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(54) Benævnelse: **Nukleotid hemisulfatsalt til behandlingen af hepatitis C virus**

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WO-A1-2016/144918
US-A1- 2010 286 083
US-A1- 2011 015 146
US-A1- 2011 257 121
US-A1- 2012 245 335
US-A1- 2016 257 706
US-A1- 2016 271 162
STEVEN S GOOD ET AL: "AT-337, AT-511 and its Salt Form, AT-527: Novel Potent and Selective Pan-genotypic Purine Nucleotide Prodrug Inhibitors of HCV Polymerase", 11 November 2016 (2016-11-11), XP055754828, Retrieved from the Internet <URL:https://ateapharma.com/wp-content/uploads/2020/08/Atea-Pharmaceuticals-AASLD-2016-FINAL-Poster.pdf> [retrieved on 20201127]
ELINA BERLIBA ET AL: "Safety, Pharmacokinetics, and Antiviral Activity of AT-527, a Novel Purine Nucleotide Prodrug, in Hepatitis C Virus-Infected Subjects with or without Cirrhosis", ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 63, no. 12, 30 September 2019 (2019-09-30), US, XP055754695, ISSN: 0066-4804, DOI: 10.1128/AAC.01201-19
CHANG ET AL.: "Discovery of PSI-353661, a Novel Purine Nucleotide Prodrug for the Treatment of HCV Infection", ACS MED. CHEM. LETT., vol. 2, no. 2, 2011, pages 130 - 135, XP055531154

Fortsættes ...

Description

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of provisional U.S. Application Nos. 62/453,437 filed February 1, 2017; 62/469,912 filed March 10, 2017; 62/488,366 filed April 21, 2017; and, 62/575,248 filed October 20, 2017.

FIELD OF THE INVENTION

[0002] The present invention is the hemi-sulfate salt of a selected nucleotide compound that has unexpected therapeutic properties to treat a host infected with hepatitis C, as well as pharmaceutical compositions and dosage forms thereof.

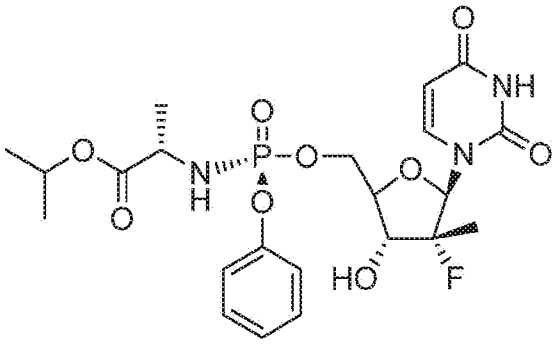
BACKGROUND OF THE INVENTION

[0003] 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 that may require a liver transplant. Approximately 71 million people worldwide are living with chronic HCV infections and approximately 399,000 people die each year from HCV, mostly from cirrhosis and hepatocellular carcinoma.

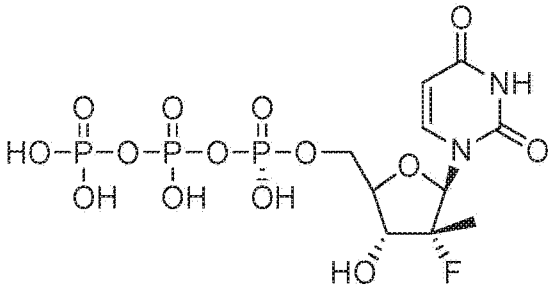
[0004] RNA polymerase is a key target for drug development against RNA single stranded viruses. The HCV non-structural protein NS5B RNA-dependent RNA polymerase is a key enzyme responsible for initiating and catalyzing viral RNA synthesis. There are two major subclasses of NS5B inhibitors: nucleoside analogs and non-nucleoside inhibitors (NNIs). Nucleoside analogs are anabolized to active triphosphates that act as alternative substrates for the polymerase and non-nucleoside inhibitors (NNIs) bind to allosteric regions on the protein. Nucleoside or nucleotide inhibitors mimic natural polymerase substrates and act as chain terminators. They inhibit the initiation of RNA transcription and elongation of a nascent RNA chain.

[0005] In addition to targeting RNA polymerase, other RNA viral proteins may also be targeted 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).

[0006] 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.



Sovaldi®



2'-Deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate

[0007] 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.

[0008] In 2014, the U.S. FDA approved Harvoni® (ledispasvir, 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.

[0009] The U.S. FDA also approved AbbVie's VIEKIRA Pak™ in 2014, a multi-pill pack containing dasabuvir (a non-nucleoside NS5B polymerase inhibitor), ombitasvir (a NS5A inhibitor), paritaprevir (a NS3/4A inhibitor), and ritonavir. The VIEKIRA Pak™ can be used with or without the ribavirin to treat Genotype 1 HCV infected patients including patients with compensated cirrhosis. VIEKIRA Pak™ does not require interferon co-therapy.

[0010] In July 2015, the U.S. FDA approved Technivie™ and Daklinza™ for the treatment of HCV genotype 4 and HCV Genotype 3, respectively. Technivie™ (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. Daklinza™ was approved for use with Sovaldi® to treat HCV genotype 3 infections. Daklinza™ is the first drug that has demonstrated safety and efficacy in treating HCV Genotype 3 without the need for co-administration of interferon or ribavirin.

[0011] 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.

[0012] 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 (Victrelis™, Merck), simeprevir (Olysio™, Johnson & Johnson), paritaprevir (AbbVie), Ombitasvir (AbbVie), the NNI Dasabuvir (ABT-333) and Merck's Zepatier™ (a single-tablet combination of the two drugs grazoprevir and elbasvir).

[0013] Additional NS5B polymerase inhibitors are currently under development. Merck is developing the uridine nucleotide prodrug MK-3682 (formerly Idenix IDX21437) and the drug is currently in Phase II combination trials.

[0014] United States patents and WO applications that describe nucleoside polymerase 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; 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; 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; 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; 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 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 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), Catabasis (WO 2013/090420) and the Regents of the University of Minnesota (WO 2006/004637).

[0015] Atea Pharmaceuticals, Inc. has disclosed β-D-2'-deoxy-2'-α-fluoro-2'-βC-substituted-2-modified-N^6-(mono- and di-methyl) purine nucleotides for the treatment of HCV in U.S. Patent No. 9,828,410 and PCT Application No. WO 2016/144918. Atea has also disclosed β-D-2'-deoxy-2'-substituted-4'-substituted-2-N^6-substituted-6-aminopurine nucleotides for the treatment of paramyxovirus and orthomyxovirus infections in US 2018/0009836 and WO 2018/009623.

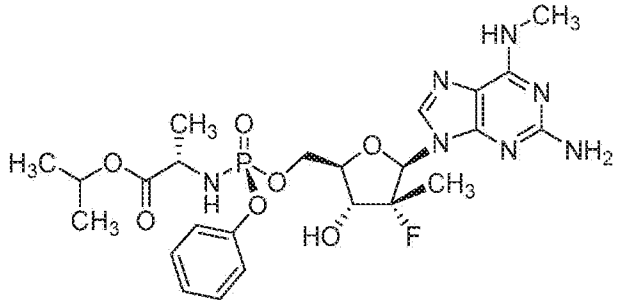
[0016] Steven S Good et al. discloses AT-337, AT-511 and its Salt Form, AT-527: Novel Potent and Selective Pangenotypic Purine Nucleotide Prodrug Inhibitors of HCV Polymerase in a poster presented at The 67th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD); 11-15 November 2016, Boston, MA.

[0017] 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 of drug resistance. More potent direct-acting antivirals could significantly shorten treatment duration and improve compliance and SVR (sustained viral response) rates for patients infected with all HCV genotypes.

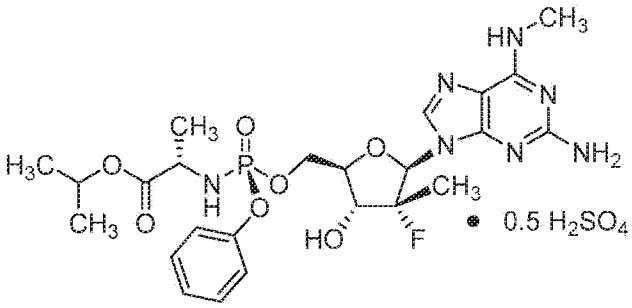
[0018] It is therefore an object of the present invention to provide compounds, pharmaceutical compositions, methods, and dosage forms to treat and/or prevent infections of HCV.

SUMMARY OF THE INVENTION

[0019] It has been surprisingly discovered 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).** These unexpected advantages could not have been predicted in advance. Compound **2** is thus a therapeutically superior composition of matter to administer in an effective amount to a host in need thereof, typically a human, for the treatment of hepatitis C. Compound **2** is referred to as the hemi-sulfate salt of isopropyl((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate. Compound **1** is disclosed in U.S. Patent No. 9,828,410.



Compound 1



Compound 2

[0020] Compound **2,** as Compound **1,** is converted to its corresponding triphosphate nucleotide (Compound **1-6)** in the cell, which is the active metabolite and inhibitor of RNA polymerase (see Scheme 1 below). Since Compound **1-6** is produced in the cell and does not leave the cell, it is not measurable in the plasma. However, the 5'-OH metabolite Compound **1-7** (see Scheme 1) is exported from the cell, and therefore is measurable in plasma and acts as a surrogate

of the concentration of intracellular active metabolite Compound **1-6.**

[0021] It has been discovered that the plasma concentration *in vivo* of surrogate Compound **1-7,** and thus intracellular Compound **1-6,** is substantially higher when Compound 2 is administered *in vivo* than when Compound **1** is administered *in vivo.* In a head-to-head comparison of dogs dosed with Compound **1** and Compound **2** (Example 19, Table 28), dosing with Compound **2** achieved an $AUC_{(0\text{-}4hrs)}$ of the ultimate guanine 5'-OH nucleoside metabolite **(1-7)** that is twice as high as the AUC following Compound **1** dosing. It is unexpected that a non-covalent salt has such an effect on plasma concentration of the parent drug (Compound **1).**

[0022] Additionally, Compound **2** selectively partitions *in vivo* to the liver over the heart (Example 19, Table 29), which is beneficial since the liver is the diseased organ in hosts infected with HCV. Dogs were dosed with Compound **1** or Compound **2** and the concentration of the active triphosphate **(1-6)** in the liver and heart was measured. The liver to heart ratio of the active triphosphate concentration was higher after dosing with Compound **2** compared to Compound **1** as shown in Table 29. Specifically, the liver/heart partitioning ratio for Compound **2** is 20 compared to a liver/heart partitioning ratio of 3.1 for Compound **1.** This data indicates, unexpectedly, that the administration of Compound **2** results in the preferential distribution of the active guanine triphosphate (Compound **1-6)** in the liver over the heart when compared to Compound **1,** which reduces potential off-target effects. It was unexpected that administration of Compound **2** would significantly reduce undesired off-target partitioning. This allows for the administration of Compound **2** at a higher dose than Compound **1,** if desired by the healthcare practitioner.

[0023] In addition, liver and heart tissue levels of the active guanine triphosphate derivative of Compound **2** (metabolite **1-6)** were measured after oral doses of Compound **2** in rats and monkeys (Example 20). High levels of the active guanine triphosphate **(1-6)** were measured in the liver of all species tested. Importantly, unquantifiable levels of the guanine triphosphate **(1-6)** were measured in monkey hearts, and this is indicative of liver-specific formation of the active triphosphate. It was thus discovered that compared to Compound 1 dosing, Compound **2** dosing improves guanine triphosphate **(1-6)** distribution.

[0024] When administered to healthy and hepatitis C infected patients, Compound **2** was well tolerated after a single oral dose and C_{max}, T_{max} and AUC_{tot} pharmacokinetic parameters were comparable in both groups (Tables 34 and 35). As described in Example 24, a single dose of Compound **2** in HCV-infected patients resulted in a significant antiviral activity. Plasma exposure of metabolite **1-7** was mostly dose-proportional over the studied range.

[0025] Individual pharmacokinetic/pharmacodynamic analyses of patients dosed with Compound **2** showed that the viral response correlated with plasma exposure of metabolite **1-7** of Compound **2** (Example 24, FIGS. 23A-23F), indicating that profound vial responses are achievable with robust doses of Compound **2.**

[0026] Example 24 confirms that, as non-limiting embodiments, single oral doses of 300 mg, 400 mg, and 600 mg result in significant antiviral activity in humans. The C_{24} trough plasma concentration of metabolite **1-7** following a 600 mg dose of Compound **2** doubled from the C_{24} trough plasma concentration of metabolite **1-7** following a 300 mg dose of Compound **2.**

[0027] FIG. 24 and Example 25 highlight the striking invention provided by Compound **2** for the treatment of hepatitis C. As shown in FIG. 24, the steady-state trough plasma levels ($C_{24,ss}$) of metabolite **1-7** following Compound **2** dosing in humans (600 mg QD (550 mg free base equivalent) and 450 mg QD (400 mg free base equivalent)) was predicted and compared to the EC_{95} of Compound **1** *in vitro* across a range of HCV clinical isolates to determine if the steady state plasma concentration is consistently higher than the EC_{95}, which would result in high efficacy against multiple clinical isolates *in vivo.* The EC_{95} for Compound **1** is the same as the EC_{95} of Compound **2.** For Compound **2** to be effective, the steady-state trough plasma level of metabolite **1-7** should exceed the EC_{95}.

[0028] As shown in FIG. 24, the EC_{95} of Compound **2** against all tesed clinical isolates ranged from approximately 18 nM to 24 nM.

[0029] As shown in FIG. 24, Compound **2** at a dose of 450 mg QD (400 mg free base equivalent) in humans provides a predicted steady state trough plasma concentration ($C_{24,ss}$) of approximately 40 ng/mL. Compound **2** at a dose of 600 mg QD (550 mg free base equivalent) in humans provides a predicted steady state trough plasma concentration ($C_{24,ss}$) of approximately 50 ng/mL.

[0030] Therefore, the predicted steady state plasma concentration of surrogate metabolite **1-7** is almost double the EC_{95} against all tested clinical isolates (even the hard to treat GT3a), which indicates superior performance.

[0031] In contrast, the EC_{95} of the standard of care nucleotide sofosbuvir (Sovaldi) ranges from 50 nM to 265 nM across all tested HCV clinical isolates, with an EC_{95} less than the predicted steady state concentration at the commercial dosage of 400 mg for only two isolates, GT2a and GT2b. The EC_{95} for the commercial dosage of 400 mg of sofosbuvir is greater than the predicted steady state concentration for other clinical isolates, GT1a, GTlb, GT3a, GT4a, and GT4d.

