ocular, buccal, and sublingual), vaginal, and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal, and epidural). Generally, tenofovir alafenamide hemifumarate is administered orally, but it can be administered by any of the other routes noted herein.

[0041] Accordingly, pharmaceutical compositions include those suitable for topical or systemic administration, including oral, rectal, nasal, buccal, sublingual, vaginal, or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal, and epidural) administration. The formulations are in unit dosage form and are prepared by any of the methods well known in the art of pharmacy.

[0042] For oral therapeutic administration, the tenofovir alafenamide hemifumarate may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such pharmaceutical compositions and preparations will typically contain at least 0.1% of tenofovir alafenamide hemifumarate. The percentage of this active compound in the compositions and preparations may, of course, be varied and may conveniently be between about 2% to about 60% or more of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful pharmaceutical compositions is preferably such that an effective dosage level will be obtained upon administration of a single-unit dosage (e.g., tablet). Other dosage formulations may provide therapeutically effective amounts of tenofovir alafenamide hemifumarate upon repeated administration of subclinically effective amounts of the same. Preferred unit dosage formulations include those containing a daily dose (e.g., a single daily dose), as well as those containing a unit daily subclinical dose, or an appropriate fraction thereof (e.g., multiple daily doses), of tenofovir alafenamide hemifumarate.

[0043] Pharmaceutical compositions suitable for oral administration may be presented as discrete units such as capsules, cachets, or tablets, each containing a predetermined amount of tenofovir alafenamide hemifumarate; as a powder or granules; as a solution or a suspension in an aqueous liquid or a nonaqueous liquid; or as an oil-in-water liquid emulsion

or a water-in-oil liquid emulsion. Tenofovir alafenamide hemifumarate may also be presented as a bolus, electuary, or paste.

[0044] Tenofovir alafenamide hemifumarate is preferably administered as part of a pharmaceutical composition or formulation. Such pharmaceutical composition or formulation comprises tenofovir alafenamide hemifumarate together with one or more pharmaceutically acceptable carriers/excipients, and optionally other therapeutic ingredients. The excipient (s)/carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the patient. Excipients include, but are not limited to, substances that can serve as a vehicle or medium for tenofovir alafenamide hemifumarate (e.g., a diluent carrier). They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet.

[0045] Accordingly, the tablets, troches, pills, capsules, and the like may also contain, without limitation, the following: a binder(s), such as hydroxypropyl cellulose, povidone, or hydroxypropyl methylcellulose; a filler(s), such as microcrystalline cellulose, pregelatinized starch, starch, mannitol, or lactose monohydrate; a disintegrating agent(s), such as croscarmellose sodium, cross-linked povidone, or sodium starch glycolate; a lubricant(s), such as magnesium stearate, stearic acid, or other metallic stearates; a sweetening agent(s), such as sucrose, fructose, lactose, or aspartame; and/or a flavoring agent(s), such as peppermint, oil of wintergreen, or a cherry flavoring. When the unit dosage form is a capsule, it may contain, in addition to materials of the above types, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, polymers, wax, shellac, or sugar and the like. Of course, any material used in preparing any unit dosage form typically will be pharmaceutically acceptable and substantially nontoxic in the amounts employed. In addition, tenofovir alafenamide hemifumarate may be incorporated into sustained-release preparations and devices.

[0046] For infections of the eye or other external tissues, e.g., mouth and skin, the pharmaceutical compositions are preferably applied as a topical ointment or cream containing tenofovir alafenamide hemifumarate in an amount of, for example, 0.01 to 10% w/w (including active ingredient in a range between 0.1% and 5% in increments of 0.1% w/w such as 0.6% w/w, 0.7% w/w, etc.), preferably 0.2 to 3% w/w and most preferably 0.5 to 2% w/w. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water cream base.

[0047] Pharmaceutical compositions suitable for topical administration in the mouth include lozenges comprising tenofovir alafenamide hemifumarate in a flavored basis, for example, sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

[0048] Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

[0049] Pharmaceutical formulations suitable for parenteral administration are sterile and include aqueous and nonaqueous

ous injection solutions that may contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions that may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials with elastomeric stoppers, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier (e.g., water for injections) immediately prior to use. Injection solutions and suspensions may be prepared from sterile powders, granules, and tablets of the kind previously described.

[0050] In addition to the ingredients particularly mentioned above, the pharmaceutical compositions/formulations may include other ingredients conventional in the art, having regard to the type of formulation in question.

