

NUCLEOSIDE ANALOGS

This application is being filed on 21 December 2009, as a PCT International Patent application in the name of Pharmasset, Inc., a U.S. national corporation, applicant for the designation of all countries except the US, and
5 Donghui Bao, a citizen of China, Wonsuk Chang, a citizen of South Korea, Dhanapalan Nagarathnam, and Michael Joseph Sofia, both citizens of the U.S., applicants for the designation of the US only.

PRIORITY CLAIM

The present application claims priority to U.S. Provisional Patent
10 Application No. 61/140,441, filed December 23, 2008, the contents of which are incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention pertains to nucleoside analogs and their use as agents for treating viral diseases. These compounds are inhibitors of RNA-dependent RNA
15 viral replication and are useful as inhibitors of HCV NS5B polymerase, as inhibitors of HCV replication and for treatment of hepatitis C infection in mammals.

BACKGROUND

Hepatitis C virus (HCV) infection is a major health problem that leads to chronic liver disease, such as cirrhosis and hepatocellular carcinoma, in a substantial
20 number of infected individuals, estimated to be 2-15% of the world's population. There are an estimated 4.5 million infected people in the United States alone, according to the U.S. Center for Disease Control. According to the World Health Organization, there are more than 200 million infected individuals worldwide, with at least 3 to 4 million people being infected each year. Once infected, about 20% of
25 people clear the virus, but the rest can harbor HCV the rest of their lives. Ten to twenty percent of chronically infected individuals eventually develop liver-destroying cirrhosis or cancer. The viral disease is transmitted parenterally by contaminated blood and blood products, contaminated needles, or sexually and vertically from infected mothers or carrier mothers to their offspring. Current
30 treatments for HCV infection, which are restricted to immunotherapy with recombinant interferon- α alone or in combination with the nucleoside analog

ribavirin, are of limited clinical benefit. Moreover, there is no established vaccine for HCV. Consequently, there is an urgent need for improved therapeutic agents that effectively combat chronic HCV infection.

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

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

Flaviviridae Viruses

The Flaviviridae family of viruses comprises at least three distinct genera:
pestiviruses, which cause disease in cattle and pigs; *flaviviruses*, which are the
primary cause of diseases such as dengue fever and yellow fever; and *hepaciviruses*,
25 whose sole member is HCV. The flavivirus genus includes more than 68 members
separated into groups on the basis of serological relatedness (Calisher et al., *J. Gen.
Virol*, 1993, 70, 37-43). Clinical symptoms vary and include fever, encephalitis and
hemorrhagic fever (*Fields Virology*, Editors: Fields, B. N., Knipe, D. M., and
Howley, P. M., Lippincott-Raven Publishers, Philadelphia, PA, 1996, Chapter 31,
30 931-959). Flaviviruses of global concern that are associated with human disease
include the Dengue Hemorrhagic Fever viruses (DHF), yellow fever virus, shock

syndrome and Japanese encephalitis virus (Halstead, S. B., *Rev. Infect. Dis.*, 1984, 6, 251-264; Halstead, S. B., *Science*, 239:476-481, 1988; Monath, T. P., *New Eng. J. Med.*, 1988, 319, 641-643).

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

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

The genetic organization of pestiviruses and hepaciviruses is very similar. These positive stranded RNA viruses possess a single large open reading frame
25 (ORF) encoding all the viral proteins necessary for virus replication. These proteins are expressed as a polyprotein that is co- and post-translationally processed by both cellular and virus-encoded proteinases to yield the mature viral proteins. The viral proteins responsible for the replication of the viral genome RNA are located within approximately the carboxy-terminal. Two-thirds of the ORF are termed
30 nonstructural (NS) proteins. The genetic organization and polyprotein processing of the nonstructural protein portion of the ORF for pestiviruses and hepaciviruses is very similar. For both the pestiviruses and hepaciviruses, the mature nonstructural

(NS) proteins, in sequential order from the amino-terminus of the nonstructural protein coding region to the carboxy-terminus of the ORF, consist of p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

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

The actual roles and functions of the NS proteins of pestiviruses and hepaciviruses in the lifecycle of the viruses are directly analogous. In both cases, the NS3 serine proteinase is responsible for all proteolytic processing of polyprotein
15 precursors downstream of its position in the ORF (Wiskerchen and Collett, *Virology*, 1991, 184, 341-350; Bartenschlager et al., *J. Virol.* 1993, 67, 3835-3844; Eckart et al. *Biochem. Biophys. Res. Comm.* 1993, 192, 399-406; Grakoui et al., *J. Virol.* 1993, 67, 2832-2843; Grakoui et al., *Proc. Natl. Acad. Sci. USA* 1993, 90, 10583-10587; Hijikata et al., *J. Virol.* 1993, 67, 4665-4675; Tome et al., *J. Virol.*,
20 1993, 67, 4017-4026). The NS4A protein, in both cases, acts as a cofactor with the NS3 serine protease (Bartenschlager et al., *J. Virol.* 1994, 68, 5045-5055; Failla et al., *J. Virol.* 1994, 68, 3753-3760; Xu et al., *J. Virol.*, 1997, 71:53 12-5322). The NS3 protein of both viruses also functions as a helicase (Kim et al., *Biochem. Biophys. Res. Comm.*, 1995, 215, 160-166; Jin and Peterson, *Arch. Biochem. Biophys.*, 1995, 323, 47-53; Warrenner and Collett, *J. Virol.* 1995, 69, 1720-1726).
25 Finally, the NS5B proteins of pestiviruses and hepaciviruses have the predicted RNA-directed RNA polymerases activity (Behrens et al., *EMBO*, 1996, 15, 12-22; Lechmann et al., *J. Virol.*, 1997, 71, 8416-8428; Yuan et al., *Biochem. Biophys. Res. Comm.* 1997, 232, 231-235; Hagedorn, PCT WO 97/12033; Zhong et al., *J. Virol.*,
30 1998, 72, 9365-9369).

Currently, there are limited treatment options for individuals infected with hepatitis C virus. The current approved therapeutic option is the use of

immunotherapy with recombinant interferon- α alone or in combination with the nucleoside analog ribavirin. This therapy is limited in its clinical effectiveness and only 50% of treated patients respond to therapy. Therefore, there is significant need for more effective and novel therapies to address the unmet medical need posed by HCV infection.

A number of potential molecular targets for drug development of direct acting antivirals as anti-HCV therapeutics have now been identified including, but not limited to, the NS2-NS3 autoprotease, the N3 protease, the N3 helicase and the NS5B polymerase. The RNA-dependent RNA polymerase is absolutely essential for replication of the single-stranded, positive sense, RNA genome and this enzyme has elicited significant interest among medicinal chemists.

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

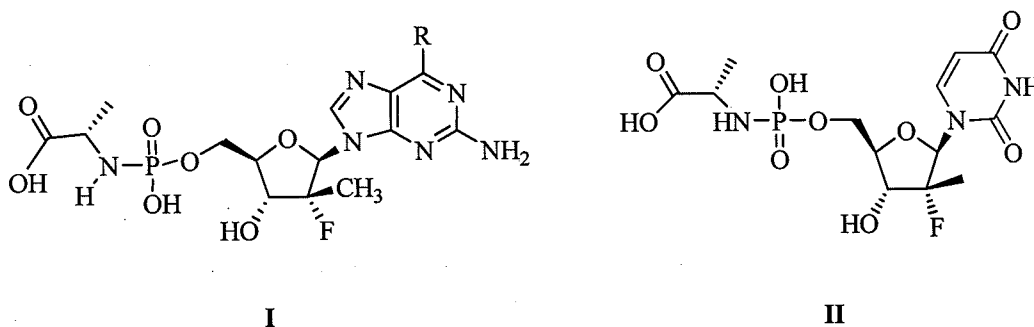
Nucleoside inhibitors of NS5B polymerase can act either as a non-natural substrate that results in chain termination or as a competitive inhibitor which competes with nucleotide binding to the polymerase. To function as a chain terminator the nucleoside analog must be taken up by the cell and converted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

In some cases, the biological activity of a nucleoside is hampered by its poor substrate characteristics for one or more of the kinases needed to convert it to the active triphosphate form. Formation of the monophosphate by a nucleoside kinase is generally viewed as the rate limiting step of the three phosphorylation events. To circumvent the need for the initial phosphorylation step in the metabolism of a nucleoside to the active triphosphate analog, the preparation of stable phosphate prodrugs has been reported. Nucleoside phosphoramidate prodrugs have been shown to be precursors of the active nucleoside triphosphate and to inhibit viral replication when administered to viral infected whole cells (McGuigan, C., et al., *J. Med. Chem.*, 1996, 39, 1748-1753; Valette, G., et al., *J. Med. Chem.*, 1996, 39, 1981-1990; Balzarini, J., et al., *Proc. National Acad Sci USA*, 1996, 93, 7295-7299; Siddiqui, A. Q., et al., *J. Med. Chem.*, 1999, 42, 4122-4128; Eisenberg, E. J., et al., *Nucleosides, Nucleotides and Nucleic Acids*, 2001, 20, 1091-1098; Lee, W.A., et al., *Antimicrobial Agents and Chemotherapy*, 2005, 49, 1898); US 2006/0241064; and WO 2007/095269.

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

SUMMARY OF THE INVENTION

The present invention is directed to a purified compound represented by formula I or a purified compound represented by formula II:



wherein R is OMe, OEt, $-N(-CH_2CH_2CH_2-)$ (azetidin-1-yl), or OH;
or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a
crystalline form thereof.