[0032] The data comparing the efficacy and pharmacokinetic steady state parameters in FIG. 24 clearly demonstrates the unexpected therapeutic importance of Compound **2** for the treatment of hepatitis C. In fact, the predicted steady-state ($C_{24,ss}$) plasma level after administration of Compound **2** is predicted to be at least 2-fold higher than the EC_{95} for all genotypes tested, and is 3- to 5-fold more potent against GT2. This data indicates that Compound **2** has potent pan-genotypic antiviral activity in humans. As shown in FIG. 24, the EC_{95} of sofosbuvir against GT1, GT3, and GT4 is greater

than 100 ng/mL. Thus surprisingly, Compound **2** is active against HCV at a dosage form that delivers a lower steady-state trough concentration (40-50 ng/mL) than the steady-state tough concentration (approximately 100 ng/mL) achieved by the equivalent dosage form of sofosbuvir.

[0033] In one embodiment, therefore, the invention includes a dosage form of Compound **2** that provides a metabolite **1-7** steady-state plasma trough concentration ($C_{24,ss}$) between approximately 15-75 ng/mL, for example, 20-60 ng/mL, 25-50 ng/mL, 40-60 ng/mL, or even 40-50 ng/mL. This is unexpected in light of the fact that the steady state concentration of the equivalent metabolite of sofosbuvir is approximately 100 ng/mL.

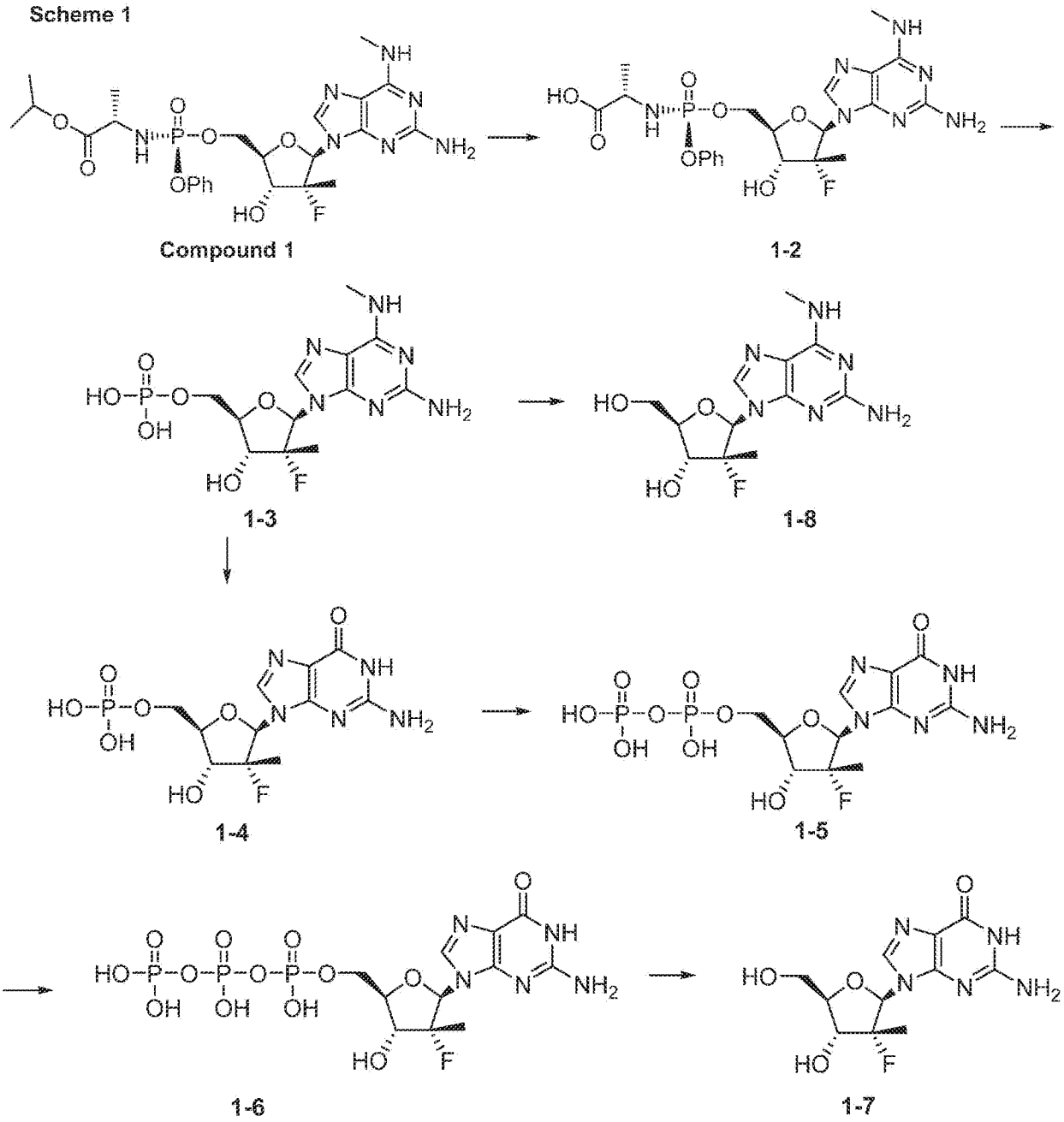
[0034] Additionally, it has been discovered that Compound **2** is an unusually stable, highly soluble, non-hygroscopic salt with activity against HCV. This is surprising because a number of salts of Compound **1** other than the hemi-sulfate salt (Compound **2),** including the mono-sulfate salt (Compound **3),** are not physically stable, but instead deliquesce or become gummy solids (Example 4), and thus are not suitable for stable solid pharmaceutical dosage forms. Surprisingly, while Compound **2** does not become gummy, it is up to 43 times more soluble in water compared to Compound **1** and is over 6 times more soluble than Compound **1** under simulated gastric fluid (SGF) conditions (Example 15).

[0035] As discussed in Example 16, Compound **2** remains a white solid with an IR that corresponds to the reference standard for 6 months under accelerated stability conditions (40 °C/75% RH). Compound **2** is stable for 9 months at ambient conditions (25 °C/60% RH) and refrigerator conditions (5 °C).

[0036] Solid dosage forms (50 mg and 100 mg tablets) of Compound **2** are also chemically stable under accelerated (40 °C/75% RH) and refrigeration conditions (5 °C) for 6 months (Example 26). Compound **2** is stable under ambient conditions (25 °C/60% RH) in a solid dosage form for at least 9 months.

[0037] Scheme 1 provides the metabolic pathway of Compound **1** and Compound **2,** which involves the initial de-esterification of the phosphoramidate (metabolite **1-1)** to form metabolite **1-2.** Metabolite **1-2** is then converted to the N^6-methyl-2,6-diaminopurine-5'-monophosphate derivative (metabolite **1-3),** which is in turn metabolized to the free 5'-hydroxyl-N^6-methyl-2,6-diaminopurine nucleoside (metabolite **1-8)** and ((2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methyl dihydrogen phosphate as the 5'-monophosphate (metabolite **1-4).** Metabolite **1-4** is anabolized to the corresponding diphosphate (metabolite **1-5)** and then the active triphosphate derivative (metabolite **1-6).** The 5'-triphosphate can be further metabolized to generate 2-amino-9-((2*R*,3*R*,4*R*,5*R*)-3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one **(1-7).** Metabolite **1-7** is measurable in plasma and is therefore a surrogate for the active triphosphate **(1-6),** which is not measurable in plasma.

Scheme 1



[0038] In one embodiment, the invention is Compound **2** for use in the treatment of hepatitis C (HCV) in a host in need thereof, optionally in a pharmaceutically acceptable carrier. In one aspect, Compound **2** is used as an amorphous solid. In another aspect, Compound **2** is used as a crystalline solid.

[0039] Described herein but not claimed is a process for the preparation of Compound **2** that includes

(i) a first step of dissolving Compound **1** in an organic solvent, for example, acetone, ethyl acetate, methanol, acetonitrile, or ether, or the like, in a flask or container;

(ii) charging a second flask or container with a second organic solvent, which may be the same as or different from the organic solvent in step (i), optionally cooling the second solvent to 0-10 degrees C, and adding dropwise H_2SO_4 to the second organic solvent to create a H_2SO_4/organic solvent mixture; and wherein the solvent for example may be methanol;

(iii) adding dropwise the H_2SO_4/solvent mixture at a molar ratio of 0.5/1.0 from step (ii) to the solution of Compound **1** of step (i) at ambient or slightly increased or decreased temperature (for example 23-35 degrees C);

(iv) stirring the reaction of step (iii) until precipitate of Compound **2** is formed, for example at ambient or slightly increased or decreased temperature;

(v) optionally filtering the resulting precipitate from step (iv) and washing with an organic solvent; and
(vi) optionally drying the resulting Compound **2** in a vacuum, optionally at elevated a temperature, for example, 55, 56, 57, 58, 59, or 60 °C.

[0040] In one embodiment, the organic solvent in step (i) is 3-methyl-2-pentanone. In one embodiment, the organic solvent in step (i) is ethyl isopropyl ketone. In one embodiment, the organic solvent in step (i) is methyl propionate. In one embodiment, the organic solvent in step (i) is ethyl butyrate.

[0041] Despite the volume of antiviral nucleoside literature and patent filings, Compound **2** has not been specifically disclosed. Accordingly, the present invention includes Compound **2,** or a pharmaceutically acceptable composition or dosage form thereof, as described herein.

[0042] Compounds, methods, dosage forms, and compositions are provided for the treatment of a host infected with a HCV virus via administration of an effective amount of Compound **2.** In certain embodiments, Compound **2** is administered at a dose of at least about 100, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700,750, 800, 850, 900, 950, or 1000 mg. In certain embodiments, Compound **2** is administered for up to 12 weeks, for up to 10 weeks, for up to 8 weeks, for up to 6 weeks, or for up to 4 weeks. In alternative embodiments, Compound **2** is administered for at least 4 weeks, for at least 6 weeks, for at least 8 weeks, for at least 10 weeks, or for at least 12 weeks. In certain embodiments, Compound **2** is administered at least once a day or every other day. In certain embodiments, Compound **2** is administered in a dosage form that achieves a steady-state trough plasma level ($C_{24,ss}$) of metabolite **1-7** between approximately 15-**75** ng/mL. In one embodiment, Compound **2** is administered in a dosage form that achieves a steady-state trough plasma level ($C_{24,ss}$) of metabolite **1-7** between approximately 20-60 ng/mL. In certain embodiments, Compound **2** is administered in a dosage form that achieves an AUC of metabolite **1-7** between approximately 1,200 ng*h/mL and 3,000 ng*h/mL. In one embodiment, Compound **2** is administered in a dosage form that achieves an AUC of metabolite **1-7** between approximately 1,500 and 2,100 ng*h/mL.

[0043] The compounds, compositions, and dosage forms 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 (hepatocellular carcinoma (HCC)), 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.

[0044] The present invention thus includes the following features:

(a) Compound **2** as described herein;
(b) Prodrugs of Compound **2** (prodrugs are not according to the present invention)
(c) Use of Compound **2** in the manufacture of a medicament for treatment of a hepatitis C virus infection;
(d) Compound **2** for use in the treatment of hepatitis C, optionally in a pharmaceutically acceptable carrier;
(e) A method for manufacturing a medicament intended for the therapeutic use for treating a hepatitis C virus infection, characterized in that Compound **2,** or a pharmaceutically acceptable salt, as described herein is used in the manufacture;
(e) A pharmaceutical formulation comprising an effective host-treating amount of Compound **2** with a pharmaceutically acceptable carrier or diluent;
(f) Processes for the preparation of therapeutic products that contain an effective amount of Compound **2;**
(g) Solid dosage forms, including those that provide an advantageous pharmacokinetic profile; and
(h) Processes for the manufacture of Compound **2,** as described herein but not claimed.

BRIEF DESCRIPTION OF THE FIGURES

[0045]

FIG. 1A is an overlay of XRPD diffractograms of samples 1-1 (amorphous Compound **1),** 1-2 (crystalline Compound **1),** and 1-3 (amorphous Compound **2)** prior to stability studies for characterization purposes as described in Example 2 and Example 5. The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts.
FIG. 1B is the HPLC chromatograph of amorphous Compound **1** (sample 1-1) to determine purity as described in Example 2. The purity of the sample was 98.7%. The x-axis is time measured in minutes and the y-axis is intensity measured in counts.
FIG. 2A is the HPLC chromatograph of crystalline Compound **1** (sample 1-2) to determine purity as described in Example 2. The purity of the sample was 99.11%. The x-axis is time measured in minutes and the y-axis is intensity measured in counts.
FIG. 2B is a DSC and TGA graph of crystalline Compound **1** (sample 1-2) prior to any stability studies for charac-

terization purposes as described in Example 2. The x-axis is temperature measured in °C, the left y-axis heat flow measured in (W/g), and the right y-axis is weight measured in percent.

FIG. 3 is an X-ray crystallography image of Compound **1** showing the absolute stereochemistry as described in Example 2.

FIG. 4A is an overlay of XRPD diffractograms of samples 1-1 (amorphous Compound **1),** 1-2 (crystalline Compound **1),** and 1-3 (amorphous Compound **2)** after storing at 25 °C and 60% relative humidity for 14 days as described in Example 2. The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts.

FIG. 4B is an overlay of XRPD diffractograms of samples 1-4, 1-5, 1-6, 1-7, and 1-9 after storing at 25 °C and 60% relative humidity for 7 days as described in Example 4. The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts.

FIG. 5A is an overlay of XRPD diffractograms of samples 1-4, 1-6, 1-7, and 1-9 after storing at 25 °C and 60% relative humidity for 14 days as described in Example 4. The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts.

FIG. 5B is the XRPD pattern of amorphous Compound **2** (sample 1-3) as described in Example 5. The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts.

FIG. 6A is the HPLC chromatograph of amorphous Compound **2** (sample 1-3) to determine purity as described in Example 5. The purity of the sample was 99.6%. The x-axis is time measured in minutes and the y-axis is intensity measured in counts.

FIG. 6B is a DSC and TGA graph for amorphous Compound **2** (sample 1-3) prior to any stability studies for characterization purposes as described in Example 5. The x-axis is temperature measured in °C, the left y-axis heat flow measured in (W/g), and the right y-axis is weight measured in percent.