[0051] In another embodiment, there is provided veterinary compositions comprising tenofovir alafenamide hemifumarate together with a veterinary carrier therefor. Veterinary carriers are materials useful for the purpose of administering the composition to cats, dogs, horses, rabbits, and other animals, and may be solid, liquid, or gaseous materials that are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These veterinary compositions may be administered orally, parenterally, or by any other desired route.

[0052] The tenofovir alafenamide hemifumarate can be used to provide controlled release pharmaceutical formulations containing a matrix or absorbent material and an active ingredient of the invention, in which the release of the active ingredient can be controlled and regulated to allow less frequent dosing or to improve the pharmacokinetic or toxicity profile of the compound. Controlled release formulations adapted for oral administration, in which discrete units comprising a compounds of the invention, can be prepared according to conventional methods.

[0053] Useful dosages of tenofovir alafenamide hemifumarate can be determined by comparing in vitro activities, and the in vivo activities in animal models. Methods for the extrapolation of effective amounts/dosages in mice and other animals to therapeutically effective amounts/dosages in humans are known in the art.

[0054] The amount of tenofovir alafenamide hemifumarate required for use in treatment will vary with several factors, including but not limited to the route of administration, the nature of the condition being treated, and the age and condition of the patient; ultimately, the amount administered will be at the discretion of the attendant physician or clinician. The therapeutically effective amount/dose of tenofovir alafenamide hemifumarate depends, at least, on the nature of the condition being treated, any toxicity or drug interaction issues, whether the compound is being used prophylactically (e.g., sometimes requiring lower doses) or against an active disease or condition, the method of delivery, and the pharmaceutical formulation, and will be determined by the clinician using conventional dose escalation studies.

[0055] In one embodiment, the oral dose of tenofovir alafenamide hemifumarate may be in the range from about 0.0001 to about 100 mg/kg body weight per day, for example, from about 0.01 to about 10 mg/kg body weight per day, from about 0.01 to about 5 mg/kg body weight per day, from about 0.5 to about 50 mg/kg body weight per day, from about 1 to about 30 mg/kg body weight per day, from about 1.5 to about 10 mg/kg body weight per day, or from about 0.05 to about 0.5 mg/kg

body weight per day. As a nonlimiting example, the daily candidate dose for an adult human of about 70 kg body weight will range from about 0.1 mg to about 1000 mg, or from about 1 mg to about 1000 mg, or from about 5 mg to about 500 mg, or from about 1 mg to about 150 mg, or from about 5 mg to about 150 mg, or from about 5 mg to about 100 mg, and may take the form of single or multiple doses.

[0056] The pharmaceutical compositions described herein may further include one or more therapeutic agents in addition to tenofovir alafenamide hemifumarate. In one specific embodiment of the invention, the additional therapeutic agent can be selected from the group consisting of HIV protease inhibiting compounds, HIV nonnucleoside inhibitors of reverse transcriptase, HIV nucleoside inhibitors of reverse transcriptase, HIV nucleotide inhibitors of reverse transcriptase, HIV integrase inhibitors, and CCR5 inhibitors.

[0057] Therapeutic methods include administering tenofovir alafenamide hemifumarate to a subject/patient in need of the same as a therapeutic or preventative treatment. Thus, tenofovir alafenamide hemifumarate may be administered to a subject/patient having a medical disorder or to a subject who may acquire the disorder. One of ordinary skill will appreciate that such treatment is given in order to ameliorate, prevent, delay, cure, and/or reduce the severity of a symptom or set of symptoms of a disorder (including a recurring disorder). The treatment may also be given to prolong the survival of a subject, e.g., beyond the survival time expected in the absence of such treatment. The medical disorders that may be treated with tenofovir alafenamide hemifumarate include those discussed herein, including without limitation, HIV infection and HBV infection.

[0058] The following are nonlimiting, illustrative Examples.

Example 1

[0059] Tenofovir alafenamide monofumarate solids (5.0 g) and 9-[(R)-2-[[[(R)-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine (GS-7339) monofumarate solids (0.75 g) were charged into 35 g MTBE at 22° C. and the mixture was stirred for 1 hour. A slurry was formed and was dried in a rotary evaporator. 58 g acetonitrile (ACN) was charged into the solids and the mixture was heated to reflux to dissolve the solids. The resulting solution was allowed to cool naturally while agitated. A slurry was formed, and the slurry was further cooled by ice-water-bath. The solids were isolated by filtration and washed with 5 g ACN. The solids were dried in a vacuum oven at 40° C. overnight. 5.52 g off-white solids were obtained. The solids were analyzed by XRPD and found to contain tenofovir alafenamide monofumarate, GS-7339 monofumarate, and tenofovir alafenamide hemifumarate.