DEFINITIONS

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

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

The term "purified," as described herein, refers to the purity of a given compound. For example, a compound is "purified" when the given compound is a major component of the composition, i.e., at least 50% w/w pure. Thus, "purified" embraces at least 50% w/w purity, at least 60% w/w purity, at least 70% purity, at least 80% purity, at least 85% purity, at least 90% purity, at least 92% purity, at least 94% purity, at least 96% purity, at least 97% purity, at least 98% purity, and at least 99% purity.

20 The term "metabolite," as described herein, refers to a compound produced
in vivo after administration to a subject in need thereof.

The term "salt," as described herein, refers to a compound produced by the protonation of a proton-accepting moiety and/or deprotonation of a proton-donating moiety. It should be noted that protonation of the proton-accepting moiety results in the formation of a cationic species in which the charge is balanced by the presence of a physiological anion, whereas deprotonation of the proton-donating moiety

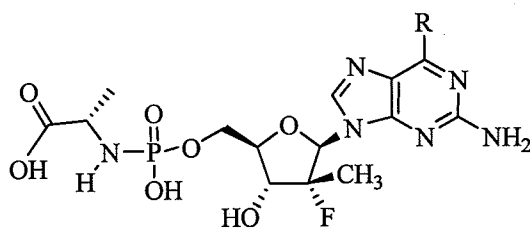
results in the formation of an anionic species in which the charge is balanced by the presence of a physiological cation.

The term "P*" means that the phosphorous atom is chiral and that it has a corresponding Cahn-Ingold-Prelog designation of "R" or "S" which have their
 5 accepted plain meanings. It is contemplated that the phosphoramidate nucleoside represented by formula I and the cyclic phosphate nucleoside represented by
 formula II can exist as a mixture of diastereomers due to the chirality at
 phosphorous. Applicants contemplate use of the mixture of diastereomers and/or
 the resolved diastereomers. In some instances, an asterisk does not appear next to
 10 the phosphoroamidate or cyclic phosphate phosphorous atom. In these instances, it
 is understood that the phosphorous atom is chiral and that one of ordinary skill
 understands this to be so unless the substituents bound to the phosphorous exclude
 the possibility of chirality at phosphorous, such as in $P(O)Cl_3$.

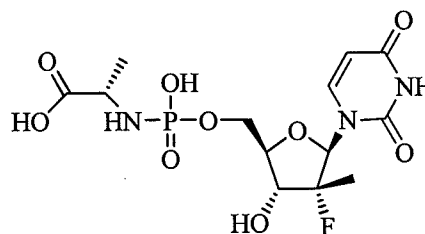
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DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a purified compound represented by
 formula I or a purified compound represented by formula II:



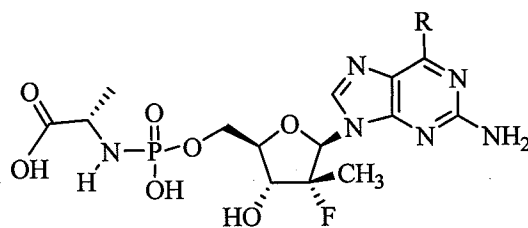
I



II

wherein R is OMe, OEt, $-N(-CH_2CH_2CH_2-)$ (azetidin-1-yl), or OH;
 20 or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a
 crystalline form thereof.

A first embodiment of the present invention is directed to a purified
 compound represented by formula I

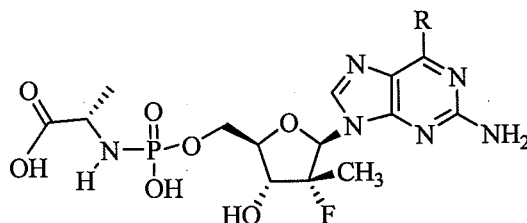


I

wherein R is OMe;

or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

- 5 A first embodiment of the present invention is directed to a purified compound represented by formula I

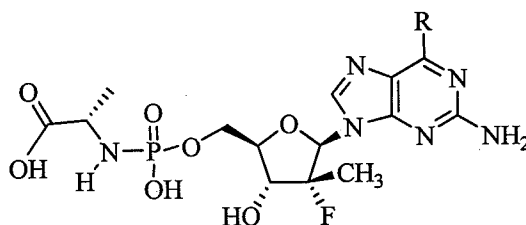


I

wherein R is OEt;

- or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a
10 crystalline form thereof.

A third embodiment of the present invention is directed to a purified compound represented by formula I

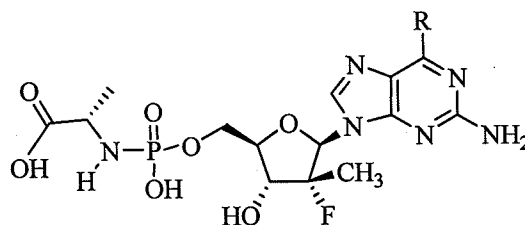


I

wherein R is OH;

or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

A fourth embodiment of the present invention is directed to a purified compound represented by formula I

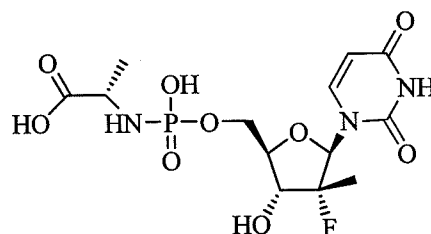


I

wherein R is $-N(-CH_2CH_2CH_2-)$ (azetidin-1-yl);

or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

A fifth embodiment of the present invention is directed to a purified compound represented by formula II



II

or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

DOSAGE, ADMINISTRATION, AND USE

A sixth embodiment of the present invention is directed to a composition for the treatment of any of the viral agents disclosed herein said composition comprising a pharmaceutically acceptable medium selected from among an excipient, carrier, diluent, or equivalent medium and a purified compound represented by formula I or

a purified compound represented by formula II, or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

It is contemplated that the formulation of the sixth embodiment can contain any of the purified compounds contemplated in the present invention either alone or
5 in combination with another purified compound of the present invention.

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

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

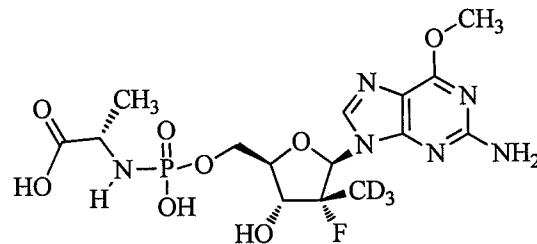
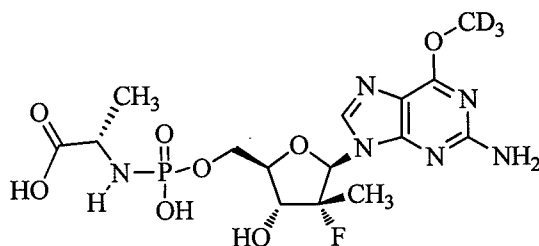
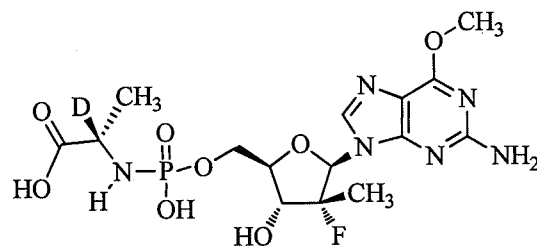
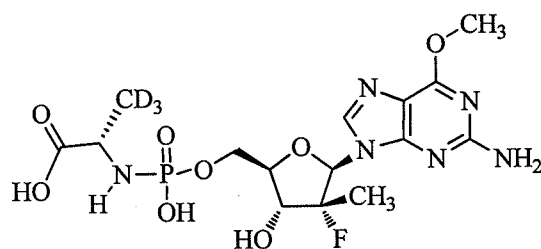
30 The term "excipient" as used herein refers to a compound that is used to prepare a pharmaceutical composition, and is generally safe, non-toxic and neither

biologically nor otherwise undesirable, and includes excipients that are acceptable for veterinary use as well as human pharmaceutical use. The purified compounds of this invention can be administered alone but will generally be administered in admixture with one or more suitable pharmaceutical excipients, diluents or carriers
5 selected with regard to the intended route of administration and standard pharmaceutical practice.

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

It is also contemplated that the compound represented by formula I or the
30 compound represented by formula II embraces deuterated analogs. The term "deuterated analogs" means a compound described herein or its salts thereof, whereby a ^1H -isotope, i.e., hydrogen (H), is substituted by a ^2H -isotope, i.e.,

deuterium (D). Deuterium substitution can be partial or complete. Partial deuterium substitution means that at least one hydrogen is substituted by at least one deuterium. For instance, for a compound represented by formula 14, one of ordinary skill can contemplate at least the following partial deuterated analogs (where " d_n " represents n-number of deuterium atoms, such as, for an isopropyl group $n = 1-7$, while for a phenyl group, $n = 1-5$). Although the methyl groups depicted below are shown as being completely deuterated, one will recognize that partial-deuterated variations are also possible, such as, $-CDH_2$ and $-CD_2H$.



10

These are but a few deuterated analogs that are synthetically accessible by procedures and reagents that are known to one of ordinary skill.