FIG. 7A is an overlay of XRPD diffractograms of crystalline samples (samples 2-2, 2-6, and 2-7) and poorly crystalline samples (samples 2-3, 2-4, 2-5, and 2-8) identified from the crystallizations of Compound 2 (Example 6). The x-axis is 2Theta measured in degrees and the y-axis intensity measured in counts.

FIG. 7B is an overlay of XRPD diffractograms of amorphous samples (samples 2-9, 2-10, and 2-11) identified from the crystallizations of Compound **2** (Example 6). The x-axis is 2Theta measured in degrees and the y-axis intensity measured in counts.

FIG. 8A is an overlay of XRPD diffractograms of samples (samples 2-2, 2-3, 2-4, 2-5, 2-6, 2-7 and 2-8) after 6 days storage at 25 °C and 60% relative humidity (Example 6). The x-axis is 2Theta measured in degrees and the y-axis intensity measured in counts.

FIG. 8B is a DSC and TGA graph for sample 2-2 (Example 6). The x-axis is temperature measured in °C, the left y-axis heat flow measured in (W/g), and the right y-axis is weight measured in percent. Experimental procedures for DSC and TGA collection are given in Example 2.

FIG. 9A is a DSC and TGA graph for sample 2-3 (Example 6). The x-axis is temperature measured in °C, the left y-axis heat flow measured in (W/g), and the right y-axis is weight measured in percent. Experimental procedures for DSC and TGA collection are given in Example 2.

FIG. 9B is a DSC and TGA graph for sample 2-4 (Example 6). The x-axis is temperature measured in °C, the left y-axis heat flow measured in (W/g), and the right y-axis is weight measured in percent. Experimental procedures for DSC and TGA collection are given in Example 2.

FIG. 10A is a DSC and TGA graph for sample 2-5 (Example 6). The x-axis is temperature measured in °C, the left y-axis heat flow measured in (W/g), and the right y-axis is weight measured in percent. Experimental procedures for DSC and TGA collection are given in Example 2.

FIG. 10B is a DSC and TGA graph for sample 2-6 (Example 6). The x-axis is temperature measured in °C, the left y-axis heat flow measured in (W/g), and the right y-axis is weight measured in percent. Experimental procedures for DSC and TGA collection are given in Example 2.

FIG. 11A is a DSC and TGA graph for sample 2-7 (Example 6). The x-axis is temperature measured in °C, the left y-axis heat flow measured in (W/g), and the right y-axis is weight measured in percent. Experimental procedures for DSC and TGA collection are given in Example 2.

FIG. 11B is a DSC and TGA graph for sample 2-8 (Example 6). The x-axis is temperature measured in °C, the left y-axis heat flow measured in (W/g), and the right y-axis is weight measured in percent. Experimental procedures for DSC and TGA collection are given in Example 2.

FIG. 12A is the XRPD pattern of amorphous Compound 4 (sample 3-12) as discussed in Example 7. The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts. No crystallization of a malonate salt was observed regardless of the solvent used.

FIG. 12B is an overlay of XRPD diffractograms of amorphous samples (samples 3-6, 3-10, 3-11, and 3-12) identified from the attempted crystallization of compound 1 with malonate salt (Example 7). The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts.

FIG. 13A is the HPLC chromatogram of sample 3-12 from the attempted crystallizations of compound **1** with malonate

salt as described in Example 7. The sample was 99.2% pure. The x-axis is time measured in minutes and the y-axis is intensity measured in mAu.

FIG. 13B is an overlay of XRPD diffractograms of solid samples obtained from the crystallization using LAG (samples 4-13, 4-12, 4-9, 4-3, and 4-1) compared to Compound **1** (sample 1-2) as described in Example 8. All the XRDP match the patterns of the crystalline acid counter ion with no additional peaks. The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts.

FIG. 14A is an overlay of XRPD diffractograms of samples obtained from utilizing ethyl acetate as a crystallization solvent (samples 6-13, 6-12, 6-11, 6-10, 6-8, 6-7, 6-6, 6-5, 6-4, and 6-2) compared to crystalline Compound 1 (sample 1-2) as described in Example 10. The XRPD patterns were generally found to match the Compound 1 pattern with the exception of samples 6-2, 6-4, and 6-5 that exhibit slight differences. The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts.

Fig. 14B is an overlay of XRPD diffractogram of sample 5-1 following a second dissolution in MEK and the addition of the antisolvent cyclohexane and pamioc acid as described in Example 9. Sample 5-1, crystallized in pamioc acid, was a solid following maturation, but the XRPD pattern matched the pattern of pamioc acid.

FIG. 15A is an overlay of XRPD diffractograms of samples obtained from utilizing ethyl acetate as a crystallization solvent (samples 6-5, 6-4, and 6-2) compared to crystalline Compound **1** (sample 1-2) as described in Example 10. The XRPD patterns were generally found to match the Compound **1** pattern with the exception of samples 6-2, 6-4, and 6-5 that exhibit slight differences. The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts and labeled with the acid used in crystallization.

FIG. 15B is the XRPD pattern for Compound **2** as described in Example 14. The x-axis is 2Theta measured in degrees and the y-axis is intensity measured in counts.

FIG. 16A is a graph of the active TP (metabolite **1-6)** concentration levels in the livers and hearts of rats, dogs, and monkeys (Example 18). The x-axis is the dosage measured in mg/kg for each species and the y-axis is the active TP concentration measured in ng/g.

FIG. 16B is a graph of the active TP (metabolite **1-6)** concentration levels in the liver and heart of dogs (n=2) measured 4 hours after a single oral dose of Compound **1** or Compound **2** (Example 19). The x-axis is the dosage of each compound measured in mg/kg and the y-axis is the active TP concentration measured in ng/g.

FIG. 17 is the plasma profile of Compound **1** and metabolite **1-7** in rats given a single 500 mg/kg oral dose of Compound **2** (Example 20) measured 72 hours post-dose. The x-axis is time measured in hours and the y-axis is plasma concentration measured in ng/mL.

FIG. 18 is the plasma profile of Compound **1** and metabolite **1-7** in monkeys given single oral doses of 30 mg, 100 mg, or 300 mg of Compound **2** (Example 20) measured 72 hours post-dose. The x-axis is time measured in hours and the y-axis is plasma concentration measured in ng/mL.

FIG. 19 is a graph of EC_{95} measured in nM of sofosbuvir and Compound **1** against HCV clinical isolates. EC_{95} values for Compound **1** are 7-33 times lower than sofosbuvir (Example 22). The x-axis is labeled with the genotype and the y-axis is EC_{95} measured in nM.

FIG. 20 is a graph of EC_{50} measured in nM of sofosbuvir and Compound **1** against laboratory strains of HCV Genotypes 1a, 1b, 2a, 3a, 4a, and 5a. Compound **1** is approximately 6-11 times more potent than sofosbuvir in Genotypes 1-5 (Example 22). The x-axis is labeled with the genotype and the y-axis is EC_{50} measured in nM.

FIG. 21 is a graph of the mean plasma concentration-time profile of Compound **1** following the administration of a single dose of Compound **2** in all cohorts of Part B of the study as described in Example 24. Compound **1** was quickly absorbed and rapidly metabolized within approximately 8 hours in all cohorts from Part B. The x-axis is the time measured in hours and the y-axis is the geometric mean plasma concentration measured in ng/mL.

FIG. 22 is a graph of the mean plasma concentration-time profile of metabolite **1-7** following the administration of a single dose of Compound **2** in all cohorts of Part B of the study as described in Example 24. Metabolite **1-7** exhibited sustained plasma concentration in all cohorts from Part B. The x-axis is the time measured in hours and the y-axis is the geometric mean plasma concentration measured in ng/mL.

FIG. 23A is an individual pharmacokinetic/pharmacodynamic analysis of a subject enrolled in the 1b cohort as described in Example 24. The graph shows plasma metabolite **1-7** exposure and HCV RNA reduction levels. The dashed line represents the minimum concentration of metabolite **1-7** required to sustain a viral response greater than the EC_{95} value against GTlb. The x-axis is time measured in hours. The left y-axis is metabolite **1-7** plasma concentration measured in ng/mL and the right y-axis is the HCV RNA reduction measured in $\log_{10}$ IU/mL.

FIG. 23B is an individual pharmacokinetic/pharmacodynamic analysis of a subject enrolled in the 1b cohort as described in Example 24. The graph shows plasma metabolite **1-7** exposure and HCV RNA reduction levels. The dashed line represents the minimum concentration of metabolite **1-7** required to sustain a viral response greater than the EC_{95} value against GT1b. The x-axis is time measured in hours. The left y-axis is metabolite **1-7** plasma concentration measured in ng/mL and the right y-axis is the HCV RNA reduction measured in $\log_{10}$ IU/mL.

FIG. 23C is an individual pharmacokinetic/pharmacodynamic analysis of a subject enrolled in the 1b cohort as

described in Example 24. The graph shows plasma metabolite **1-7** exposure and HCV RNA reduction levels. The dashed line represents the minimum concentration of metabolite **1-7** required to sustain a viral response greater than the EC_{95} value against GT1b. The x-axis is time measured in hours. The left y-axis is metabolite **1-7** plasma concentration measured in ng/mL and the right y-axis is the HCV RNA reduction measured in log_{10} IU/mL.

FIG. 23D is an individual pharmacokinetic/pharmacodynamic analysis of a subject enrolled in the 3b cohort as described in Example 24. Each graph shows plasma metabolite **1-7** exposure and HCV RNA reduction levels. The dashed line represents the minimum concentration of metabolite **1-7** required to sustain a viral response greater than the EC_{95} value against GT1b. The x-axis is time measured in hours. The left y-axis is metabolite **1-7** plasma concentration measured in ng/mL and the right y-axis is the HCV RNA reduction measured in log_{10} IU/mL.

FIG. 23E is an individual pharmacokinetic/pharmacodynamic analysis of a subject enrolled in the 3b cohort as described in Example 24. Each graph shows plasma metabolite **1-7** exposure and HCV RNA reduction levels. The dashed line represents the minimum concentration of metabolite **1-7** required to sustain a viral response greater than the EC_{95} value against GT1b. The x-axis is time measured in hours. The left y-axis is metabolite **1-7** plasma concentration measured in ng/mL and the right y-axis is the HCV RNA reduction measured in log_{10} IU/mL.

FIG. 23F is an individual pharmacokinetic/pharmacodynamic analysis of a subject enrolled in the 3b cohort as described in Example 24. Each graph shows plasma metabolite **1-7** exposure and HCV RNA reduction levels. The dashed line represents the minimum concentration of metabolite **1-7** required to sustain a viral response greater than the EC_{95} value against GT1b. The x-axis is time measured in hours. The left y-axis is metabolite **1-7** plasma concentration measured in ng/mL and the right y-axis is the HCV RNA reduction measured in log_{10} IU/mL.

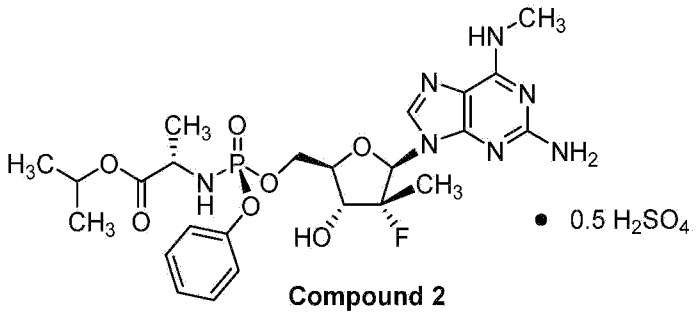
FIG. 24 is a graph of the EC_{95} values of Compound **1** and sofosbuvir against clinical isolates of GT1, GT2, GT3, and GT4 HCV-infected patients. The dashed horizontal line (- - - -) represents the steady-state trough concentration ($C_{24,ss}$) of sofosbuvir nucleoside following a dose of 400 mg QD of sofosbuvir. The full horizontal line (-) represents the steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** following 600 mg of Compound **2** (equivalent to 550 mg of Compound **1**). The dotted horizontal line (--------) represents the steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** following 450 mg of Compound **2** (equivalent to 400 mg of Compound **1**). As discussed in Example 25, the predicted steady-state trough plasma level ($C_{24,ss}$) of metabolite **1-7** following 600 mg and 450 mg of Compound **2** exceeds the *in vitro* EC_{95} of Compound **1** against all tested clinical isolates. The steady state trough plasma level ($C_{24,ss}$) of sofosbuvir only exceeds the EC_{95} at GT2 clinical isolates. The x-axis is labeled with the clinical isolates and the table under the x-axis lists the EC_{95} values for Compound 1 and sofosbuvir. The y-axis is the EC_{95} against the clinical isolates measured in ng/mL. EC_{95} is expressed as nucleoside equivalent. Sofosbuvir and Compound **2** were administered daily (QD).

FIG. 25 is a flow diagram showing the manufacturing process of 50 mg and 100 mg tablets of Compound **2** as described in Example 26. In step 1, microcrystalline cellulose, Compound **2,** lactose monohydrate, and croscarmellose sodium are filtered through a 600 μM screen. In step 2, the contents from step 1 are loaded into a V-blender and mixed for 5 minutes at 25 rpm. In step 3, magnesium stearate is filtered through a 600 μM screen. In step 4, magnesium stearate is loaded into the V-blender containing the contents from step 2 (microcrystalline cellulose, Compound **2,** lactose monohydrate, and croscarmellose sodium) and mixed for 2 minutes at 25 rpm. The common blend is then divided for the production of 50 mg tablets and 100 mg tablets. To produce 50 mg tablets, the blend from step 4 is compressed with 6 mm round standard concave tooling. To produce 100 mg tablets, the blend from step 4 is compressed with 8 mm round standard concave tooling. The tablets are then packaged into HOPE bottles induction-sealed with PP caps with desiccant.

FIG. 26 is the hemi-sulfate salt that exhibits advantageous pharmacological properties over its corresponding free base for the treatment of an HCV virus.

DETAILED DESCRIPTION OF THE INVENTION

[0046] The invention disclosed herein is a compound, composition, and solid dosage form 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 the hemi-sulfate salt of isopropyl((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (Compound **2**) as described herein, optionally in a pharmaceutically acceptable carrier. In one embodiment, Compound **2** is an amorphous solid. In yet another embodiment, Compound **2** is a crystalline solid.



Compound 2

[0047] The compound, compositions, and dosage forms can also be used to treat conditions related to or occurring as a result of an 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 (e.g, hepatocellular carcinoma), cirrhosis, acute hepatitis C, fulminant hepatitis C, chronic persistent hepatitis C, and anti-HCV-based fatigue.