Example 2

Preparation of Tenofovir Alafenamide Hemifumarate via Selective Crystallization

[0060] 9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine as a slurry in ACN (9.7 kg slurry, 13.8 wt %, a diastereomeric mixture of 1.0 kg (2.10 mol, 1 mol equiv) of 9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine and 0.35 kg of 9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]

phenoxyphosphinyl]methoxy]propyl]adenine was charged into a reactor and rinsed forward with dichloromethane (5 kg). The mixture was concentrated under vacuum to about 3 L with jacket temperature below 40° C. The concentrate was then coevaporated with ACN (6 kg) under vacuum to about 3 L with jacket temperature below 40° C. The concentrate was diluted with ACN (8.5 kg) and warmed to 40-46° C. The warm mixture was filtered into a second reactor and the filtrate was cooled to 19-25° C.

[0061] To the above solution was charged fumaric acid (0.13 kg, 1.12 mol, 0.542 mole equiv) followed by ACN (1 kg), and the mixture was heated to 67-73° C. The hot mixture was transferred into a reactor via a polishing filter, and then adjusted to 54-60° C. Seed crystals (5 g) of the hemifumarate form of tenofovir alafenamide were charged (for example, the mixture can be seeded with tenofovir alafenamide hemifumarate formed in Example 1 or a subsequent production), and the resulting mixture was agitated at 54-60° C. for about 30 minutes. The mixture was cooled over a minimum of 4 hours to 0-6° C., and then agitated at 0-6° C. for a minimum of 1 hour. The resulting slurry was filtered and rinsed with chilled (0-6° C.) ACN (2 kg). The product was dried under vacuum below 45° C. until loss on drying (LOD) and organic volatile impurities (OVI) limits were met (LOD≤1.0%, dichloromethane content≤0.19%, acetonitrile content≤0.19%) to afford the final compound of the hemifumarate form of tenofovir alafenamide as a white to off-white powder (typical yield is about 0.95 kg). ¹H NMR (400 MHz, d6 DMSO): δ 1.06 (d, J=5.6 Hz, 3H), 1.12-1.16 (m, 9H), 3.77 (dd, J=10.4, 11.6 Hz, 1H), 3.84-3.90 (m, 2H), 3.94 (m, 1H), 4.14 (dd, J=6.8, 14.8 Hz, 1H), 4.27 (m, 1H), 4.85 (heptet, J=6.0 Hz, 1H), 5.65 (t, J=11.2 Hz, 1H), 6.63 (s, 1H), 7.05 (d, J=7.6 Hz, 2H), 7.13 (t, J=7.2 Hz, 1H), 7.24 (s, 2H), 7.29 (t, J=7.6 Hz, 2H), 8.13 (t, J=13.6 Hz, 2H), ³¹P NMR (162 MHz, d6 DMSO): δ 23.3.

Example 3

Preparation of Tenofovir Alafenamide Hemifumarate

[0062] To a jacketed reactor equipped with overhead agitator, was charged 9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine (10 g), fumaric acid (1.22 g), and ACN (100 mL). The mixture was heated to 70-75° C. to dissolve the solids. Any undissolved particulates were removed by filtration through a cartridge filter. The filtered solution was cooled to 60-65° C., and seeded with 1% (by weight) of tenofovir alafenamide hemifumarate. The slurry was aged for 30 minutes and cooled to 0-5° C. over 2 hours. The temperature was maintained for 1-18 hours, and the resulting slurry was filtered and washed with 2 ml of cold ACN (0-5° C.). The solids were dried under vacuum at 50° C. to provide the hemifumarate form of tenofovir alafenamide, which was characterized as described below.

Characterization of Tenofovir Alafenamide Hemifumarate from Example 3

[0063] Tenofovir alafenamide hemifumarate from Example 3 consists of 9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine and one-half an equivalent of fumaric acid. Tenofovir alafenamide hemifumarate is anhydrous, nonhygroscopic, and has a DSC onset endotherm of about 131° C.

X-ray Powder Diffraction

[0064] The XRPD pattern of tenofovir alafenamide hemifumarate was obtained in the following experimental setting: 45 KV, 45 mA, Kα1=1.5406 Å, scan range 2.-40°, step size 0.0084°, counting time: 8.25 s. The XRPD pattern for tenofovir alafenamide hemifumarate is shown in FIG. 1. The characteristic peaks include: 6.9±0.2°, 8.6±0.2°, 10.0±0.2°, 11.0±0.2°, 12.2±0.2°, 15.9±0.2°, 16.3±0.2°, 20.2±0.2°, and 20.8±0.2°.