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

form preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like. Examples of solid formulations are exemplified in EP 0524579; US 6,635,278; US 2007/0099902; US 7,060,294; US 2006/0188570; 5 US 2007/0077295; US 2004/0224917; US 7,462,608; US 2006/0057196; US 6,267,985; US 6,294,192; US 6,569,463; US 6,923,988; US 2006/0034937; US 6,383,471; US 6,395,300; US 6,645,528; US 6,932,983; US 2002/0142050; US 2005/0048116; US 2005/0058710; US 2007/0026073; US 2007/0059360; and US 2008/0014228, each of which is incorporated by reference.

10 Liquid formulations also are suitable for oral administration include liquid formulation including emulsions, syrups, elixirs and aqueous suspensions. These include solid form preparations which are intended to be converted to liquid form preparations shortly before use. Emulsions may be prepared in solutions, for example, in aqueous propylene glycol solutions or may contain emulsifying agents 15 such as lecithin, sorbitan monooleate, or acacia. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well known suspending agents.

The purified compounds of the present invention may be formulated for 20 administration as suppositories. A low melting wax, such as a mixture of fatty acid glycerides or cocoa butter is first melted and the active component is dispersed homogeneously, for example, by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and to solidify.

The purified compounds of the present invention may be formulated for 25 vaginal administration. Pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Suitable formulations along with pharmaceutical carriers, diluents and excipients are described in *Remington: The Science and Practice of Pharmacy 1995*, 30 edited by E. W. Martin, Mack Publishing Company, 19th edition, Easton, Pennsylvania, which is hereby incorporated by reference. A skilled formulation

scientist may modify the formulations within the teachings of the specification to provide numerous formulations for a particular route of administration without rendering the compositions of the present invention unstable or compromising their therapeutic activity.

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

- Additionally, the purified compounds of the present invention may be formulated in conjunction with liposomes or micelles. As to liposomes, it is contemplated that the purified compounds can be formulated in a manner as disclosed in U.S. Patent Nos. 5,013,556; U.S. 5,213,804; 5,225,212;
- 15 5,891,468; 6,224,903; 6,180,134; 5,192,549; 5,316,771; 4,797,285; 5,376,380; 6,060,080; 6,132,763; 6,653,455; 6,680,068; 7,060,689; 7,070,801; 5,077,057; 5,277,914; 5,549,910; 5,567,434; 5,077,056; 5,154,930; 5,736,155; 5,827,533; 5,882,679; 6,143,321; 6,200,598; 6,296,870; 6,726,925; and 6,214,375, each of
- 20 which is incorporated by reference. As to micelles, it is contemplated that the purified compounds can be formulated in a manner as disclosed in U.S. Patent Nos. 5,145,684 and 5,091,188, both of which are incorporated by reference.

- A seventh embodiment of the present invention is directed to a use of a purified compound represented by formula I or a purified compound represented by
- 25 formula II, or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof, in the manufacture of a medicament for the treatment of any condition the result of an infection by any one of the following viral agents: hepatitis C virus, West Nile virus, yellow fever virus, degue virus, rhinovirus, polio virus, hepatitis A virus, bovine viral diarrhea virus and Japanese encephalitis virus.

- 30 The term "medicament" means a substance used in a method of treatment and/or prophylaxis of a subject in need thereof, wherein the substance includes, but

is not limited to, a composition, a formulation, a dosage form, and the like, comprising the compound of formula I. It is contemplated that the use of the compound represented by formula I in the manufacture of a medicament, for the treatment of any of the antiviral conditions disclosed herein, either alone or in
5 combination with another compound of the present invention. A medicament includes, but is not limited to, any one of the compositions contemplated by the seventh embodiment of the present invention.

An eighth embodiment of the present invention is directed to a method of treatment and/or prophylaxis in a subject in need thereof said method comprises
10 administering a therapeutically effective amount of a purified compound represented by formula I or a purified compound represented by formula II, or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof to the subject.

A first aspect of the eighth embodiment is directed to a method of treatment and/or prophylaxis in a subject in need thereof said method comprises administering
15 a therapeutically effective amount of at least two compounds falling within the scope of a purified compound represented by formula I or a purified compound represented by formula II, or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof to the subject.

A second aspect of the eighth embodiment is directed to a method of treatment and/or prophylaxis in a subject in need thereof said method comprises alternatively or concurrently administering a therapeutically effective amount of at least two compounds falling within the scope of a purified compound represented by
20 formula I or a purified compound represented by formula II, or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof to
25 the subject.

It is intended that a subject in need thereof is one that has any condition the result of an infection by any of the viral agents disclosed herein, which includes, but is not limited to, hepatitis C virus, West Nile virus, yellow fever virus, dengue virus,
30 rhinovirus, polio virus, hepatitis A virus, bovine viral diarrhea virus or Japanese

encephalitis virus, flaviviridae viruses or pestiviruses or hepaciviruses or a viral agent causing symptoms equivalent or comparable to any of the above-listed viruses.

The term "subject" means a mammal, which includes, but is not limited to, cattle, pigs, sheep, chicken, turkey, buffalo, llama, ostrich, dogs, cats, and humans, preferably the subject is a human. It is contemplated that in the method of treating a subject thereof of the ninth embodiment can be any of the compounds contemplated herein, either alone or in combination with another compound of the present invention.

The term "therapeutically effective amount" as used herein means an amount required to reduce symptoms of the disease in an individual. The dose will be adjusted to the individual requirements in each particular case. That dosage can vary within wide limits depending upon numerous factors such as the severity of the disease to be treated, the age and general health condition of the patient, other medicaments with which the patient is being treated, the route and form of administration and the preferences and experience of the medical practitioner involved. For oral administration, a daily dosage of between about 0.001 and about 10 g, including all values in between, such as 0.001, 0.0025, 0.005, 0.0075, 0.01, 0.025, 0.050, 0.075, 0.1, 0.125, 0.150, 0.175, 0.2, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, and 9.5, per day should be appropriate in monotherapy and/or in combination therapy. A particular daily dosage is between about 0.01 and about 1 g per day, including all incremental values of 0.01 g (i.e., 10 mg) in between, a preferred daily dosage about 0.01 and about 0.8 g per day, more preferably about 0.01 and about 0.6 g per day, and most preferably about 0.01 and about 0.25 g per day, each of which including all incremental values of 0.01 g in between. Generally, treatment is initiated with a large initial "loading dose" to rapidly reduce or eliminate the virus following by a decreasing the dose to a level sufficient to prevent resurgence of the infection. One of ordinary skill in treating diseases described herein will be able, without undue experimentation and in reliance on personal knowledge, experience and the disclosures of this application, to ascertain a therapeutically effective amount of the compounds of the present invention for a given disease and patient.

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

10 A third aspect of the eighth embodiment, is directed to a method of treatment and/or prophylaxis in a subject in need thereof said method comprises administering to the subject a therapeutically effective amount of a purified compound represented by formula I or a purified compound represented by formula II, or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof
15 and a therapeutically effective amount of another antiviral agent; wherein the administration is concurrent or alternative. It is understood that the time between alternative administration can range between 1-24 hours, which includes any sub-range in between including, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 23 hours. Examples of "another antiviral agent" include, but are
20 not limited to: HCV NS3 protease inhibitors (see WO 2008010921, WO 2008010921, EP 1881001, WO 2007015824, WO 2007014925, WO 2007014926, WO 2007014921, WO 2007014920, WO 2007014922, US 2005267018, WO 2005095403, WO 2005037214, WO 2004094452, US 2003187018, WO 200364456, WO 2005028502, and WO 2003006490); HCV NS5B Inhibitors (see
25 US 2007275947, US20072759300, WO2007095269, WO 2007092000, WO 2007076034, WO 200702602, US 2005-98125, WO 2006093801, US 2006166964, WO 2006065590, WO 2006065335, US 2006040927, US 2006040890, WO 2006020082, WO 2006012078, WO 2005123087, US 2005154056, US 2004229840, WO 2004065367, WO 2004003138, WO 2004002977, WO
30 2004002944, WO 2004002940, WO 2004000858, WO 2003105770, WO 2003010141, WO 2002057425, WO 2002057287, WO 2005021568, WO 2004041201, US 20060293306, US 20060194749, US 20060241064, US 6784166, WO 2007088148, WO 2007039142, WO 2005103045, WO 2007039145, WO

2004096210, and WO 2003037895); HCV NS4 Inhibitors (see WO 2007070556 and WO 2005067900); HCV NS5a Inhibitors (see US 2006276511, WO 2006120252, WO 2006120251, WO 2006100310, WO 2006035061); Toll-like receptor agonists (see WO 2007093901); and other inhibitors (see WO 2004035571, WO 5 2004014852, WO 2004014313, WO 2004009020, WO 2003101993, WO 2000006529); and compounds disclosed in U.S. Patent Application No. 12/053,015, filed March 21, 2008 (the contents of which are incorporated by reference).

A fourth aspect of the eighth embodiment, is directed to a method of treatment and/or prophylaxis in a subject in need thereof said method comprises 10 alternatively or concurrently administering a therapeutically effective amount of a purified compound represented by formula I or a purified compound represented by formula II, or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof to the subject. It is understood that the time between alternative administration can range between 1-24 hours, which includes any sub- 15 range in between including, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 23 hours.