[0048] The active compounds and compositions can also be used to treat the range of HCV genotypes. At least six distinct genotypes of HCV, each of which have multiple subtypes, have been identified globally. Genotypes 1-3 are prevalent worldwide, and Genotypes 4, 5, and 6 are more limited geographically. Genotype 4 is common in the Middle East and Africa. Genotype 5 is mostly found in South Africa. Genotype 6 predominately exists in Southeast Asia. Although the most common genotype in the United States is Genotype 1, defining the genotype and subtype can assist in treatment type and duration. For example, different genotypes respond differently to different medications and optimal treatment times vary depending on the genotype infection. Within genotypes, subtypes, such as Genotype 1a and Genotype 1b, respond differently to treatment as well. Infection with one type of genotype does not preclude a later infection with a different genotype.

[0049] As described in Example 22, Compound **2** is active against the range of HCV genotypes, including Genotypes 1-5. In one embodiment, Compound **2** is used to treat HCV Genotype 1, HCV Genotype 2, HCV Genotype 3, HCV Genotype 4, HCV Genotype 5, or HCV Genotype 6. In one embodiment, Compound **2** is used to treat HCV Genotype 1a. In one embodiment, Compound **2** is used to treat HCV Genotype 1b. In one embodiment, Compound **2** is used to treat HCV Genotype 2a. In one embodiment, Compound **2** is used to treat HCV Genotype 2b. In one embodiment, Compound **2** is used to treat HCV Genotype 3a. In one embodiment, Compound **2** is used to treat HCV Genotype 4a. In one embodiment, Compound **2** is used to treat HCV Genotype 4d.

[0050] In one embodiment, Compound **1** or Compound **2** is used to treat HCV Genotype 5a. In one embodiment, Compound **1** or Compound **2** is used to treat HCV Genotype 6a. In one embodiment, Compound **1** or Compound **2** is used to treat HCV Genotype 6b, 6c, 6d, 6e, 6f, 6g, 6h, 6i, 6j, 6k, 6l, 6m, 6n, 6o, 6p, 6q, 6r, 6s, 6t, or 6u.

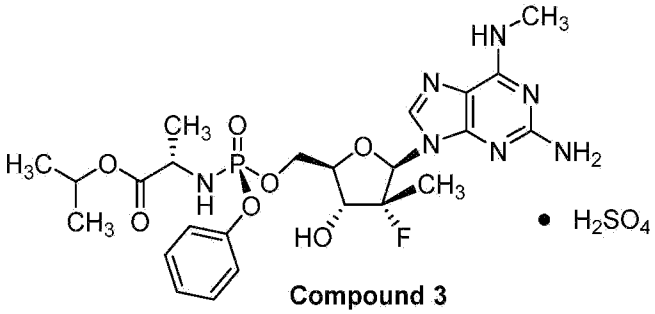
[0051] As discussed in Example 25 and shown in FIG. 24, the predicted steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** following a dose of 450 mg (400 mg free base) and a dose of 600 mg (550 mg free base) of Compound **2** is approximately 40 ng/mL to 50 ng/mL. This $C_{24,ss}$ level exceeded the EC_{95} of Compound **1** at HCV Genotypes 1a, 1b, 2a, 2b, 3a, 4a, and 4d. This data confirms that Compound **2** has potent-pan genotypic activity. This is surprising because Compound **2** achieves a smaller steady-state trough concentration ($C_{24,ss}$) than the steady-state trough concentration ($C_{24,ss}$) of the nucleoside metabolite of sofosbuvir following equivalent sofosbuvir dosing. The steady-state trough concentration ($C_{24,ss}$) of the corresponding nucleoside metabolite of sofosbuvir is approximately 100 ng/mL, but this level only exceeds the EC_{95} of sofosbuvir against GT2 clinical isolates (FIG. 24). Compound **2** is more potent than sofosbuvir against GT1, GT2, GT3, and GT4, and therefore allows a dosage form that delivers a smaller steady-state trough concentration of its metabolite which is nonetheless efficacious against all tested genotypes of HCV. In one embodiment, a dosage form of Compound **2** is delivered that achieves a metabolite **1-7** steady-state trough concentration ($C_{24,ss}$) between approximately 15-75 ng/mL. In one embodiment, a dosage form of Compound **2** is delivered that achieves a metabolite **1-7** steady-state trough concentration ($C_{24,ss}$) between approximately 20-60 ng/mL, 20-50 ng/mL, or 20-40 ng/mL.

[0052] In one embodiment, the compound, formulations, or solid dosage forms that include the compound 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.

[0053] In particular, it has been discovered that Compound **2** is active against HCV and exhibits superior drug-like and pharmacological properties compared to its free base (Compound **1**). Surprisingly, Compound **2** is more bioavailable and achieves a higher AUC than Compound **1** (Example 19) and Compound **2** is more selective for the target organ, the liver, than Compound **1** (Example 19).

[0054] Compound **2** is also advantageous over Compound **1** in terms of solubility and chemical stability. This is surprising because the mono-sulfate salt of isopropyl((*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 (Compound **3)** is unstable and exhibits the appearance of a sticky gum, while Compound **2,** the hemi-sulfate salt, is a stable white solid. The hemisulfate salt, both as a solid and in a solid dosage form, is very stable over 9 months and is not hydroscopic.



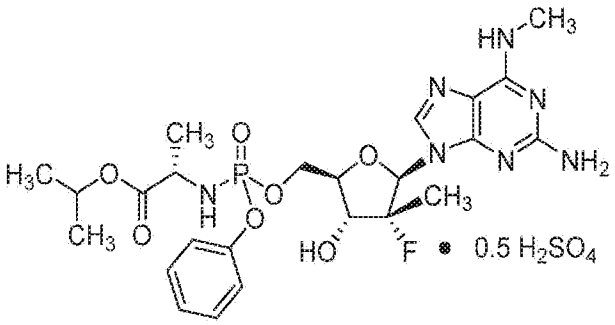
Compound 3

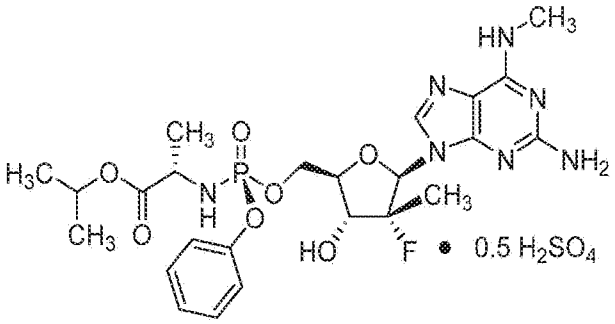
[0055] Despite the volume of antiviral nucleoside literature and patent filings, Compound **2** has not been specifically disclosed.

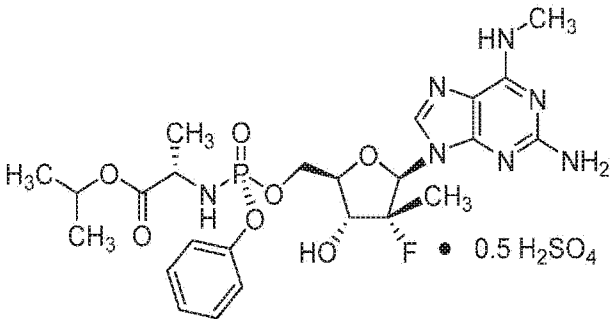
[0056] Compound **2** has S-stereochemistry at the phosphorus atom which has been confirmed with X-ray crystallography (FIG. 3, Example 2). In alternative embodiments, Compound **2** can be used in the form of any desired ratio of phosphorus R- and S-enantiomers, including up to pure enantiomers. In some embodiments, Compound **2** is used in a form that is 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, an enantiomerically enriched Compound **2** is at least 90% free of the opposite enantiomer. In addition, in an alternative embodiment, the amino acid of the phosphoramidate can be in the D- or L-configuration, or a mixture thereof, including a racemic mixture.

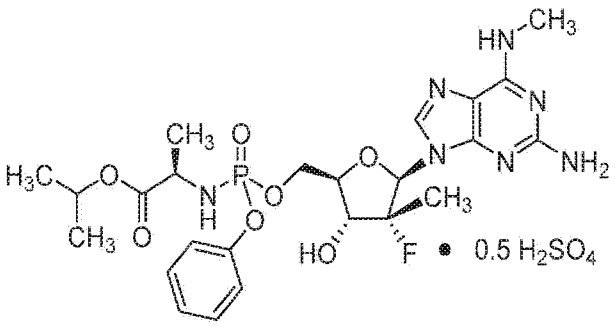
[0057] Unless otherwise specified, the compounds described herein are provided in the β-D-configuration. In an alternative embodiment, the compounds can be provided in a β-L-configuration. Likewise, any substituent group that exhibits chirality can be provided in racemic, enantiomeric, diastereomeric form, or any mixture thereof. Where a phosphoramidate exhibits chirality, 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 stereo configurations are described herein. The invention is defined by claim 1. In another embodiment, at least one of the hydrogens of Compound **2** (the nucleotide or the hemi-sulfate salt) can be replaced with deuterium.

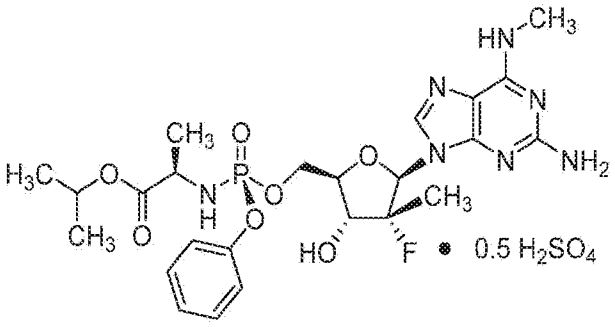
[0058] These alternative configurations do not form part of the invention and are only intended for illustrative purposes, include, but are not limited to,

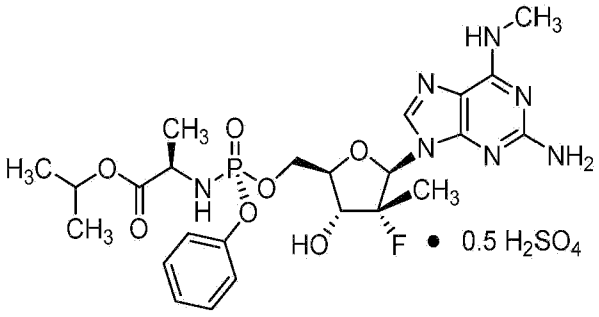












I. Hemi-sulfate salt of isopropyl((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-(methylamino)-9*H*-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (Compound 2)

[0059] The active compound of the invention is Compound **2**, which can be provided in a pharmaceutically acceptable composition or solid dosage form thereof. In one embodiment, Compound **2** is an amorphous solid. In yet a further embodiment, Compound **2** is a crystalline solid.

Synthesis of Compound 2

[0060] Described herein but not claimed is a process for the preparation of Compound **2** that includes

(i) a first step of dissolving Compound **1** in an organic solvent, for example, acetone, ethyl acetate, methanol, acetonitrile, or ether, or the like, in a flask or container;
(ii) charging a second flask or container with a second organic solvent, which may be the same as or different from the organic solvent in step (i), optionally cooling the second solvent to 0-10 degrees C, and adding dropwise H_2SO_4 to the second organic solvent to create a H_2SO_4/organic solvent mixture; and wherein the solvent for example may be methanol;
(iii) adding dropwise the H_2SO_4/solvent mixture at a molar ratio of 0.5/1.0 from step (ii) to the solution of Compound **1** of step (i) at ambient or slightly increased or decreased temperature (for example 23-35 degrees C);
(iv) stirring the reaction of step (iii) until precipitate of Compound **2** is formed, for example at ambient or slightly increased or decreased temperature;
(v) optionally filtering the resulting precipitate from step (iv) and washing with an organic solvent; and
(vi) optionally drying the resulting Compound **2** in a vacuum, optionally at elevated a temperature, for example, 55, 56, 57, 58, 59, or 60 °C.

[0061] In certain embodiments, step (i) above is carried out in acetone. Further, the second organic solvent in step (ii) may be for example methanol and the mixture of organic solvents in step (v) is methanol/acetone.

[0062] In one embodiment, Compound **1** is dissolved in ethyl acetate in step (i). In one embodiment, Compound **1** is dissolved in tetrahydrofuran in step (i). In one embodiment, Compound **1** is dissolved in acetonitrile in step (i). In an additional embodiment, Compound **1** is dissolved in dimethylformamide in step (i).

[0063] In one embodiment, the second organic solvent in step (ii) is ethanol. In one embodiment, the second organic solvent in step (ii) is isopropanol. In one embodiment, the second organic solvent in step (ii) is *n*-butanol.

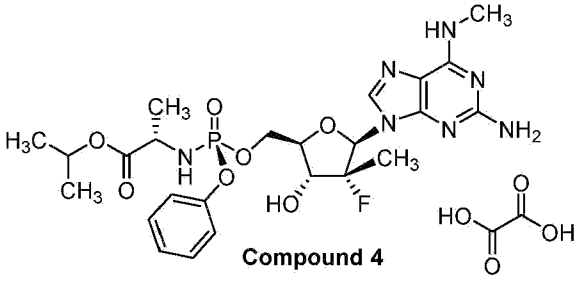
[0064] In one embodiment, a mixture of solvents are used for washing in step (v), for example, ethanol/acetone. In one embodiment, the mixture of solvent for washing in step (v) is isopropanol/acetone. In one embodiment, the mixture of solvent for washing in step (v) is n-butanol/acetone. In one embodiment, the mixture of solvent for washing in step (v) is ethanol/ethyl acetate. In one embodiment, the mixture of solvent for washing in step (v) is isopropanol/ethyl acetate. In one embodiment, the mixture of solvent for washing in step (v) is n-butanol/ethyl acetate. In one embodiment, the mixture of solvent for washing in step (v) is ethanol/ tetrahydrofuran. In one embodiment, the mixture of solvent for washing in step (v) is isopropanol/ tetrahydrofuran. In one embodiment, the mixture of solvent for washing in step (v) is n-butanol/ tetrahydrofuran. In one embodiment, the mixture of solvent for washing in step (v) is ethanol/ acetonitrile. In one embodiment, the mixture of solvent for washing in step (v) is isopropanol/ acetonitrile. In one embodiment, the mixture of solvent for washing in step (v) is n-butanol/ acetonitrile. In one embodiment, the mixture of solvent for washing in step (v) is ethanol/ dimethylformamide. In one embodiment, the mixture of solvent for washing in step (v) is isopropanol/ dimethylformamide. In one embodiment, the mixture of solvent for washing in step (v) is n-butanol/ dimethylformamide.