Single-Crystal X-Ray Diffraction

[0065] The crystal size was 0.32×0.30×0.20 mm³. The sample was held at 123 K and the data was collected using a radiation source with a wavelength of 0.71073 Å in the theta range of 1.59 to 25.39°. Conditions of, and data collected from the single-crystal X-ray diffraction are shown in Table 1.

TABLE 1

Single-Crystal X-ray Diffraction			
Empirical formula	C ₂₃ H ₃₁ N ₆ O ₇ P		
Formula weight	534.50		
Temperature	123(2) K		
Crystal size	0.32 × 0.30 × 0.20 mm ³		
Theta range for data collection	1.59 to 25.39°		
Wavelength	0.71073 Å		
Crystal system	Trigonal		
Space group	P4(2)2(1)2		
Unit cell dimensions	a = 18.1185(12) Å	α = 90°	
	b = 18.1185(12) Å	β = 90°	
	c = 17.5747(11) Å	γ = 90°	
Volume	5769.4(6) Å ³		
Z	8		
Density (calculated)	1.231 g/cm ³		

DSC Analysis

[0066] The DSC analysis was conducted using 2.517 mg of tenofovir alafenamide hemifumarate. It was heated at 10° C./min over the range of 40-200° C. The onset endotherm was found to be about 131° C. (FIG. 2).

TGA Data

[0067] The TGA data were obtained using 4.161 mg of tenofovir alafenamide hemifumarate. It was heated at 10° C./min over the range of 25-200° C. The sample lost 0.3% weight before melting (FIG. 3). It was determined to be an anhydrous form.

DVS Analysis

[0068] DVS analysis was conducted using 4.951 mg of tenofovir alafenamide hemifumarate. The material was kept at 25° C. in nitrogen at humidities ranging from 10% to 90% relative humidity; each step was equilibrated for 120 minutes. The sorption isotherm is shown at FIG. 4. The material was found to be nonhygroscopic, and to absorb 0.65% water at a relative humidity of 90%.

Purging of Diastereomeric Impurity

[0069] In the prior syntheses of tenofovir alafenamide, one of the major impurities is typically the diastereomer 9-[(R)-2-[[[(R)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine. The hemifumarate form of tenofovir alafenamide from Example 3 has an excep-

tional capability to purge this diastereomeric impurity, as compared with the capability of the monofumarate form (described in U.S. Pat. No. 7,390,791). The data in Table 2 (below) demonstrates that tenofovir alafenamide hemifumarate (Batch 2) purged the diastereomeric impurity to less than one-tenth of the starting concentration, whereas the monofumarate form of tenofovir alafenamide (Batch 1) only slightly purged the diastereomeric impurity.

TABLE 2

Purging Capability Comparison					
Batch	Starting Material	Solvent	Fumaric acid charge (mole equivalent)	Product obtained	Diastereomeric Impurity in Product
1	9.3%	ACN	0.9	Monofumarate form	7.6%
2	10.0%	ACN	0.5	Hemifumarate form	0.65%

Chemical Stability

[0070] Chemical stability of the hemifumarate form of tenofovir alafenamide was compared with the monofumarate form. As shown in Table 3 (below), under identical conditions, the hemifumarate form of tenofovir alafenamide was chemically more stable and exhibited better long-term storage stability, with significantly less degradation (% Total Deg. Products) than the monofumarate form. Conditions evaluated include temperature, relative humidity (RH), and the open or closed state of the container cap.

TABLE 3

Chemical Stability Comparison					
Storage Condition	Time Points (weeks)	Monofumarate form		Hemifumarate form	
		% TA* Area Normalized	% Total Deg. Products	% TA Area Normalized	% Total Deg. Products
40° C./	0	97.1	0.69	98.4	0.05
75% RH	1	97.0	0.87	98.4	0.14
Cap	2	96.6	1.18	98.5	0.14
Closed	4	96.4	1.49	98.4	0.25
	8	95.4	2.36	98.0	0.49
40° C./	0	97.1	0.69	98.4	0.05
75% RH	1	96.9	0.90	98.5	0.15
Cap	2	96.6	1.10	98.5	0.14
Open	4	96.2	1.67	98.4	0.26
	8	95.0	2.74	98.1	0.50
70° C.	0	97.1	0.69	98.4	0.05
Cap	2	96.2	1.83	98.5	0.22
Closed	4	93.3	4.78	98.4	0.33

*TA is tenofovir alafenamide

Thermodynamic Stability

[0071] Stable form screening of tenofovir alafenamide hemifumarate showed that it is thermodynamically stable in most solvents, such as ACN, toluene, ethyl acetate, methyl tert-butyl ether (MTBE), acetone, THF, and 2-methyl THF. A similar stable form screening of the monofumarate form showed that this form is not thermodynamically stable in the

above-listed solvents. When suspended in these solvents, the monofumarate form of tenofovir alafenamide fully converts to the hemifumarate form in THF and 2-methyl THF, and partially converts to the hemifumarate form in ACN, ethyl acetate, MTBE, and acetone, as well as at ambient temperatures.