It is contemplated that the another antiviral agent such as interferon- α , interferon- β , pegylated interferon- α , ribavirin, levovirin, viremagine, another nucleoside HCV polymerase inhibitor, a HCV non-nucleoside polymerase inhibitor, 20 a HCV protease inhibitor, a HCV helicase inhibitor or a HCV fusion inhibitor. When the active compound or its derivative or salt are administered in combination with another antiviral agent the activity may be increased over the parent compound. When the treatment is combination therapy, such administration may be concurrent or sequential with respect to that of the nucleoside derivatives. "Concurrent 25 administration" as used herein thus includes administration of the agents at the same time or at different times. Administration of two or more agents at the same time can be achieved by a single formulation containing two or more active ingredients or by substantially simultaneous administration of two or more dosage forms with a single active agent.

30 It should be noted that for the purified compounds disclosed herein may form metabolites upon administration to a subject in need thereof. For instance, the phosphoramidate moiety may undergo hydrolysis to form a monophosphate

derivative, which may then undergo phosphorylation to provide the diphosphate and the triphosphate derivative, which under physiological conditions would be present in the form of physiological salts. As metabolites or metabolite salts are contemplated herein, it is also contemplated as an alternative embodiment a method of treatment in a patient in need thereof contacting at least one metabolite obtained from the purified compound represented by formula I or at least one metabolite obtained from the purified compound represented by formula II with at least one hepatitis C virus infected cell.

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

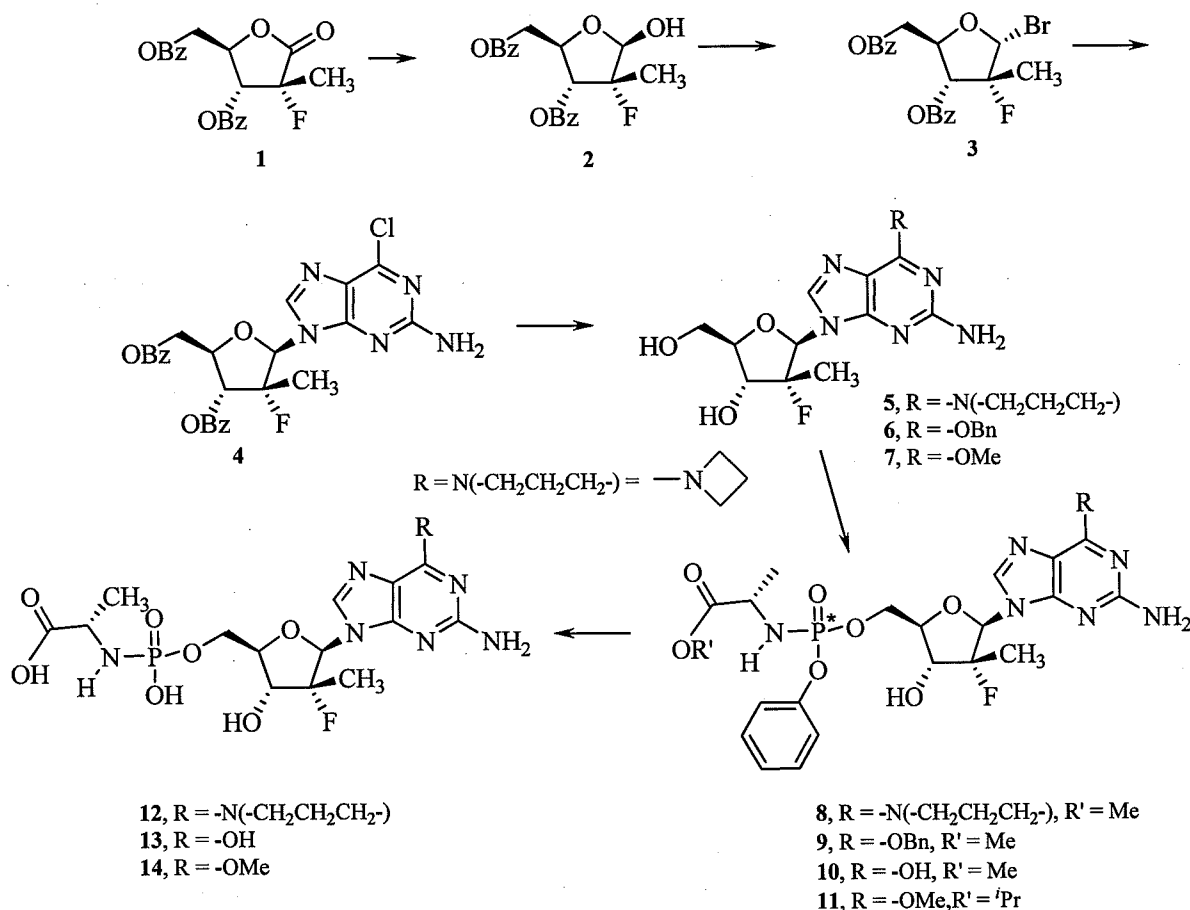
EXAMPLES

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

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

UPLC analysis: Acquity UPLC BEH C18 1.7 μ m 2.1 $\times$ 50 mm column; 0 to 2 min, 5 to 95% (0.1% formic acid in acetonitrile) in 0.1% formic acid in water; 2 to 2.4 min, 95 to 5% (0.1% formic acid in acetonitrile) in 0.1% formic acid in water; 2.4 to 3 min, 5% (0.1% formic acid in acetonitrile) in 0.1% formic acid in water.

The following scheme is intended to serve as a visual aid for the following discussion and is not intended to limit the scope of the appended claims. A general procedure for preparing phosphoramidate nucleosides is disclosed in U.S. Patent Application No. 12/053,015, filed March 21, 2008, at pages 651-655, the contents of which are incorporated by reference.



Compound (1) can be obtained by a process disclosed at page 5 in U.S.

- 5 Published Application No. 2008/0139802 (which corresponds to WO 2008/045419),
 at pages 11-13 in WO 2006/012440, and at pages 20-22 and 30-31 in WO
 2006/031725, each of which is hereby incorporated by reference.

10 **Synthesis of ((2R,3R,4R,5R)-3-(benzoyloxy)-4-fluoro-5-hydroxy-4-methyltetrahydrofuran-2-yl)methyl benzoate (2)**

- 15 To a 5 L of dry three-neck round-bottomed flask fit with a mechanical stirrer,
 addition funnel and thermometer was charged the lactone ((2R,3R,4R)-3-(
 (benzoyloxy)-4-fluoro-4-methyl-5-oxotetrahydrofuran-2-yl)methyl benzoate) (1,
 379 g, 1.018 mol). The solid was dissolved in anhydrous THF (1.75 L) and cooled
 to -30°C under a nitrogen atmosphere. A solution of lithium tri-tert-
 butoxyaluminumhydride (1.0 M in THF, 1.527 L) was added to the lactone solution

while stirring over 1 h and maintaining the -30°C temperature. After finishing the addition, the temperature was slowly increased and the reaction was followed by TLC (lactol R_f 0.4, 30% EtOAc in hexanes). The reaction was complete after 1h 15 min (temperature reached -10°C). The reaction was quenched by addition of Ethyl acetate (900 mL) via addition funnel. Sat. NH_4Cl (40 mL) was added at 0°C . The cloudy mixture was decanted into a 10 L round-bottomed flask. The solid residue left behind was filtered and washed with ethyl acetate (2x200 mL). The filtrate was combined with the decanted solution and the combined solution was concentrated under reduced pressure. The oily residue was dissolved in ethyl acetate (2 L) and washed with 3 N HCl (600 mL). The aqueous layer was back-extracted with ethyl acetate (3x400 mL). The combined organic layer was washed with water (3x800 mL), sat. NaHCO_3 (400 mL) and brine (400 mL). The organic solution was dried over MgSO_4 , filtered and concentrated under reduced pressure to afford a light brown oily residue. The residue was purified by plug column (2.2 kg of 40-63 micron silica gel, packed in a 6 L sintered glass funnel, 22 cm length of silica gel, diameter 15 cm) using suction and a step-gradient of 5%, 10%, 20%, and 30% ethyl acetate in hexanes –ca 5 L of each). The product containing fractions were combined and concentrated under reduced pressure to a colorless, very thick liquid (310.4 g).

The liquid slowly solidified after adding crystalline beta product as seeds (ca 100 mg spread out) under vacuum (0.2 mmHg) at 50°C . The process of solidification was complete in 20 hours at 50°C with or without vacuum. The white solid thus collected (293.8 g, 77%) has a mp of $79-80^{\circ}\text{C}$ and ratio of β/α is 20:1 based on NMR.

$^1\text{H-NMR}$ ($\text{DMSO}-d_6$) β -isomer, $\delta = 5.20$ (dd, 1 H, OH); α -isomer, $\delta = 5.40$ (dd, 1 H, OH). (β -lactol) ($\text{DMSO}-d_6$): δ 7.99 (m, 2 H, arom.), 7.93 (m, 2 H, arom.), 7.70 (m, 1 H, arom.), 7.61 (m, 1 H, arom.), 7.55 (m, 2 H, arom.), 7.42 (m, 2 H, arom.), 7.32 (dd, 1 H, C1-H), 5.54 (dd, 1 H, C3-H), 5.20 (dd, 1 H, OH), 4.55-4.50 (m, 1 H, C5-Ha), 4.46-4.40 (m, 2 H, C5-Hb and C4-H), 1.42 (d, 3 H, CH_3).