II. Metabolism of Isopropyl((*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 (Compound 2)

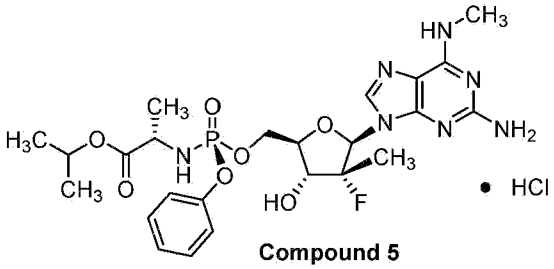
[0065] The metabolism of Compound **1** and Compound **2** involves the production of a 5'-monophosphate and the subsequent anabolism of the N^6-methyl-2,6-diaminopurine base **(1-3)** to generate ((2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methyl dihydrogen phosphate **(1-4)** as the 5'-monophosphate. The monophosphate is then further anabolized to the active triphosphate species: the 5'-triphosphate **(1-6).** The 5'-triphosphate can be further metabolized to generate 2-amino-9-((2*R*,3*R*,4*R*,5*R*)-3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)-1 ,9-dihydro-6H-purin-6-one **(1-7).** Alternatively, 5'-monophophate 1-2 can be metabolized to generate the purine base **1-8.** The metabolic pathway for isopropyl((*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 is illustrated in Scheme 1 (shown above).

III. Additional Salts of Compound 1 (not according to the present invention)

[0066] In alternative embodiments, the present invention provides Compound **1** as an oxalate salt (Compound **4)** or an HCl salt (Compound **5).**



Compound 4



Compound 5

[0067] Both the 1:1 oxalate salt and the 1:1 HCl salt form solids with reasonable properties for solid dosage forms for the treatment of a host such as a human with hepatitis C. However, the oxalate salt may be less desired, and perhaps not suitable, if the patient is susceptible to kidney stones. The HCl salt is more hydroscopic than the hemisulfate salt. Thus, the hemisulfate salt remains the most desired salt form of Compound **1** with unexpected properties.

IV. Definitions

[0068] 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.

[0069] The terms "coadminister" and "coadministration" or combination therapy are used to describe the administration of Compound **2** 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. 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.

[0070] 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

[0071] The present invention includes compounds and the use of compound **2** 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 (^{2}H) and tritium (^{3}H) 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 pharmacokinetic or pharmacodynamic, including at the 5-position of the molecule.

[0072] 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 breakdown 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 (^{1}H), deuterium (^{2}H) and tritium (^{3}H). Thus, reference herein to a compound encompasses all potential isotopic forms unless the context clearly dictates otherwise.

[0073] 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 (^{1}H), is substituted by a H-isotope, i.e., deuterium (^{2}H). 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.

V. Methods of Treatment or Prophylaxis (not according to the present invention)

[0074] Treatment, as used herein, refers to the administration of Compound **2** to a host, for example a human that is or may become infected with a HCV virus.

[0075] The term "prophylactic" or preventative, when used, refers to the administration of Compound **2** 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, Compound **2** is administered to a host who has been exposed to and thus is at risk of infection by a hepatitis C virus infection.

[0076] 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), 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, typically a human, with an effective amount of Compound **2** as described herein, optionally in combination with at least one additional bioactive agent, for example, an additional anti-HCV agent, further in combination with a pharmaceutically acceptable carrier additive and/or excipient.

[0077] In yet another aspect, the present invention is a method for prevention or prophylaxis of 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, 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 to a patient at risk with an effective amount Compound **2** 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.

[0078] In an alternative embodiment, Compound **2** is provided as the hemisulfate salt of a phosphoramidate of Compound **1** other than the specific phosphoramidate described in the compound illustration. A wide range of phosphoramidates are known to those skilled in the art that include various esters and phospho-esters, any combination of which can be used to provide an active compound as described herein in the form of a hemisulfate salt.

VI. Pharmaceutical Compositions and Dosage Forms

[0079] In an aspect of the invention, pharmaceutical compositions according to the present invention comprise an anti-HCV virus effective amount of Compound **2** as 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 one embodiment, the invention includes a solid dosage form of Compound **2** in a pharmaceutically acceptable carrier.

[0080] In an aspect of the invention, pharmaceutical compositions according to the present invention comprise an anti-HCV effective amount of Compound **2** described herein, optionally in combination with a pharmaceutically acceptable carrier, additive, or excipient, further optionally in combination with at least one other antiviral agent, such as an anti-HCV agent.

[0081] The invention includes pharmaceutical compositions that include an effective amount to treat a hepatitis C virus infection of Compound **2** of the present invention 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 Compound **2** of the present invention or prodrug, in a pharmaceutically acceptable carrier or excipient.

[0082] 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 pharmacokinetic 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.

[0083] Compound **2** according to the present invention can be formulated in a mixture with a pharmaceutically accept-

able carrier. In general, it is preferable to administer the pharmaceutical composition in orally-administrable form, an in particular, a solid dosage form such as a pill or tablet. 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 another vehicle, for example, this can be easily accomplished by minor modifications (salt formulation, esterification, etc.) that are well within the ordinary skill in the art. It is also well within the routineers' skill to modify the route of administration and dosage regimen of Compound **2** in order to manage the pharmacokinetic of the present compounds for maximum beneficial effect in patients, as described in more detail herein.

[0084] 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, may be used to achieve the desired effect. 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 person of ordinary skill in the art 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.

[0085] The amount of Compound **2** included within the therapeutically active formulation according to the present invention is an effective amount to achieve the desired outcome according to the present invention, for example, 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 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.

[0086] 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, Compound **2** will be administered in a solid dosage form in an amount ranging from about 250 micrograms up to about 800 milligrams or more at least once a day, for example, at least about 5, 10, 20, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, or 800 milligrams or more, once, twice, three, or up to four times a day according to the direction of the healthcare provider. Compound 2 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. More generally, Compound **2** can be administered in a tablet, capsule, injection, intravenous formulation, suspension, liquid, emulsion, implant, particle, sphere, cream, ointment, suppository, inhalable form, transdermal form, buccal, sublingual, topical, gel, mucosal, and the like.

[0087] When a dosage form herein refers to a milligram weight dose, it refers to the amount of Compound **2** (i.e., the weight of the hemi-sulfate salt) unless otherwise specified to the contrary.

[0088] In certain embodiments, the pharmaceutical composition is in a dosage form that contains from about 1 mg to about 2000 mg, from about 10 mg to about 1000 mg, from about 100 mg to about 800 mg, from about 200 mg to about 600 mg, from about 300 mg to about 500 mg, or from about 400 mg to about 450 mg of Compound **2** in a unit dosage form. In certain embodiments, the pharmaceutical composition is in a dosage form, for example in a solid dosage form, that contains up to about 10, about 50, about 100, about 125, about 150, about 175, about 200, about 225, about 250, about 275, about 300, about 325, about 350, about 375, about 400, about 425, about 450, about 475, about 500, about 525, about 550, about 575, about 600, about 625, about 650, about 675, about 700, about 725, about 750, about 775, about 800, about 825, about 850, about 875, about 900, about 925, about 950, about 975, or about 1000 mg or more of Compound **2** in a unit dosage form. In one embodiment, Compound **2** is administered in a dosage form that delivers at least about 300 mg. In one embodiment, Compound **2** is administered in a dosage form that delivers at least about 400 mg. In one embodiment, Compound **2** is administered in a dosage form that delivers at least about 500 mg. In one embodiment, Compound **2** is administered in a dosage form that delivers at least about 600 mg. In one embodiment, Compound **2** is administered in a dosage form that delivers at least about 700 mg. In one embodiment, Compound **2** is administered in a dosage form that delivers at least about 800 mg. In certain embodiments, Compound **2** is administered at least once a day for up to 12 weeks. In certain embodiments, Compound **2** is administered at least once a day for up to 10 weeks. In certain embodiments, Compound **2** is administered at least once a day for up to 8 weeks. In certain embodiments, Compound **2** is administered at least once a day for up to 6 weeks. In certain embodiments, Compound **2** is administered at least once a day for up to 4 weeks. In certain embodiments, Compound **2** is administered at least once a day for at least 4 weeks. In certain embodiments, Compound **2** is administered at least once a day for at least 6 weeks. In certain embodiments, Compound **2** is administered at least once a day for at least 8 weeks. In certain

embodiments, Compound **2** is administered at least once a day for at least 10 weeks. In certain embodiments, Compound **2** is administered at least once a day for at least 12 weeks. In certain embodiments, Compound **2** is administered at least every other day for up to 12 weeks, up to 10 weeks, up to 8 weeks, up to 6 weeks, or up to 4 weeks. In certain embodiments, Compound **2** is administered at least every other day for at least 4 weeks, at least 6 weeks, at least 8 weeks, at least 10 weeks, or at least 12 weeks. In one embodiment, at least about 600 mg of Compound **2** is administered at least once a day for up to 6 weeks. In one embodiment, at least about 500 mg of Compound **2** is administered at least once a day for up to 6 weeks. In one embodiment, at least about 400 mg of Compound **2** is administered at least once a day for up to 6 weeks. In one embodiment, at least 300 mg of Compound **2** is administered at least once a day for up to 6 weeks. In one embodiment, at least 200 mg of Compound **2** is administered at least once a day for up to 6 weeks. In one embodiment, at least 100 mg of Compound **2** is administered at least once a day for up to 6 weeks.

[0089] Metabolite **1-6** is the active triphosphate of Compound **2,** but metabolite **1-6** is not measurable in plasma. A surrogate for metabolite **1-6** is metabolite **1-7.** Metabolite **1-7** is a nucleoside metabolite measurable in plasma and is therefore an indication of the intracellular concentrations of metabolite **1-6.** For maximum HCV antiviral activity, a dosage form of Compound **2** must achieve a metabolite **1-7** steady-state trough concentration ($C_{24,ss}$) that exceeds the EC_{95} value of Compound **2.** As shown in FIG. 24, the EC_{95} of Compound **1** against clinical isolates of GT1, GT2, GT3, and GT4 is less than 25 ng/mL (Compound **1** EC_{95} and Compound **2** EC_{95} values are the same). In one embodiment, a dosage form of Compound **2** is delivered that achieves a steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** that is between approximately 15 to 75 ng/mL. In one embodiment, a dosage form of Compound **2** is delivered that achieves a steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** that is between approximately 20 to 60 ng/mL. In one embodiment, a dosage form of Compound **2** is delivered that achieves a steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** that is between approximately 30 to 60 ng/mL. In one embodiment, a dosage form of Compound **2** is delivered that achieves a steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** that is between approximately 20 to 50 ng/mL. In one embodiment, a dosage form of Compound **2** is delivered that achieves a steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** that is between approximately 30 to 50 ng/mL. In one embodiment, a dosage form of Compound **2** is delivered that achieves a steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** that is between approximately 20 to 45 ng/mL. In one embodiment, a dosage form of Compound **2** is delivered that achieves a steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** that is between approximately 20 to 30 ng/mL. In one embodiment, a dosage form of Compound **2** is delivered that achieves a steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** that is between approximately 20 to 35 ng/mL. In one embodiment, a dosage form of Compound **2** is delivered that achieves a steady-state trough concentration ($C_{24,ss}$) of metabolite **1-7** that is between approximately 20 to 25 ng/mL. Approximate dosage forms are + 10% of the steady-state trough concentration.

[0090] In one embodiment, Compound **2** is dosed at an amount that achieves a metabolite **1-7** AUC (area under the curve) of between approximately 1,200 and 3,000 ng/mL. In one embodiment, Compound **2** is dosed at an amount that achieves a metabolite **1-7** AUC of between approximately 1,500 and 3,000 ng/mL. In one embodiment, Compound **2** is dosed at an amount that achieves a metabolite **1-7** AUC of between approximately 1,800 and 3,000 ng/mL. In one embodiment, Compound **2** is dosed at an amount that achieves a metabolite **1-7** AUC of between approximately 2,100 and 3,000 ng/mL. In a preferred embodiment, Compound **2** is dosed at amount that achieves a metabolite **1-7** AUC of approximately 2,200 ng*h/mL. Approximate dosage forms are + 10% of the AUC.

[0091] In the case of the co-administration of Compound **2** in combination with another anti-HCV compound as otherwise described herein, the amount of Compound **2** according to the present invention to be administered in ranges from about 0.01 mg/kg of the patient to about 800 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 800 mg/kg. Examples of dosage amounts of the second active agent are amounts ranging from about 250 micrograms up to about 750 mg or more at least once a day, for example, at least about 5, 10, 20, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, or 800 milligrams or more, up to four times a day. In certain preferred embodiments, Compound **2** 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 pharmacokinetic of the two agents in the patient. These dosage ranges generally produce effective blood level concentrations of active compound in the patient.

[0092] 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.

[0093] Administration of Compound **2** 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/pharmacokinetic 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.

[0094] To prepare the pharmaceutical compositions according to the present invention, a therapeutically effective amount of Compound **2** 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.

[0095] 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.

[0096] 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.

[0097] In typical embodiments according to the present invention, Compound **2** and the compositions described are used to treat, prevent or delay a HCV infection or a secondary disease state, condition or complication of HCV.

VIL Combination and Alternation Therapy

[0098] It is well recognized that drug-resistant variants of viruses can emerge after prolonged treatment with an antiviral agent. Drug resistance sometimes 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 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 pharmacokinetic, 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 Compound **2** is an NS5B polymerase inhibitor, 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;
(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;
(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
(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 Compound 2 of the invention, alone or with multiple drugs from this lists, are

(i) protease inhibitors such as telaprevir (Incivek®), boceprevir (Victrelis™), simeprevir (Olysio™), paritaprevir (ABT-450), glecaprevir (ABT-493), ritonavir (Norvir), 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, ledispasvir, velpatasvir (Epclusa), elbasvir (MK-8742), grazoprevir (MK-5172), and Ombitasvir (ABT-267);
(iii) NS5B inhibitors such as AZD-7295, Clemizole, dasabuvir (Exviera), ITX-5061, PPI-461, PPI-688, sofosbuvir

(Sovaldi®), MK-3682, and mericitabine;
(iv) NS5B inhibitors such as ABT-333, and MBX-700;
(v) Antibody such as GS-6624;
(vi) Combination drugs such as Harvoni (ledipasvir/sofosbuvir); Viekira Pak (ombitasvir/paritaprevir/ritonavir/dasabuvir); Viekirax (ombitasvir/paritaprevir/ritonavir); G/P (paritaprevir and glecaprevir); Technivie (ombitasvir/ paritaprevir/ritonavir) and Epclusa (sofosbuvir/velpatasvir) and Zepatier (elbasvir and grazoprevir).