Thermal Stability

[0072] As shown by the DSC data, the hemifumarate form of tenofovir alafenamide has a melting point that is about 10° C. higher than that of the monofumarate form, indicating that the hemifumarate form has improved thermal stability as compared with the monofumarate form.

[0073] All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

What is claimed is:

1. Tenofovir alafenamide hemifumarate.
2. Tenofovir alafenamide hemifumarate, wherein the ratio of fumaric acid to tenofovir alafenamide is 0.5 ± 0.1 .
3. The hemifumarate of claim 2, wherein the ratio of fumaric acid to tenofovir alafenamide is 0.5 ± 0.05 .
4. The hemifumarate of claim 2, wherein the ratio of fumaric acid to tenofovir alafenamide is 0.5 ± 0.01 .
5. The hemifumarate of claim 2, wherein the ratio of fumaric acid to tenofovir alafenamide is about 0.5.
6. The hemifumarate of claim 1 that is a solid.
7. Tenofovir alafenamide hemifumarate, wherein an X-ray powder diffraction (XRPD) pattern comprises 2theta values of $6.9 \pm 0.2^\circ$ and $8.6 \pm 0.2^\circ$.
8. The hemifumarate of claim 7, wherein the XRPD pattern comprises 2theta values of $6.9 \pm 0.2^\circ$, $8.6 \pm 0.2^\circ$, $11.0 \pm 0.2^\circ$, $15.9 \pm 0.2^\circ$, and $20.2 \pm 0.2^\circ$.
9. The hemifumarate of claim 1 that has a differential scanning calorimetry (DSC) onset endotherm of $131 \pm 2^\circ$ C.
10. The hemifumarate of claim 9 that has a DSC onset endotherm of $131 \pm 1^\circ$ C.
11. A pharmaceutical composition comprising the hemifumarate of claim 1 and a pharmaceutically acceptable excipient.
12. The pharmaceutical composition of claim 11, further comprising an additional therapeutic agent.
13. The pharmaceutical composition of claim 12, wherein the additional therapeutic agent is selected from the group consisting of human immunodeficiency virus (HIV) protease inhibiting compounds, HIV nonnucleoside inhibitors of reverse transcriptase, HIV nucleoside inhibitors of reverse transcriptase, HIV nucleotide inhibitors of reverse transcriptase, HIV integrase inhibitors, and CCR5 inhibitors.
14. A method for treating a human immunodeficiency virus (HIV) infection comprising administering to a subject in need thereof a therapeutically effective amount of the hemifumarate of claim 1.
15. A method for treating an HIV infection comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition of claim 11.
16. The method for treating an HIV infection of claim 14, further comprising administering to the subject one or more additional therapeutic agents selected from the group consist-

ing of HIV protease inhibiting compounds, HIV nonnucleoside inhibitors of reverse transcriptase, HIV nucleoside inhibitors of reverse transcriptase, HIV nucleotide inhibitors of reverse transcriptase, HIV integrase inhibitors, and CCR5 inhibitors.

17. A method for treating a hepatitis B virus (HBV) infection comprising administering to a subject in need thereof a therapeutically effective amount of the hemifumarate of claim 1.

18. A method for treating an HBV infection comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition of claim 11.

19. A method for preparing a pharmaceutical composition comprising combining the hemifumarate of claim 1 and a pharmaceutically acceptable excipient to provide the pharmaceutical composition.

20. A method for preparing tenofovir alafenamide hemifumarate comprising subjecting a solution comprising: a) a suitable solvent; b) fumaric acid; c) tenofovir alafenamide; and d) one or more seeds of tenofovir alafenamide hemifu-

marate, to conditions that provide for the crystallization of the fumaric acid and the tenofovir alafenamide.

21. The method of claim 20, wherein the solvent comprises acetonitrile.

22. The method of claim 20, wherein the solution is subjected to a temperature in the range of from about 0° C. to about 75° C.

23. The method for treating an HIV infection of claim 14, wherein the hemifumarate is administered in multiple daily doses.

24. The method for treating an HIV infection of claim 14, wherein the hemifumarate is administered in a single daily dose.

25. The method for treating an HBV infection of claim 17, wherein the hemifumarate is administered in multiple daily doses.

26. The method for treating an HBV infection of claim 17, wherein the hemifumarate is administered in a single daily dose.

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