Synthesis of ((2R,3R,4R,5R)-3-(benzoyloxy)-5-bromo-4-fluoro-4-methyltetrahydrofuran-2-yl)methyl benzoate (3)

Anhydrous dichloromethane (5.6 L) was charged into a reactor and cooled to
5 -22°C or below. Triphenylphosphine (205.4 g, 0.783 mol) was added to the cold
solvent and the suspension was stirred to form a solution. The lactol (2, 209.4 g,
0.559 mol) in solid form was added to the cold solution and stirred for 15 mins.
Carbon tetrabromide (278.2 g, 0.839 mol) was added portion-wise while maintaining
the temperature of the solution between -22°C to -20 °C under a flow of nitrogen gas
10 (approx. 30 min). After finishing the addition of CBr₄, the temperature was slowly
raised to -17°C over 20 mins. The reaction was judged to be >95% complete by
TLC (R_fs 0.61 (α), 0.72 (β), 0.36 lactol; 20% EtOAc in hexanes). The reaction
solution was immediately transferred to a vessel containing 230 g of flash
chromatography grade silica gel (40-63 microns). The stirred mixture was
15 immediately passed through a pad of silica gel (680 g) in a 2.5 L sintered glass
Buchner funnel. The filtrate was concentrated under reduced pressure to about 800
mL and the ratio of α/β isomers of the crude product was 10:1 as determined by ¹H-
NMR. (CDCl₃) δ = 6.35, (s, α C1-H), 6.43, (d, β C1-H). The residue was purified
by plug column chromatography using 2.1 kg of silica gel in a 6 L sintered glass
20 Buchner funnel and eluted (via suction) with a stepwise gradient elution of 1%, 5%,
8% 12% EtOAc in hexane (ca 4 L each) to remove non-polar impurities followed by
12%, 25% EtOAc in hexane (6 L total) to elute the product. The product containing
fractions were combined into two fractions, concentrated under reduced pressure,
dried under vacuum (0.1 mmHg, ambient temp., 20 h) to colorless oils. Main
25 fraction (197 g, 89% α/β = 20:1). The alpha isomer crystallized from a small
portion of the oil upon standing at 0°C for several weeks to give large, thin plates,
mp 59-61°C. The pure beta isomer crystallized from a mixture of alpha and beta
product oil from an earlier less selective run to give needles, mp 77-79°C.

¹H-NMR (β-bromide) (CDCl₃): δ = 8.08 (m, 2 H, arom.), 8.04 (m, 2 H,
30 arom.), 7.62 (m, 1 H, arom.), 7.54-7.45 (m, 3 H, arom.), 7.35 (m, 2 H, arom.), 6.43
(d, 1 H, C1-H), 6.04 (dd, 1 H, C3-H), 4.78-4.73 (m, 2 H, C4-H and C5-Ha), 4.63-
4.58 (m, 1 H, C5-Hb), 1.76 (d, 3 H, CH₃). α-bromide, α/β = 20:1) (CDCl₃): δ 8.13
(m, 2 H, arom.), 8.02 (m, 2 H, arom.), 7.63-7.56 (m, 2 H, arom.), 7.50-7.42 (m, 4 H,

arom.), 6.34 (s, 1 H, C1-H), 5.29 (dd, 1 H, C3-H), 4.88 (m, 1 H, C4-H), 4.78 (dd, 1 H, C5-Ha), 4.63 (dd, 1 H, C5-Hb), 1.72 (d, 3 H, CH₃).

Synthesis of (2R,3R,4R,5R)-5-(2-amino-6-chloro-9H-purin-9-yl)-2-

5 (benzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl benzoate (4)

To a 12 L of three-neck round-bottomed flask was charged 6-chloro-2-aminopurine (225.4 g, 1.329 mol). Anhydrous tert-BuOH (4.5 L) was added and the solution was stirred with a mechanical stirrer at ambient temperature. Potassium
10 tert-butoxide (solid, 151.6 g, 1.35 mol) was added portion-wise under a flow of nitrogen gas while stirring. The mixture was stirred at RT for an additional 30 min. To a 5 L round-bottomed flask was loaded the α -bromide (**3**, 197 g, 0.451 mol) and 3 L of anhydrous acetonitrile at ambient temperature. The bromide solution was added to the purine base suspension over 1 min at ambient temperature. The 5L flask
15 was rinsed with acetonitrile (2x1L) to transfer bromide completely to the reaction mixture. The mixture was heated gradually to 50°C over 2 h with a heating mantle and controller, and stirred for 20 h. The reaction was almost complete as shown by TLC beta (R_f 0.28, 30% EtOAc in hexanes). The reaction was quenched by the addition of sat. NH₄Cl (200 mL) to form a suspension. The suspended solid¹ was
20 removed by filtration through a 3 cm pad of Celite in a 2.5 L porcelain Buchner funnel. The solid was washed with toluene (3x100 mL). The combined filtrate was neutralized by adding 6 N HCl solution until pH 7 (approx 220 mL). The mixture was concentrated under reduced pressure. When the volume of mixture was reduced to about one-third volume, additional precipitated solid was removed by filtration in
25 a similar manner. The filtrate was further concentrated to a volume of about 800 mL. The residue was loaded onto a plug column (1.6 kg flash grade silica gel in a 6 L sintered glass Buchner funnel) and eluted (via suction) with a gradient of 10% ethyl acetate in hexanes (6 L) to remove non-polar impurities, 30% ethyl acetate in hexanes to afford a small amount of lactol (6 L), and then 40%~45% ethyl acetate in
30 hexanes (4 L) to elute the main amount of product. The product containing fractions were combined, concentrated under reduced pressure and dried under vacuum (0.2 mmHg, 24 h, ambient temp.) to a white foam solid (150.7 g, $\beta/\alpha = 14:1$ by NMR.

¹H-NMR. (CDCl₃) beta: δ = 1.33 (d, 22.4 Hz, 2'-C-CH₃), alpha: 1.55 (d, 22 Hz, 2'-C-CH₃).

The product mixture foam was dissolved in methanol (700 mL) at ambient temperature. Upon standing, a solid slowly formed over 2 h. The suspension was cooled in a freezer to -5°C for 17 h. The resulting white solid was collected by filtration and washed with cold MeOH (-5°C, 3x60 mL) and ethyl ether (3x100 mL).

- 5 The solid was dried under vacuum (0.2 mmHg, 24 h, ambient temp.) to afford 110.5 g of β -product with excellent de (β/α 99.8:1 by HPLC). The filtrate was partially concentrated (ca. 400 mL) and then diluted with more MeOH (400 mL) while heating to 60°C. The solution was cooled down to ambient temperature, seeded and the cooled to -5°C. The second crop was collected, washed and dried in a similar
- 10 manner to give more product as a white solid (12.26 g) with similar diastereomeric purity. The mother liquor was concentrated to dryness under reduced pressure (ca. 25 g). The residue was a mixture of β and α -isomers. It was subjected to automated silica gel column chromatography (Analogix, 240 g cartridge, 40% to 50% ethyl acetate in hexanes) to afford 14.52 g of product foam which was recrystallized from
- 15 MeOH, washed and dried in a similar manner to afford an additional 8.46 g of product in high purity.

The three solids were judged to be of similar purity and they were combined to give 131.2 g of white crystalline product 4, (55% from bromosugar, 49% from lactol). Mp 160.5-162.0°C. HPLC purity 99.5% including 0.20 % alpha.

- 20 $^1\text{H-NMR}$ (pure β -anomer, CDCl_3): δ = 8.03 (m, 2 H, arom.), 7.93 (m, 2 H, arom.), 7.88 (s, 1 H, C8-H), 7.60 (m, 1 H, arom.), 7.50 (m, 1 H, arom.), 7.44 (m, 2 H, arom.), 7.33 (m, 2 H, arom.), 6.44 (dd, 1 H, C1'-H), 6.12 (d, 1 H, C3'-H), 5.35 (s, 2 H, NH_2), 5.00 (dd, 1 H, C5'-Ha), 4.76 (m, 1 H, C4'-H), 4.59 (dd, 1 H, C5'-Hb), 1.33 (d, 3 H, CH_3).

- 25 $^1\text{H-NMR}$ (α -isomer, CDCl_3): δ = 8.11-8.09 (m, 3 H, arom. and C8-H), 8.01 (m, 2 H, arom.), 7.63 (m, 1 H, arom.), 7.55 (m, 1 H, arom.), 7.48 (m, 2 H, arom.), 7.39 (m, 2 H, arom.), 6.35 (d, 1 H, C1'-H), 5.76 (dd, 1 H, C3'-H), 5.18 (s, 2 H, NH_2), 4.93-4.89 (m, 1 H, C4'-H), 4.75-4.71 (m, 1 H, C5'-Ha), 4.58-4.54 (m, 1 H, C5'-Hb), 1.55 (d, 3 H, CH_3).