[0099] If Compound **2** 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 decetylace inhibitors.

EXAMPLES

General Methods

[0100] ^{1}H, ^{19}F and ^{31}P NMR spectra were recorded on a 400 MHz Fourier transform Brücker spectrometer. Spectra were obtained DMSO-d_6 unless stated otherwise. 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. The reactions were generally carried out under a dry nitrogen atmosphere using Sigma-Aldrich anhydrous solvents. All common chemicals were purchased from commercial sources.

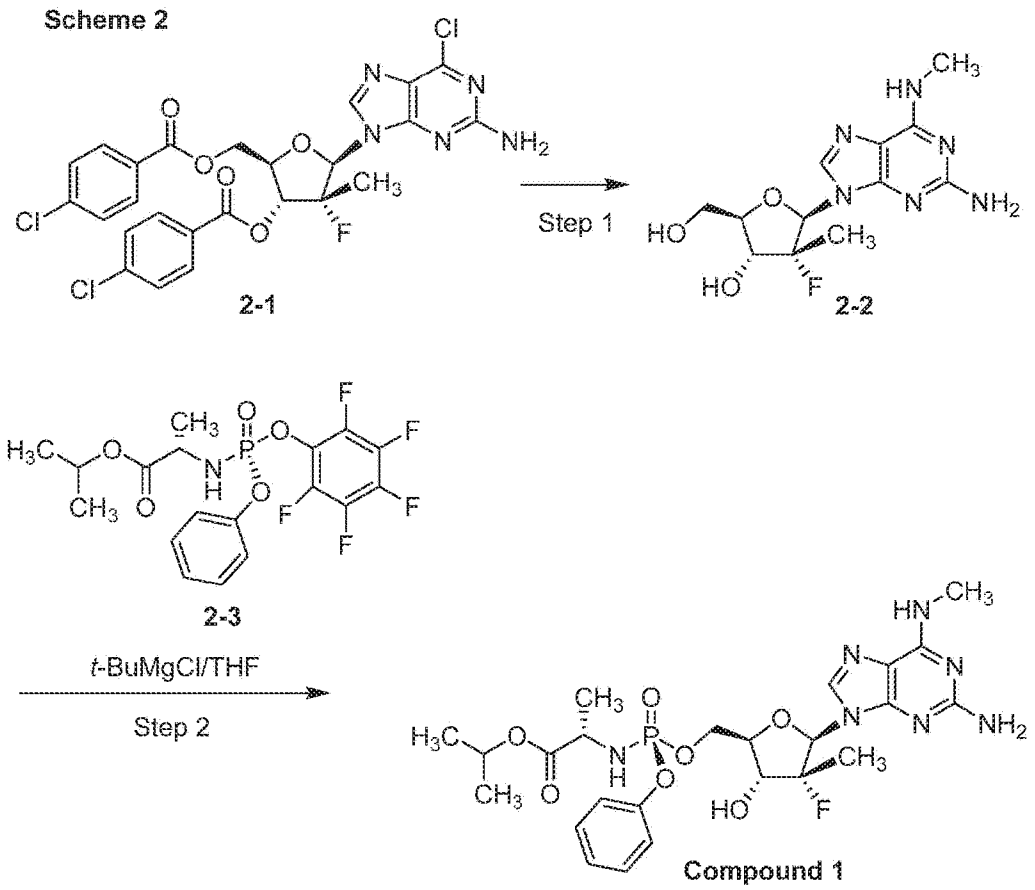
[0101] The following abbreviations are used in the Examples:

AUC: Area under the Curve
C_{24}: Concentration of the drug in plasma at 24 hours
$C_{24,ss}$: Concentration at 24 hours after dosing at steady state
C_{max}: Maximum concentration of the drug achieved in plasma
DCM: Dichloromethane
EtOAc: Ethyl acetate
EtOH: Ethanol
HPLC: High pressure liquid chromatography
NaOH: Sodium hydroxide
Na_2SO_4: Sodium sulphate (anhydrous)
MeCN: Acetonitrile
$MeNH_2$: Methylamine
MeOH: Methanol
Na_2SO_4: Sodium sulfate
$NaHCO_3$: Sodium bicarbonate
NH_4Cl: Ammonium chloride
NH_4OH: Ammonium hydroxide
PE: Petroleum ether
Ph_3P: Triphenylphosphine
RH: relative humidity
Silica gel (230 to 400 mesh, Sorbent)
t-BuMgCl: *t*-Butyl magnesium chloride
T_{max}: Time at which C_{max} is achieved
THF: Tetrahydrofuran (THF), anhydrous
TP: Triphosphate

Example 1. Synthesis of Compound 1

[0102]

Scheme 2



Step 1: Synthesis 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 (2-2)

[0103] A 50 L flask was charged with methanol (30 L) and stirred at 10 + 5 °C. NH_2CH_3 (3.95 Kg) was slowly ventilated into the reactor at 10 ± 5 °C. Compound **2-1** (3.77 kg) was added in batches at 20 + 5 °C and stirred for 1 hour to obtain a clear solution. The reaction was stirred for an additional 6-8 hours, at which point HPLC indicated that the intermediate was less than 0.1% of the solution. The reactor was charged with solid NaOH (254 g), stirred for 30 minutes and concentrated at 50 ± 5 °C (vacuum degree: -0.095). The resulting residue was charged with EtOH (40 L) and re-slurried for 1 hour at 60 °C. The mixture was then filtered through celite and the filter cake was re-slurried with EtOH (15 L) for 1 hour at 60 °C. The filtrate was filtered once more, combined with the filtrate from the previous filtration, and then concentrated at 50 + 5 °C (vacuum degree: -0.095). A large amount of solid was precipitated. EtOAc (6 L) was added to the solid residue and the mixture was concentrated at 50 ± 5 °C (vacuum degree: -0.095). DCM was then added to the residue and the mixture was re-slurried at reflux for 1 hour, cooled to room temperature, filtered, and dried at 50 + 5 °C in a vacuum oven to afford compound **2-2** as an off-white solid (1.89 Kg, 95.3%, purity of 99.2%).

[0104] **Analytic Method for Compound 2-2:** The purity of compound **2-2** (15 mg) was obtained using an Agilent 1100 HPLC system with a Agilent Poroshell 120 EC-C18 4.6*150mm 4-Micron column with the following conditions: 1 mL/min flow rate, read at 254 nm, 30 °C column temperature, 15 μL injection volume, and a 31 minute run time. The sample was dissolved in acetonitrile - water (20:80) (v/v). The gradient method is shown below.

Time (min)	A% (0.05 TFA in water)	B% (Acetonitrile)
0	95	5
8	80	20
13	50	50
23	5	95
26	5	95

(continued)

Time (min)	A% (0.05 TFA in water)	B% (Acetonitrile)
26.1	95	5
31	95	5

Step 2: Synthesis of isopropyl((*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 (Compound 1)

[0105] Compound **2-2** and compound **2-3** (isopropyl ((perfluorophenoxy)(phenoxy)phosphoryl)-*L*-alaninate) were dissolved in THF (1 L) and stirred under nitrogen. The suspension was then cooled to a temperature below -5 °C and a 1.7 M solution of *t*-BuMgCl solution (384 mL) was slowly added over 1.5 hours while a temperature of 5-10 °C was maintained. A solution of NH_4Cl (2 L) and water (8 L) was added to the suspension at room temperature followed by DCM. The mixture was stirred for 5 minutes before a 5% aqueous solution of K_2CO_3 (10 L) was added and the mixture was stirred for 5 additional minutes before filtering through diatomite (500 g). The diatomite was washed with DCM and the filtrate was separated. The organic phase was washed with a 5% aqueous K_2CO_3 solution (10 L x 2), brine (10 L x 3), and dried over Na_2SO_4 (500 g) for approximately 1 hour. Meanwhile, this entire process was repeated 7 times in parallel and the 8 batches were combined. The organic phases were filtered and concentrated at 45 + 5 °C (vacuum degree of 0.09 Mpa). EtOAc was added and the mixture was stirred for 1 hour at 60 °C and then at room temperature for 18 hours. The mixture was then filtered and washed with EtOAc (2 L) to afford crude Compound **1.** The crude material was dissolved in DCM (12 L), heptane (18 L) was added at 10-20 °C, and the mixture was allowed to stir for 30 minutes at this temperature. The mixture was filtered, washed with heptane (5 L), and dried at 50 + 5 °C to afford pure Compound **1** (1650 g, 60%).

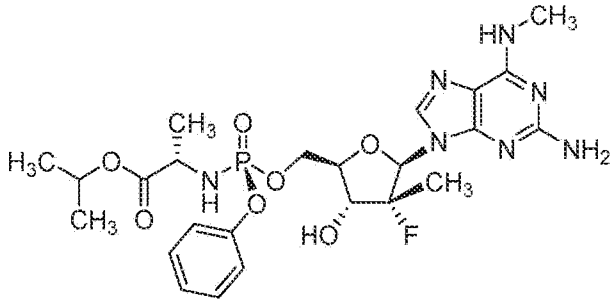
[0106] **Analytic Method for Compound 1:** The purity of Compound **1** (25 mg) was obtained using an Agilent 1100 HPLC system with a Waters XTerra Phenyl 5μm 4.6*250mm column with the following conditions: 1 mL/min flow rate, read at 254 nm, 30 °C column temperature, 15 μL injection volume, and a 25 minute run time. The sample was dissolved in acetonitrile - water (50:50) (v/v). The gradient method is shown below.

Time (min)	A% (0.1% H_3PO_4 in water)	B% (Acetonitrile)
0	90	10
20	20	80
20.1	90	10
25	90	10

Example 2. Characterization of Amorphous and Crystalline Compound 1

[0107] Amorphous Compound **1** and crystalline Compound **1** were initially analyzed by XRPD, ^{1}HNMR, and HPLC. The XRPD patterns for both compounds are shown in FIG. 1A and the HPLC traces to determine purity are shown in FIGS. 1B and 2A, respectively. Table 1 is a list of peaks from the XRPD of crystalline Compound **1** and Table 2 is a list of relative retention times (RTT) from the HPLC traces. Amorphous Compound **1** was 98.61% pure and crystalline Compound 1 was 99.11% pure. Both compounds were a white solid. FIG. 2B is the TGA and DSC graphs of crystalline Compound **1.** For crystalline Compound **1,** an endotherm was observed at 88.6 °C and there was a 7.8% mass loss from 80 - 110 °C.

[0108] A sample of Compound **1** was recrystallized from EtOAc/hexane and drawn with ORTEP. The absolute structure of Compound **1** was confirmed by the recrystallization of a single crystal. FIG. 3 is the ORTEP drawing of Compound **1.** Crystal data and measurement data are shown in Table 3. The absolute stereochemistry of Compound 1 based on the X-ray crystallography is shown below:



[0109] DSC data were collected on a TA Instruments Q2000 equipped with a 50 position autosampler. The calibration for thermal capacity was carried out using sapphire and the calibration for energy and temperature was carried out using certified indium. Typically approximately 3 mg of each sample, in a pin-holed aluminum pan, was heated at 10 °C/min from 25 °C to 200 °C. A purge of dry nitrogen at 50 ml/min was maintained over the sample. The instrument control software was Advantage for Q Series v2.8.0.394 and Thermal Advantage v5.5.3 and the data were analyzed using Universal Analysis v4.5A.

[0110] TGA data were collected on a TA Instruments Q500 TGA, equipped with a 16 position auto- sampler. The instrument was temperature calibrated using certified Alumel and Nickel. Typically 5 - 10 mg of each sample was loaded onto a pre-tared aluminum DSC pan and heated at 10 °C/min from ambient temperature to 350 °C. A nitrogen purge at 60 ml/min was maintained over the sample. The instrument control software was Advantage for Q Series v2.5.0.256 and Thermal Advantage v5.5.3 and the data were analyzed using Universal Analysis v4.5.

Amorphous Compound 1 (1-1):

[0111] ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.01 - 1.15 (m, 9 H), 1.21 (d, *J*=7.20 Hz, 3 H), 2.75 - 3.08 (m, 3 H), 3.71 - 3.87 (m, 1 H), 4.02 - 4.13 (m, 1 H), 4.22 - 4.53 (m, 3 H), 4.81 (s, 1 H), 5.69 - 5.86 (m, 1 H), 6.04 (br d, *J*=19.33 Hz, 4 H), 7.12 - 7.27 (m, 3 H), 7.27 - 7.44 (m, 3 H), 7.81 (s, 1 H)

Crystalline Compound 1 (1-2):

[0112] ^{1}H NMR (400 MΣiz, *DMSO-d*$_6$) δ ppm 0.97 - 1.16 (m, 16 H), 1.21 (d, *J*=7.07 Hz, 3 H), 2.87 (br s, 3 H), 3.08 (s, 2 H), 3.79 (br d, *J*=7.07 Hz, 1 H), 4.08 (br d, *J*=7.58 Hz, 1 H), 4.17 - 4.55 (m, 3 H), 4.81 (quin, *J*=6.25 Hz, 1 H), 5.78 (br s, 1 H), 5.91 - 6.15 (m, 4 H), 7.10 - 7.26 (m, 3 H), 7.26 - 7.44 (m, 3 H), 7.81 (s, 1 H)

Table 1. Peak list for crystalline Compound 1

Angle / °2θ	d spacing / Å	Intensity / Counts	Intensity / %
6.03	14.64	1005	39.0
7.36	12.00	315	12.2
7.94	11.13	1724	66.9
9.34	9.47	2500	97.0
9.51	9.29	860	33.4
9.77	9.05	1591	61.8
11.08	7.98	2576	100.0
12.02	7.36	171	6.6
12.95	6.83	319	12.4
13.98	6.33	241	9.4
14.30	6.19	550	21.4
14.69	6.03	328	12.7
15.20	5.82	2176	84.5
15.94	5.56	1446	56.1

(continued)