30

Synthesis of (2R,3R,4R,5R)-5-(2-amino-6-(azetidin-1-yl)-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (5)

To a 350 mL of dry seal pressure flask (Chemglass) were added
5 (2R,3R,4R,5R)-5-(2-amino-6-chloro-9H-purin-9-yl)-2-(benzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl benzoate (**4**, 3.6 g, 6.85 mmol) and 150 mL of absolute ethanol. Azetidine hydrochloride (2.56 g, 27.4 mmol) was added and then followed by triethylamine (4.16 g, 41.1 mmol). The suspension was stirred and heated to 70°C while sealed for 5 hours. All the starting material was consumed but
10 the benzoyl groups remained as shown by TLC. Sodium methoxide (7.8 mL, 34.3 mmol, 25% solution in methanol) was added to the mixture and heated at 50°C. The reaction was complete after 3.5 h. The reaction mixture was allowed to cool to room temperature and neutralized by addition of glacial acetic acid (0.41 g, 6.85 mmol). The mixture was concentrated under reduced pressure and then the residue was
15 triturated with ethyl acetate. The resulting solid was removed by filtration and the solid was washed with EtOAc (2x 15 mL). The filtrate was concentrated under reduced pressure and the residue was purified via column chromatography (Analogix, 120 g cartridge, gradient of 0 to 15% MeOH in DCM). The pure product containing fractions were combined, concentrated under reduced pressure and dried
20 (50°C, 0.2 mmHg, 17 h) to a light pink colored foam solid (2.15 g, 6.35 mmol, 93%).

¹H-NMR (DMSO-*d*₆) δ = 8.00 (s, 1 H, C8-H), 6.03 (s, 2 H, NH₂), 6.00 (d, 1 H, C1'-H), 5.64 (d, 1 H, 3'-OH), 5.24 (t, 1 H, 5'-OH), 4.24-4.10 (m, 5 H, N-CH₂ of azetidine, C3'-H), 3.90-3.81 (m, 2 H, C4'-H and C5'-H_a), 3.69-3.64 (m, 1 H, C5'-H_b), 2.37 (penta, 2 H, center CH₂ of azetidine), 1.05 (d, 3 H, C2'-CH₃),
25

Synthesis of (2R,3R,4R,5R)-5-(2-amino-6-(benzyloxy)-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (6)

30 To a 500 mL of dry round-bottomed flask were added (2R,3R,4R,5R)-5-(2-amino-6-chloro-9H-purin-9-yl)-2-(benzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl benzoate (**4**, 8.0 g, 15.2 mmol) and anhydrous benzyl alcohol (128 mL). To another 250 mL of dry round-bottomed flask were charged NaH (60% in mineral oil, 2.44 g, 60.8 mmol) and anhydrous DMF (40 mL). The

suspension was stirred at 0°C in an ice-water bath. Benzyl alcohol (27 mL) was added drop-wise via a syringe. A solution was slowly formed and it was transferred to the nucleoside suspension quickly under a nitrogen atmosphere at room temperature. The mixture was heated to 50°C and stirred. The reaction was complete after 3 h and cooled to ambient temperature. It was neutralized by the addition of 4 N HCl to *ca.* pH=7 (12 mL). The solution was concentrated under reduced pressure (4 mbar, 90°C bath). The cloudy residue was diluted with dichloromethane (100 mL) and washed with water (3x 30 mL), brine (30 mL) and dried over Na₂SO₄. The suspension was filtered and the filtrate was concentrated under reduced pressure to an oily residue. This was purified by column chromatography (Analogix, 0 to 8% gradient of MeOH in DCM). The product eluted at 4% MeOH in DCM. The product containing fractions were combined, concentrated under reduced pressure and dried (50°C, 0.2 mmHg, 17 h) to a white foam solid (4.57 g, 11.7 mmol, 77.2%).

¹H-NMR (DMSO-*d*₆) δ = 8.18 (s, 1 H, 8-H), 7.53-7.51 (m, 2 H, arom-H), 7.43-7.34 (m, 3 H, arom-H), 6.66 (s, 2 H, NH₂), 6.05 (d, 1 H, C1'-H), 5.67 (d, 1 H, 3'-OH), 5.48 (dd, 2 H, CH₂ of Benzyl), 5.25 (t, 1 H, 5'-OH), 4.18 (dt, 1 H, C3'-H), 3.92-3.82 (m, 2 H, C4'-H and C5'-H_a), 3.71-3.66 (m, 1 H, C5'-H_b), 1.07 (d, 3 H, C2'-CH₃).

Synthesis of (2R,3R,4R,5R)-5-(2-amino-6-methoxy-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (7)

To a suspension of 4 (500 mg, 0.951 mmol) in MeOH (10 ml) was added NaOMe in MeOH (25%, 0.90 ml). The mixture was heated to 50°C for 3 h. Then, it was neutralized with AcOH. It was concentrated and EtOAc (30 ml) was added. The insoluble solid was filtered off. The filtrate was concentrated and purified by flash column chromatography on silica gel using 0 to 15% MeOH in CH₂Cl₂ as eluents. Compound 7 (203 mg, 68.3%) was obtained as a white solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.17 (s, 1H), 6.61 (s, 2H), 6.05 (d, *J* = 17.6 Hz, 1H), 5.67 (d, *J* = 7.2 Hz, 1H), 5.25 (t, *J* = 4.8 Hz, 1H), 4.18 (dt, *J* = 25.6, 8.0 Hz, 1H), 3.96 (s, 3H), 3.91 (dm, *J* = 9.2 Hz, 1H), 3.85 (ddd, *J* = 2.0, 5.2, 12.8 Hz, 1H), 3.69 (ddd, *J* = 2.8, 3.2, 12.0 Hz, 1H), 1.06 (d, *J* = 22.8 Hz, 3H); *t*R = 0.52 (99.1%); LRMS (ESI) [M+H]⁺ calculated for C₁₂H₁₇FN₅O₄ 314.3, found 314.2.

Synthesis of (2S)-methyl 2-((((2R,3R,4R,5R)-5-(2-amino-6-(azetidin-1-yl)-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphorylamino)-propanoate (8)

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To a 100 mL dry round-bottomed flask were added phenyl dichlorophosphate (1.72 g, 8.15 mmol) and anhydrous dichloromethane (17 mL). The amino ester (1.42 g, 10.2 mmol) was added and the suspension was cooled to -5°C. *N*-Methylimidazole (3.34 g, 40.7 mmol) was added via a syringe in one portion and the solution was stirred at -5°C for 1 h under a nitrogen atmosphere. The nucleoside (**5**, 1.38 g, 4.07 mmol) (foam solid) was then added in one portion and the solution was allowed to warm up over 1 h to ambient temperature. After 4 h at ambient temperature, TLC (5% MeOH in DCM) indicated an incomplete reaction (about 30% SM remained) but also a growing less polar impurity. The reaction was quenched by the addition of sat NH₄Cl (20 mL) and diluted with dichloromethane (20 mL). The organic layer was separated and washed with water (5x 30 mL), brine (20 mL) and dried over Na₂SO₄. The product containing solution was filtered and concentrated under reduced pressure to a crude oily residue, 3.26 g. This was purified by column chromatography (Analogix, 40 g cartridge, gradient of MeOH in DCM from 0% to 10%). The product eluted at 4% MeOH in DCM. The pure product containing fractions were combined, concentrated under reduced pressure and dried (50°C, 0.2 mmHg, 17 h) to a white foam solid (1.322 g, 2.28 mmol, 56%). HPLC purity 99.25%. NMR spectra of product showed it is a mixture of two diastereoisomers with a ratio of 55:45.

¹H-NMR (DMSO-*d*₆) δ = 7.80 (s, 1 H, 8-H of one isomer), 7.80 (s, 1 H, 8-H of another isomer), 7.38-7.33 (m, 2 H, arom-H), 7.22-7.14 (m, 3 H, arom-H), 6.09 (s, 2 H, NH₂), 6.12-6.02 (m, 2 H, C1'-H and NH), 5.83 (d, 1 H, 3'-OH of one isomer), 5.77 (d, 1 H, 3'-OH of another isomer), 4.46-4.05 (m, 8 H, NCH₂ of azetidine, α-H of aminoester, C3'-H, C4'-H, C5'-H_a), 3.89-3.79 (m, 1 H, C5'-H_b), 3.56 (s, 3 H, OCH₃ of aminoester in one isomer), 3.54 (s, 3 H, OCH₃ of aminoester in another isomer), 2.37 (penta, 2 H, center CH₂ of azetidine), 1.21 (d, 3 H, α-CH₃ of aminoester in one isomer), 1.19 (d, 3 H, α-CH₃ of aminoester in another isomer), 1.08 (d, 3 H, C2'-CH₃).

³¹P NMR (DMSO-*d*₆): δ 4.85 (one isomer), 4.77 (other isomer).

Synthesis of (2S)-methyl 2-((((2R,3R,4R,5R)-5-(2-amino-6-(benzyloxy)-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphorylamino)-propanoate (9)

5

To a 100 mL of dry round-bottomed flask are charged phenyl dichlorophosphate (3.29 g, 15.58 mmol) and anhydrous dichloromethane (24 mL). The aminoester tosylate (white powder) is added and the solution was cooled to -5°C under nitrogen. *N*-Methylimidazole (4.92 g, 59.94 mmol) is added via a dry
10 syringe in one portion and the resulted colorless clear solution is stirred at -5°C for one hour. Then the nucleoside (6) solid is added (2.334 g, 5.99 mmol) to the solution under nitrogen in one portion and the mixture is allowed to warm to ambient temperature to give a colorless solution. Reaction progress is monitored by TLC (5% methanol in dichloromethane). The reaction is still quenched by the
15 addition of dichloromethane (30 mL) and 1 *N* HCl (60 mL). The organic layer is separated and the aqueous layer is extracted with dichloromethane (2x20 mL). The combined organic layer is washed with water (2x40 mL), sat NaHCO₃ (30 mL), water, and brine. The organic layer is dried over Na₂SO₄. After removal of solid by filtration, the filtrate is concentrated under reduced pressure to a gummy residue
20 (7.28 g). The residue is purified via column chromatography (Analogix, 80 g cartridge, gradient of 0 to 10% MeOH in DCM). The product is eluted at 2% MeOH in DCM. The product containing fractions are combined and are concentrated under reduced pressure and are dried (50°C, 0.2 mmHg, 17 h) to a white foam solid. A portion of the starting nucleoside (0.257 g) is also recovered. Yield is based on
25 consumed starting material.

Synthesis of (2S)-methyl 2-((((2R,3R,4R,5R)-5-(2-amino-6-hydroxy-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphorylamino)-propanoate (10)

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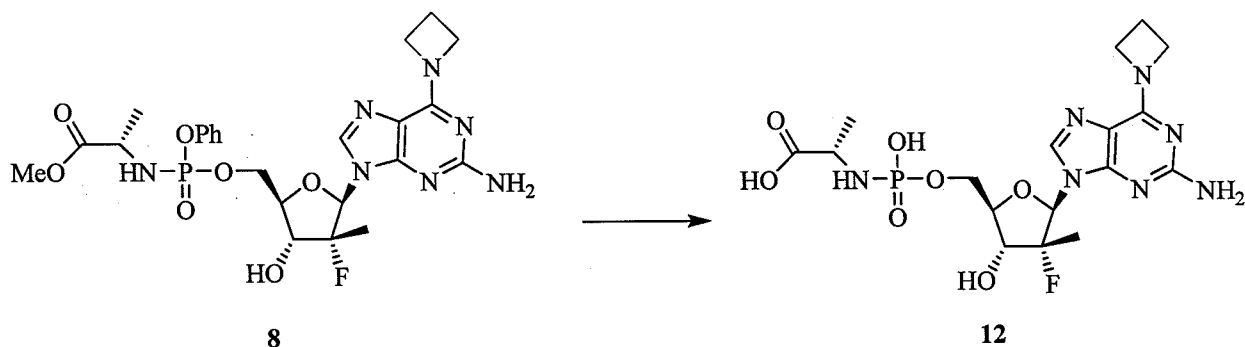
To a 250 mL of dry round-bottomed flask with starting material (9, 1.92 g, 2.8 mmol) is added anhydrous absolute ethanol (50 mL). Palladium on charcoal (10%, 120 mg) is added. The atmosphere in the flask is exchanged with hydrogen and the mixture is stirred under 1 atm of hydrogen gas for 3.5 h at room temperature. The reaction is judged complete by TLC and the Pd on charcoal is removed by

filtration and is washed with ethanol (2x 10 mL). The filtrate is concentrated under reduced pressure to a solid residue. The solid is mixed with silica gel (10 g) and is purified by column chromatography (Analogix, 40 g cartridge, gradient of 1% to 16% MeOH in DCM). The product containing fractions are combined, concentrated under reduced pressure and are dried (50°C, 0.2 mmHg, 17 h) to a white powder (1.43 g, 86%). HPLC purity 99.55%.

Synthesis of (2S)-isopropyl 2-((((2R,3R,4R,5R)-5-(2-amino-6-methoxy-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy) phosphorylamino)-propanoate (11)

To a solution of the **7** (50 mg, 0.16 mmol) and *N*-methylimidazole (0.10 ml, 1.3 mmol) in THF (1.5 ml) was added the phosphorochloridate in THF (1.0 M, 0.48 ml) at 0°C dropwise. The reaction was slowly warmed to room temperature and stirred for 1 h. Then water (0.1 ml) and EtOAc (5 ml) was added. The organic solution was washed with sat. aq. sodium citrate mono basic (2 ml x 2), sat. aq. NaHCO₃ (2 mL x 1), dried (MgSO₄) and concentrated. The crude oil was purified by flash column chromatography on silica gel using 0 to 8% iPrOH in CH₂Cl₂ as eluents to give the phosphoramidate (36 mg, 39%, 2:1 mixture of diastereomers) as a white solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.97 (s, 0.66H), 7.95 (s, 0.34H), 7.39-7.30 (m, 2H), 7.25-7.13 (m, 3H), 6.63 (bs, 2H), 6.11 (d, *J* = 18.8 Hz, 0.34H), 6.09 (d, *J* = 19.2 Hz, 0.66H), 6.06-5.88 (m, 1H), 5.90-5.78 (m, 1H), 4.80 (septet, *J* = 6.8 Hz, 1H), 4.45-4.24 (m, 3H), 4.16-4.05 (m, 1H), 3.96 (s, 3H), 3.84-3.70 (m, 1H), 1.28-1.03 (m including d at 1.20 with *J* = 7.2 Hz, 12H); ³¹P NMR (162 MHz, DMSO-*d*₆) δ 4.91, 4.72; *t*_R = 1.16 (33.1%), 1.18 (63.9%); LRMS (ESI) [M + H]⁺ calculated for C₂₄H₃₃FN₆O₈P 583.5, found 583.4.

Synthesis of (2S)-2-((((2R,3R,4R,5R)-5-(2-amino-6-(azetidin-1-yl)-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(hydroxy)phosphorylamino)propanoic acid (12)



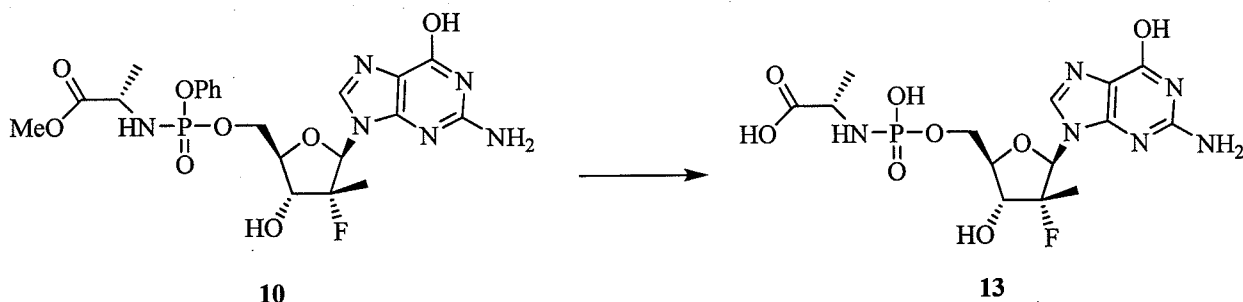
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Compound **8** (150 mg, 0.269 mmol) was suspended in triethylamine (2 mL) and water (0.5 mL), and heated at 60°C for 20 h. Volatile components were then evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 0 to 15% NH₄OH in iPrOH as eluents. The product was obtained as a white solid (101 mg, 80%): ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.93 (s, 1H), 6.10 (bs, 2H), 5.97 (d, *J* = 17.2 Hz, 1H), 4.44-3.86 (m, 10H), 2.37 (quintet, *J* = 7.6 Hz, 2H), 1.10 (d, *J* = 6.8 Hz, 3H), 1.05 (d, *J* = 22.4 Hz, 3H); ³¹P NMR (162 MHz, DMSO-*d*₆) δ 8.72; *t*_R = 0.55 (97.4%); LRMS (ESI) [*M* + *H*]⁺ calculated for C₁₇H₂₆FN₇O₇P 490.4, found 490.4.

15

Synthesis of (2S)-2-((((2R,3R,4R,5R)-5-(2-amino-6-hydroxy-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(hydroxy)phosphorylamino)propanoic acid (13)

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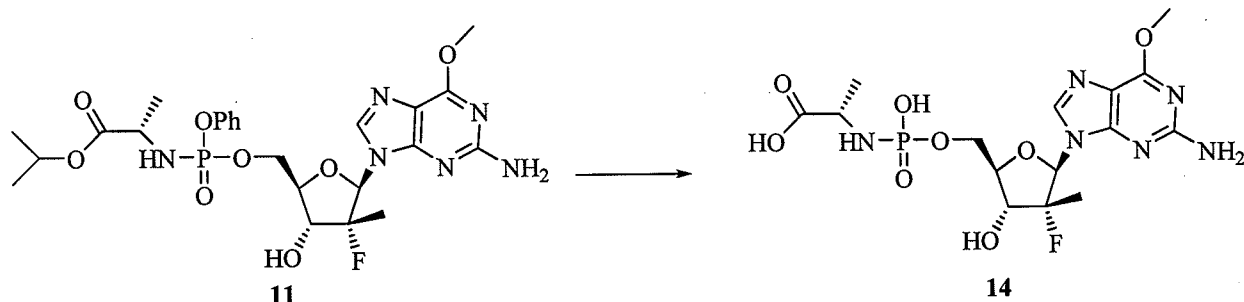
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Compound **10** (110 mg, 0.204 mmol) was suspended in triethylamine (2 mL) and water (0.5 mL), and heated at 60°C for 20 h. The volatile components were then evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 0 to 20% NH₄OH in iPrOH as eluents.

The product was obtained as a white solid (74 mg, 81%): ^1H NMR (400 MHz, DMSO- d_6) δ 7.88 (s, 1H), 6.68 (bs, 2H), 5.93 (d, J = 18.0 Hz, 1H), 4.42-3.83 (m, 6H), 1.11 (d, J = 7.2 Hz, 3H), 1.07 (d, J = 22.4 Hz, 3H); ^{31}P NMR (162 MHz, DMSO- d_6) δ 8.90; t_R = 0.50 (98.7%); LRMS (ESI) $[\text{M} + \text{H}]^+$ calculated for $\text{C}_{14}\text{H}_{21}\text{FN}_6\text{O}_8\text{P}$ 451.3, found 451.3.