Angle / °2θ	d spacing / Å	Intensity / Counts	Intensity / %
16.75	5.29	1009	39.2
17.29	5.13	700	27.2
17.72	5.00	1213	47.1
18.11	4.89	1565	60.8
18.46	4.80	302	11.7
18.89	4.69	385	14.9
19.63	4.52	636	24.7
20.37	4.36	1214	47.1
20.74	4.28	1198	46.5
21.24	4.18	640	24.8
22.31	3.98	961	37.3
22.88	3.88	806	31.3
23.43	3.79	355	13.8
24.08	3.69	573	22.2
24.49	3.63	159	6.2
25.00	3.56	351	13.6
25.36	3.51	293	11.4
26.09	3.41	235	9.1
26.26	3.39	301	11.7
26.83	3.32	696	27.0
27.35	3.26	436	16.9
27.46	3.25	363	14.1
28.07	3.18	200	7.8
28.30	3.15	195	7.6
28.82	3.10	599	23.3
29.85	2.99	217	8.4
30.26	2.95	186	7.2
30.75	2.91	333	12.9
31.12	2.87	149	5.8
31.85	2.81	238	9.2
33.28	2.69	261	10.1
34.77	2.58	171	6.6
35.18	2.55	175	6.8
36.83	2.44	327	12.7
37.41	2.40	172	6.7

Table 2. Relative Retention Times from HPLC chromatographs of Amorphous Compound **1** and Crystalline Compound **1**

Amorphous Compound 1		Crystalline Compund 1	
RRT	**Area %**	**RRT**	**Area %**
0.48	0.15	0.48	0.17
0.51	0.04	0.48	0.17
0.48	0.15	0.94	0.12
0.51	0.04	**1.00**	**99.11**
0.94	0.13	1.04	0.22
0.98	0.21	1.37	0.07
1.00	**98.61**		
1.04	0.29		
1.37	0.31		

Table 3. Crystal and Data Measurement of Compound 1

Bond Precision	C-C = 0.0297A, Wavelength = 1.54184	
Cell	a=10.1884(3) b=28.6482(9) c=12.9497(5) alpha=90 beta=113.184(4) gamma=90	
Temperature	150K	
	Calculated	**Reported**
Volume	3474.5(2)	3474.5(2)
Space Group	P21	P 1 21 1
Hall Group	P 2yb	P 2yb
Moiety Formula	C24 H34 F N7 O7 P	2(C24 H34 F N7 O7 P)
Sum Formula	C24 H34 F N7 O7 P	C48 H68 F2 N14 O14 P2
Mr	582.55	1165.10
Dx, g cm^{-1}	1.114	1.114
Z	4	2
Mu (mm^{-1})	1.139	1.139
F000	1228.0	1228.0
F000'	1233.21	
h, k, l_{max}	12,34,15	12,34,15
N_{ref}	12742 [6510]	8259
T_{min}, T_{max}	0.790, 0.815	0.808, 1.000
$T_{min'}$	0.716	
Correction Method	# Reported T Limits: T_{min} = 0.808 T_{max} = 1.00	
AbsCorr	MULTI-SCAN	
Data completeness	1.27/0.65	
Theta (max)	68.244	

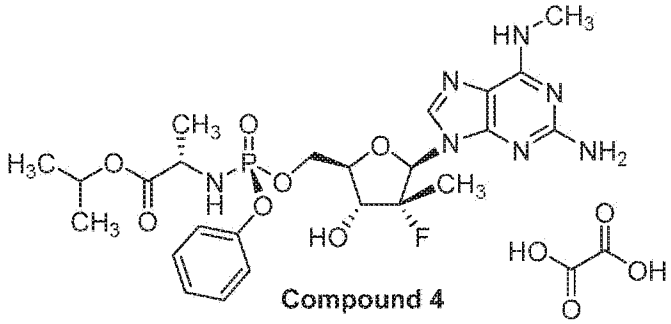
(continued)

Cell	a=10.1884(3) alpha=90	b=28.6482(9) beta=113.184(4)	c=12.9497(5) gamma=90
Temperature	150K		
	Calculated	**Reported**	
R (reflections)	0.2091 (7995)		
wR2 (reflections)	0.5338 (8259)		
S	2.875		
Npar	716		

[0113] This initial characterization was followed by storage at 25 °C / 60% relative humidity (RH) for 14 days with analysis by HPLC and XRPD after 7 and 14 days. FIG. 4A is the XRPD after 14 days at 25 °C / 60% (RH). Amorphous Compound **1** (sample 1-1) remained poorly crystalline, whereas crystalline Compound **1** (sample 1-2) retained its crystallinity, but both compounds were stable after 14 days at 25 °C / 60% (RH).

Example 3. Formation of Oxalate Salt Compound 4

[0114] Initially, the oxalate salt of Compound **1,** Compound **4,** was formed by mixing the oxalic salt with solvent (5 vol, 100 μL) and allowing any solution to evaporate at room temperature. Any suspension was matured (room temperature - 50 °C) for 3 hours and crystallinity was accessed.



Compound 4

[0115] Table 4 shows the different solvents used in the production of Compound **4.** All solvents except for two (cyclohexane and *n*-heptane) afforded crystalline products. Despite the high crystallinity and solubility of Compound **4,** oxalate salts are not acceptable for clinical development due to the potential formation of kidney stones and other salts of compound 1 were explored.

Table 4. Formation of Oxalate Compound 4

Solvent	**Observation post acid addition at room temperature**	**Observation after maturation/ evaporation**
EtOH	Solution	**OXA - Form 1**
IPA	Solution	**OXA - Form 1**
Acetone	Solution	**OXA - Form 1**
MEK	Solution	**OXA - Form 1**
EtOAc	Suspension	**OXA - Form 1**
*i*PrOAc	Suspension	**OXA - Form 1**
THF	Solution	**OXA - Form 1**
Toluene	Solution	**OXA - Form 1**

(continued)

Solvent	Observation post acid addition at room temperature	Observation after maturation/ evaporation
MeCN	Solution	**OXA - Form 1**
IPA:10%water	Solution	**OXA - Form 1**
TBME	Suspension	**OXA - Form 1**
Cyclohexane	Suspension	Amorphous
n-Heptane	Suspension	Amorphous

Example 4. Salt Compounds of Amorphous Compound 1

[0116] Since the oxalate salt compound **4** (Example 3) could not be carried forward in clinical trials due to its potential to form kidney stones, amorphous salts of Compound **1** were formed with the counter ions listed in Table 5. Compound **1** was dissolved in *t*-butanol (20 vol, 6 ml) and the solution was treated with the acid counter-ions (1 equivalent for each sample except sample 1-9 which had 0.5 equivalent of sulfate). The samples were then frozen with the solvent removed by lyophilization. The residual solid in samples 1-4, 1-5, 1-6, 1-7, 1-8, and 1-9 was initially analyzed by XRPD and HPLC.

Table 5. Amorphous salt formation details

Sample ID	Sample details	Stock solution details	Observation	NMR
1-4	HCl (1:1)	THF 1M	White solid	3 fewer protons ~0.3 eq *t*-BuOH
1-5	Sulfuric (1:1)	THF 1M	White solid	3 fewer protons ~0.3 eq *t*-BuOH
1-6	Fumaric (1:1)	MeOH:THF (1:1) 0.5M	Glassy solid	1.05 eq fumaric acid 0.84 eq *t*-BuOH
1-7	Benzoic (1:1)	THF 1M	White solid	1.0 eq benzoic acid 0.34 eq *t*-BuOH
1-8	Succinic (1:1)	MeOH 1M	Sticky white solid	~ 1.1 eq succinic acid 0.37 eq *t*-BuOH
1-9	Sulfuric (0.5:1 acid: API)	THF 1M	White solid	3 fewer protons ~0.3 eq *t*-BuOH
^{1}HNMR spectrum were taken for all samples.				

Sample 1-4, HCl (1:1) salt:

[0117] ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 0.93 - 1.39 (m, 16 H), 2.97 (br s, 2 H), 3.70 - 3.88 (m, 1 H), 4.10 (br s, 1 H), 4.18 - 4.49 (m, 3 H), 4.70 - 4.88 (m, 1 H), 5.71 - 5.94 (m, 1 H), 6.07 (br d, *J*=19.07 Hz, 2 H), 7.14 - 7.27 (m, 3 H), 7.29 - 7.44 (m, 2 H), 7.83 - 8.19 (m, 1 H)

Sample 1-5, Sulfuric (1:1) salt:

[0118] ^{1}H NMR (400 MHz, *DMS-d*$_6$) δ ppm 0.97 - 1.38 (m, 15 H), 2.96 (br s, 2 H), 4.06 - 4.18 (m, 1 H), 4.19 - 4.49 (m, 3 H), 4.66 - 4.91 (m, 1 H), 5.70 - 5.95 (m, 1 H), 5.96 - 6.16 (m, 2 H), 7.10 - 7.27 (m, 3 H), 7.30 - 7.43 (m, 2 H), 7.88 - 8.19 (m, 1 H)

Sample 1-6, Fumaric (1:1) salt:

[0119] ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 0.95 - 1.31 (m, 21 H), 2.87 (br s, 3 H), 3.79 (br d, *J*=7.20 Hz, 1 H), 4.01 - 4.13 (m, 1 H), 4.16 - 4.23 (m, 1 H), 4.16 - 4.24 (m, 1 H), 4.20 (s, 1 H), 4.18 - 4.23 (m, 1 H), 4.24 - 4.52 (m, 1 H), 4.24

- 4.52 (m, 1 H), 4.24 - 4.49 (m, 1 H), 4.72 - 4.88 (m, 1 H), 5.68 - 5.86 (m, 1 H), 6.04 (br d, *J*=19.33 Hz, 4 H), 6.63 (s, 1 H), 6.61 - 6.66 (m, 1 H), 7.12 - 7.27 (m, 3 H), 7.27 - 7.45 (m, 3 H), 7.81 (s, 1 H), 13.16 (br s, 2 H)

Sample 1-7, Benzoic (1:1) salt:

[0120] ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 0.96 - 1.30 (m, 15 H), 2.87 (br s, 3 H), 3.79 (br d, *J*=7.07 Hz, 1 H), 4.07 (br s, 1 H), 4.20 (s, 1 H), 4.25 - 4.52 (m, 3 H), 4.81 (s, 1 H), 5.71 - 5.85 (m, 1 H), 6.04 (br d, *J*=19.33 Hz, 4 H), 7.08 - 7.27 (m, 3 H), 7.27 - 7.43 (m, 3 H), 7.45 - 7.57 (m, 2 H), 7.63 (s, 1 H), 7.81 (s, 1 H), 7.95 (dd, *J*=8.27, 1.33 Hz, 2 H), 12.98 (br s, 1 H)

Sample 1-8, Succinic (1:1) salt:

[0121] ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 0.98 - 1.28 (m, 15 H), 2.42 (s, 5 H), 2.87 (br s, 3 H), 3.57 - 3.62 (m, 1 H), 3.70 - 3.86 (m, 1 H), 4.02 - 4.14 (m, 1 H), 4.20 (s, 1 H), 4.24 - 4.51 (m, 3 H), 4.70 - 4.88 (m, 1 H), 5.69 - 5.86 (m, 1 H), 6.04 (br d, *J*=19.33 Hz, 4 H), 7.12 - 7.27 (m, 3 H), 7.27 - 7.44 (m, 3 H), 7.81 (s, 1 H), 11.95 - 12.58 (m, 2 H)

Sample 1-9, Sulfuric (0.5:1) salt:

[0122] ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 1.02 - 1.31 (m, 15 H), 2.94 (br s, 3 H), 3.79 (br d, *J*=7.20 Hz, 2 H), 4.09 (br s, 1 H), 4.22 - 4.48 (m, 3 H), 4.72 - 4.90 (m, 1 H), 5.71 - 5.92 (m, 1 H), 6.07 (br d, *J*=19.07 Hz, 2 H), 7.12 - 7.28 (m, 3 H), 7.31 - 7.44 (m, 2 H), 7.75 - 8.19 (m, 1 H).

[0123] The samples were then subjected to storage at 25 °C / 60% relative humidity (RH) for 14 days with analysis by HPLC and XRPD after 7 (FIG. 4B) and 14 days (FIG. 5A). All prepared salts remained amorphous and the observations are shown in Table 6. The mono sulfate (sample 1-5) and succinate salts (sample 1-8) were found to be physically unstable and deliquesced or became a gum during the course of the study. Both the fumarate (sample 1-6) and benzoate salts (sample 1-7) were found to be glassy solids. The HCl salt (sample 1-4) was found to retain its physical appearance. Surprisingly, the hemi-sulfate salt (sample 1-9) also retained its physical appearance as a white solid in contrast to mono-sulfate compound (sample 1-5), which was a sticky gum. Results are shown in Table 6. The mono HCl salt (sample 1-4) and the hemi-sulfate salt (sample 1-9) were found to be physically and chemically stable after 2 weeks storage at 25 °C / 60% relative humidity (RH). Although both salts were stable over the two weeks, the hemi-sulfate salt was superior to the HCl salt because the HCl salt was hygroscopic, rendering it less useful compared to the hemi-sulfate salt for long-term storage or use.

Table 6. Stability of samples after 7 and 14 days at 25 °C / 60% RH

Sample ID	Time exposed to 25 °C / 60% RH (days)					
	0		7		14	
	HPLC	Observation	HPLC	Observation	HPLC	Observation
1-1	98.6	White solid	98.7	White solid	98.5	White solid
1-2	99.1	White solid	99.2	White solid	99.0	White solid
1-3	99.7	White solid	99.6	White solid	99.4	White solid
1-4	98.7	White solid	98.8	White solid	98.6	White solid
1-5	98.4	White solid	55.7	Sticky white solid	-	Sticky gum
1-6	98.7	Glassy solid	98.6	Clear glassy solid	98.4	White glassy solid
1-7	98.8	White solid	98.8	Clear glassy solid	98.7	Clear glassy solid
1-8	98.7	Sticky white solid	-	Deliquesced / sticky oil	-	Deliquesced
1-9	98.7	White solid	98.1	White solid	96.4	White solid

Example 5. Characterization of Amorphous Compound 2

[0124] Amorphous Compound **2** was initially analyzed by XRPD, ^{1}HNMR, DSC, TGA, and HPLC. The XRPD pattern for amorphous Compound **2** overlaid with amorphous Compound **1** and crystalline Compound **1** is shown in FIG. 1A and the XRPD pattern of amorphous Compound **2** alone is shown in FIG. 5B. Table 7 is a peak list from the XRPD

pattern shown in FIG. 5B. The HPLC trace to determine purity is shown in FIG. 6A. Table 8 is a list of relative retention times (RTT) from the HPLC trace shown in FIG. 6A. Amorphous Compound **2** was 99.68% pure. FIG. 6B is a TGA and DSC graph of amorphous Compound **2.** Experimental details for the TGA and DSC experiments are given in Example 2.