Synthesis of (2S)-2-((((2R,3R,4R,5R)-5-(2-amino-6-methoxy-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(hydroxy)phosphorylamino)propanoic acid (14)

10



Compound 11 (110 mg, 0.189 mmol) was suspended in triethylamine (2 mL) and water (0.5 mL), and heated at 60°C for 48 h. The volatile components were then evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 0 to 20% NH_4OH in $i\text{PrOH}$ as eluents. The product was obtained as a white solid (88 mg, 97%): ^1H NMR (400 MHz, DMSO- d_6) δ 8.08 (s, 1H), 6.67 (bs, 2H), 6.03 (d, J = 18.0 Hz, 1H), 4.44-3.70 (m including s at 3.96, 8H), 3.39 (m, 1H), 1.10 (d, J = 7.2 Hz, 3H), 1.05 (d, J = 22.8 Hz, 3H); ^{31}P NMR (162 MHz, DMSO- d_6) δ 8.52; t_R = 0.61 (96.3%); LRMS (ESI) $[\text{M} + \text{H}]^+$ calculated for $\text{C}_{15}\text{H}_{23}\text{FN}_6\text{O}_8\text{P}$ 465.3, found 465.3.

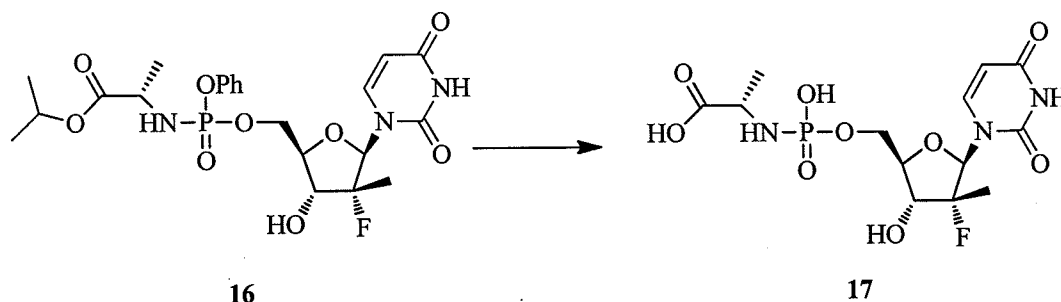
Synthesis of (2S)-2-((((2R,3R,4R,5R)-5-(2-amino-6-ethoxy-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(hydroxy)phosphorylamino)propanoic acid (15)

25

It is contemplated that compound 15 is prepared in a manner analogous to compound 14.

Synthesis of (2S)-2-((((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(hydroxy)phosphorylamino)propanoic acid (17)

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Compound 16 ((2S)-isopropyl 2-((((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl-amino)propanoate) was prepared according to the procedures disclosed at pages 652–669 of U.S. Patent Application No. 12/053,015 (Attorney Docket No. 60137.0034USU1) filed March 21, 2008 the identified subject matter of which is incorporated by reference in its entirety. (Compound 16 is identified as compound 25 at page 674 of 12/053,015.)

Compound 17 was synthesis from compound 15 by the following procedure.

Compound 16 (300 mg, 0.57 mmol) was suspended in triethylamine (6 mL) and water (1.5 mL), and heated at 60°C for 30 h. The volatile components were then evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 50-70% isopropyl alcohol in dichloromethane followed by 0-20% ammonium hydroxide in isopropyl alcohol as eluents. The product (2) was obtained as a white solid (210 mg, 92% yield): ¹H NMR (400 MHz, DMSO-d₆) δ 8.01 (d, J = 8.0 Hz, 1H), 5.97 (d, J = 18.8 Hz, 1H), 5.55 (d, J = 8.0 Hz, 1H), 4.02-3.80 (m, 4H), 3.34 (dq, J = 7.2, 10.4 Hz, 1H), 1.25 (d, J = 22.4 Hz, 3H), 1.10 (d, J = 6.8 Hz, 3H); ³¹P NMR (162 MHz, DMSO-d₆) δ 7.91; MS (ESI) (M + H)⁺ 412.3.

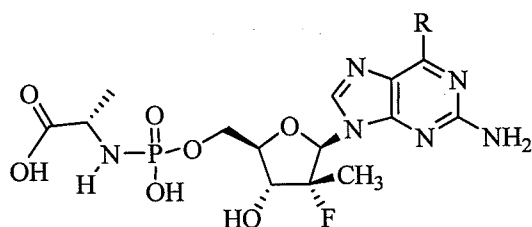
It is contemplated that the purified compounds disclosed herein are active against hepatitis C virus.

The contents of U.S. Patent Application No. 12/053,015, filed March 21, 2008 (see also WO 2008/121634), U.S. Patent Application No. 12/479,075, filed June 5, 2009, and U.S. Provisional Patent Application Nos. 61/060,683, filed June 11, 2008, 61/140,423 and 61/140,317, each of which being filed December 23, 2008 are hereby incorporated by reference in their entirety. Moreover, the patent and non-patent references disclosed herein are incorporated by reference. In the event that the incorporated subject matter contains a term that conflicts with a term disclosed in

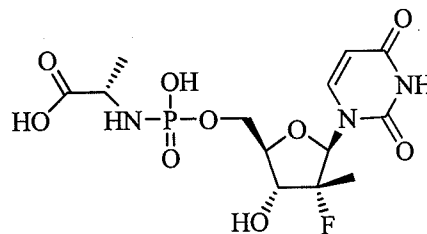
the present application text, the meaning of the term contained in the present application controls provided that the overall meaning of the incorporated subject matter is not lost.

CLAIMS

1. A purified compound represented by formula I or a purified compound represented by formula II, a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof:



I

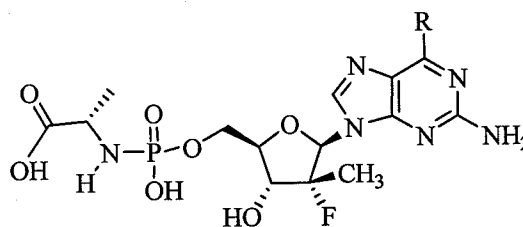


II

wherein R is OMe, OEt, $-N(-CH_2CH_2CH_2-)$ (azetidin-1-yl), or OH;
or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

10

2. A purified compound represented by formula I

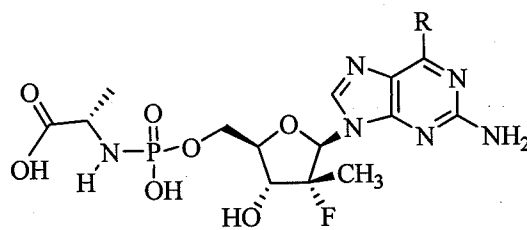


I

wherein R is OMe;
or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

15

3. A purified compound represented by formula I



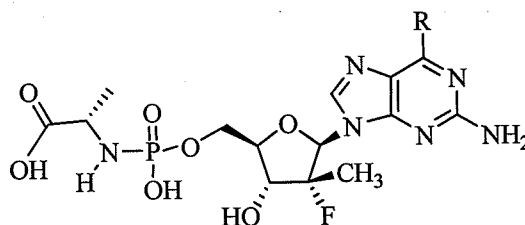
I

wherein R is OEt;

or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

5

4. A purified compound represented by formula I



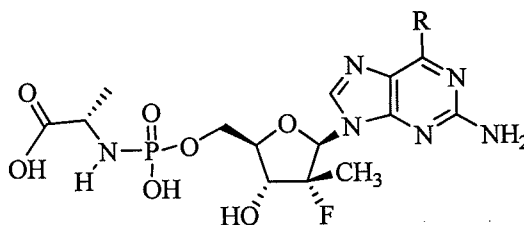
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wherein R is OH;

or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

10

5. A purified compound represented by formula I

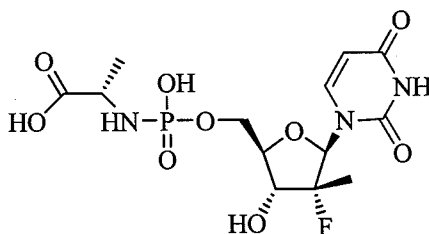


I

wherein R is $-N(-CH_2CH_2CH_2-)$ (azetidin-1-yl);

or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

6. A purified compound represented by formula II



II

or a salt, a pharmaceutically acceptable salt, a hydrate, a solvate, or a crystalline form thereof.

7. A composition for the treatment and/or prophylaxis of any of the viral agents disclosed herein said composition comprising a pharmaceutically acceptable medium selected from among an excipient, carrier, diluent, and equivalent medium and the compound as claimed in any one of claims 1-6.

8. A use of the compound as claimed in any one of claims 1-6 in the manufacture of a medicament for the treatment of any condition the result of an infection by hepatitis C virus, West Nile virus, yellow fever virus, dengue virus, rhinovirus, polio virus, hepatitis A virus, bovine viral diarrhea virus or Japanese encephalitis virus.

9. A method of treatment in a subject in need thereof, which comprises administering a therapeutically effective amount of the compound as claimed in any one of claims 1-6.

10. A method of treatment in a subject in need thereof, which comprises contacting at least one compound as claimed in any one of claims 1-6 with at least one hepatitis C virus infected cell.