Table 7. Peak list for Amorphous Compound **2**

Angle / °2θ	d spacing / Å	Intensity / Counts	Intensity / %
4.20	21.03	486	81.8
4.67	18.91	482	81.0
5.16	17.10	595	100.0
9.13	9.68	547	92.0

Table 8. HPLC chromatogram of Amorphous Compound 2

Amorphous Compound 2	
RRT	**Area %**
0.48	0.02
0.48	0.02
0.67	0.01
0.94	0.13
1.00	**99.68**
1.04	0.06

Amorphous Compound 2:

[0125] ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 0.93 - 1.29 (m, 13 H), 2.94 (br s, 3 H), 3.79 (td, *J*=10.04, 7.07 Hz, 2 H), 4.05 - 4.19 (m, 1 H), 4.19 - 4.50 (m, 3 H), 4.81 (quin, *J*=6.25 Hz, 1 H), 5.71 - 5.94 (m, 1 H), 5.97 - 6.16 (m, 2 H), 7.14 - 7.28 (m, 3 H), 7.31 - 7.44 (m, 2 H), 7.82 - 8.09 (m, 1 H)

Example 6. Crystallization of Amorphous Compound 2

[0126] Since the hemi-sulfate salt was found to remain as a solid after the 14 day stability study as shown in Table 6, preliminary tests studying crystallization conditions using 11 different solvents was conducted. Amorphous Compound **2** was suspended in 5 volumes of solvent at 25 °C (sample 2-1, 2-2, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, and 2-11). To those samples that were not free flowing (2-1, 2-2, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, and 2-10), an additional 5 volumes of solvent was added. The samples were then matured at 25 - 50 °C (1 °C/min between temperatures and 4 hour at each temperature) for 6 days except for sample 2-1, which was observed to be a clear solution after 1 day and was allowed to evaporate under ambient conditions. The results are shown in Table 9. Crystalline patterns resulted from crystallization with isobutanol (sample 2-1), acetone (sample 2-2), EtOAc (sample 2-6), and *i*PrOAc (sample 2-7). Two poorly crystalline samples were also identified from crystallization with MEK (sample 2-4) and MIBK (sample 2-5). The XRPD patterns are shown in FIG. 7A.

Table 9. Crystallization Conditions of Compound 2

Sample ID	Solvent	Observation after 5 volumes	Observation after 10 volumes	Observation after 1 day maturation	XRPD
2-1	IPA	Solid - not free flowing	Free flowing suspension	Solution, evaporated at RT yielding a gum	Gum
2-2	Isobutanol	Solid - not free flowing	Free flowing suspension	Suspension	Crystalline - Pattern 2

(continued)

Sample ID	Solvent	Observation after 5 volumes	Observation after 10 volumes	Observation after 1 day maturation	XRPD
2-3	Acetone	Solid - not free flowing	Free flowing suspension	Suspension	Crystalline - Pattern 3
2-4	MEK	Solid - not free flowing	Free flowing suspension	Suspension	Poorly crystalline - Pattern 4
2-5	MIBK	Solid - not free flowing	Free flowing suspension	Suspension	Poorly crystalline - Pattern 4
2-6	EtOAc	Solid - not free flowing	Free flowing suspension	Suspension	Crystalline - Pattern 1
2-7	iPrOAc	Solid - not free flowing	Free flowing suspension	Suspension	Crystalline - Pattern 1
2-8	THF	Solid - not free flowing	Free flowing suspension	Suspension	Poorly crystalline
2-9	TBME	Free flowing suspension	-	Suspension	Amorphous
2-10	Toluene	Solid - not free flowing	Free flowing suspension	Suspension	Amorphous
2-11	Heptane	Free flowing suspension	-	Suspension	Amorphous

[0127] The seven samples (Samples 2-2, 2-3, 2-4, 2-5, 2-6, 2-7 and 2-8) were analyzed by DSC, TGA, ^{1}H-NMR and IC (Table 10, FIG. 8A, FIG. 8B, FIG. 9A, FIG. 9B, FIG. 10A, FIG. 10B, FIG. 11A, and FIG. 11B) as well as by XRPD following 6 days storage at 25 °C / 60% relative humidity (RH) (all samples remained crystalline / poorly crystalline following stability). All samples retained roughly half an equivalent of sulfate, but contained a relatively large amount of residual solvent. An overlay of the X-ray diffractograms of amorphous samples 2-9, 2-10, and 2-11 is shown in FIG. 7B.

Table 10. Characterization of crystalline Compound 2 samples

Sample ID	Solvent	DSC	TGA	^{1}HNMR	IC (corrected for TGA)
2-2	Isobutanol	Endo 113.8 °C	8.3% ambient-140 °C	1.1 eq isobutanol	0.45 eq
2-3	Acetone	Endo 30-95 °C Endo 100-145 °C	7.6% ambient - 140 °C	0.5 eq acetone	0.46 eq
2-4	MEK	Broad complex endo 30-115 °C Endo 115-145 °C	8.5 % ambient - 140 °C	0.8 eq MEK	0.45 eq
2-5	MIBK	Broad endo 30-105 °C Endo 114.7 °C	5.2% ambient - 110 °C	0.2 eq MIBK	0.46 eq
2-6	EtOAc	Sharp endo 113.6 °C	2.0% ambient-100 °C	0.9 eq EtOAc	0.46 eq
2-7	iPrOAc	Endo 30-90 °C	1.6% ambient-90 °C	0.8 eq iPrOAc	0.45 eq

(continued)

Sample ID	Solvent	DSC	TGA	^{1}HNMR	IC (corrected for TGA)
2-8	THF	Endo 30-100 °C Sharper endo 115.6 °C	4.2% ambient-130 °C	0.7 eq THF	0.45 eq
^{1}HNMR spectrum were taken for all samples and listed below.					

Sample 2-2:

[0128] ^{1}H NMR (400 MHz, *DMSO-d*6) δ ppm 0.83 (d, *J*=6.69 Hz, 7 H), 0.99 - 1.26 (m, 14 H), 1.61 (dt, *J*=13.26, 6.63 Hz, 1 H), 3.73 - 3.87 (m, 2 H), 4.03 - 4.18 (m, 1 H), 4.18 - 4.51 (m, 4 H), 4.66 - 4.92 (m, 1 H), 4.70 - 4.90 (m, 1 H), 4.72 - 4.88 (m, 1 H), 5.81 (br s, 1 H), 5.93 - 6.11 (m, 2 H), 7.10 - 7.26 (m, 3 H), 7.14 - 7.26 (m, 1 H), 7.30 - 7.41 (m, 2 H), 7.94 (br s, 1 H)

Sample 2-3:

[0129] ^{1}H NMR (400 MHz, *DMSO-d*6) δ ppm 1.00 - 1.26 (m, 13 H), 2.09 (s, 3 H), 3.74 - 3.87 (m, 2 H), 4.10 (br d, *J*=7.70 Hz, 1 H), 4.22 - 4.50 (m, 3 H), 4.81 (quin, *J*=6.28 Hz, 1 H), 5.71 - 5.90 (m, 1 H), 5.96 - 6.15 (m, 2 H), 7.12 - 7.26 (m, 3 H), 7.31 - 7.41 (m, 2 H), 7.79 - 8.07 (m, 1 H)

Sample 2-4:

[0130] ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 0.91 (t, *J*=7.33 Hz, 3 H), 1.01 - 1.28 (m, 13 H), 2.08 (s, 2 H), 3.72 - 3.89 (m, 2 H), 4.10 (br d, *J*=8.08 Hz, 1 H), 4.23 - 4.47 (m, 3 H), 4.81 (quin, *J*=6.25 Hz, 1 H), 5.69 - 5.89 (m, 1 H), 5.94 - 6.13 (m, 2 H), 7.14 - 7.25 (m, 3 H), 7.32 - 7.41 (m, 2 H), 7.79 - 8.11 (m, 1 H)

Sample 2-5:

[0131] ^{1}H NMR (400 MHz, *DMSO-d*6) δ ppm 0.86 (d, *J*=6.69 Hz, 1 H), 0.98 - 1.33 (m, 13 H), 2.02 - 2.09 (m, 1 H), 4.03 - 4.17 (m, 1 H), 4.22 - 4.50 (m, 3 H), 4.81 (quin, *J*=6.25 Hz, 1 H), 5.81 (br s, 1 H), 5.93 - 6.15 (m, 2 H), 7.11 - 7.27 (m, 3 H), 7.31 - 7.41 (m, 2 H), 7.77 - 8.21 (m, 1 H)

Sample 2-6:

[0132] ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 0.98 - 1.28 (m, 15 H), 2.00 (s, 3 H), 3.99 - 4.14 (m, 3 H), 4.21 - 4.49 (m, 3 H), 4.81 (quin, *J*=6.22 Hz, 1 H), 5.82 (br s, 1 H), 5.93 - 6.14 (m, 2 H), 7.11 - 7.26 (m, 3 H), 7.29 - 7.42 (m, 2 H), 7.79 - 8.17 (m, 1 H)

Sample 2-7:

[0133] ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 0.92 - 1.28 (m, 17 H), 1.97 (s, 2 H), 4.04 - 4.16 (m, 1 H), 4.20 - 4.51 (m, 3 H), 4.71 - 4.93 (m, 2 H), 5.82 (br s, 1 H), 5.95 - 6.14 (m, 2 H), 7.11 - 7.28 (m, 3 H), 7.31 - 7.43 (m, 2 H), 7.75 - 8.21 (m, 1 H)

Sample 2-8:

[0134] ^{1}H NMR (400 MHz, *DMSO-d*6) δ ppm 0.81 - 1.11 (m, 13 H), 1.19 (s, 1 H), 1.53 - 1.66 (m, 1 H), 3.87 - 4.01 (m, 1 H), 4.06 - 4.32 (m, 3 H), 4.64 (quin, *J*=6.25 Hz, 1 H), 5.55 - 5.75 (m, 1 H), 5.77 - 5.97 (m, 2 H), 6.94 - 7.10 (m, 3 H), 7.13 - 7.26 (m, 2 H), 7.66 - 7.96 (m, 1 H)

Example 7. Failure to Crystallize Amorphous Malonate Salt (Compound 4)

[0135] As shown in Example 3, a crystalline oxalate salt was identified when determining appropriate salts for Compound 1, but oxalate salt Compound 4 could not be carried forward in clinical trials due to its potential for causing kidney stones. Therefore, crystallization of the chemically related malonate salt (Compound 5) was attempted using the same

11 solvents as for the hemi-sulfate salt. Compound 1 (12 × 50 mg, samples 3-1, 3-2, 3-3, 3-4, 3-5, 3-6, 3-7, 3-8, 3-9, 3-10, 3-11, and 3-12) was dissolved in *t*-butanol (20 vol) and the solutions were then treated with 1 equivalence of a malonic acid stock solution (1 M in THF). The samples were then frozen with the solvent removed by lyophilisation. To samples 3-1, 3-2, 3-3, 3-4, 3-5, 3-6, 3-7, 3-8, 3-9, 3-10, and 3-11, relevant solvent (5 volumes) was added at room temperature. Any resulting solutions were allowed to evaporate under ambient conditions, while gums or solids were matured at 25 - 50 °C (1 °C/min between temperatures and 4 hour at each temperature) for 5 days. The solids were analyzed by XRPD (FIG. 12B), but all samples were found to either form a gum or were amorphous (FIG. 12B). Results are shown in Table 11. The one solid (amorphous) sample (3-12) was analyzed by ^{1}H-NMR and HPLC, and was found to contain around 1 equivalence of malonic acid (peaks overlap) as well as 0.6 eq. *t*-BuOH. The compound was 99.2% pure (FIG. 13A). FIG. 12A is an XRDP of sample 3-12 and FIG. 13A is the HPLC chromatograph of sample 3-12.

Sample 3-12:

[0136] ^{1}H NMR (400 MHz, *DMSO-d6*) δ ppm 0.81 - 1.11 (m, 13 H), 1.19 (s, 1 H), 1.53 - 1.66 (m, 1 H), 3.87 - 4.01 (m, 1 H), 4.06 - 4.32 (m, 3 H), 4.64 (quin, *J*=6.25 Hz, 1 H), 5.55 - 5.75 (m, 1 H), 5.77 - 5.97 (m, 2 H), 6.94 - 7.10 (m, 3 H), 7.13 - 7.26 (m, 2 H), 7.66 - 7.96 (m, 1 H)

Table 11. Crystallization Conditions of Amorphous Malonate Salt Compound 4

Sample ID	Solvent	Observation after 5 volumes	Observation after 5 days maturation / evaporation	XRPD
3-1	IPA	Clear solution*	Clear gum	-
3-2	Isobutanol	Clear solution*	Clear gum	-
3-3	Acetone	Clear solution*	Clear gum	-
3-4	MEK	Clear solution*	Clear gum	-
3-5	NIBK	Solution & some gum	Clear gum	-
3-6	EtOAc	Clear solution*	Clear gum & crystal-like appearance	Amorphous
3-7	iPrOAc	Gum	Clear gum	-
3-8	THF	Clear solution*	Clear gum	-
3-9	TBME	Thick suspension	Clear gum	-
3-10	Toluene	White gum / solid	White gum	Amorphous
3-11	Heptane	White solid (static)	White gum	Amorphous
3-12	-	(White solid - no solvent)	(Sticky white solid - ambient conditions)	Amorphous
*Evaporated at room temperature				

Example 8. Failure of Adequate Salt Formation using Liquid Assisted Grinding (LAG)

[0137] A liquid assisted grinding (LAG) study to determine appropriate salts other than hemi-sulfate was performed using the 14 acidic counter ions in Table 12.

Table 12. Counter-ion stock solutions used in LAG Crystallization

Counter-ion	Solvent (1 M)
Pamoic	DMSO
Malonic	THF
D-Glucuronic	Water
DL-Mandelic	THF
D-Gluconic	THF
Glycolic	THF

(continued)

Counter-ion	Solvent (1 M)
L-Lactic	THF
Oleic	THF
L-Ascorbic	Water
Adipic	THF (heat)
Caproic	THF
Stearic	THF
Palmitic	THF
Methanesulfonic	THF

[0138] Compound **1** (30 mg) was placed in HPLC vials with two 3 mm ball bearings. The materials were wetted with solvent (15 μl ethanol, sample 4-1, 4-2, 4-3, 4-4, 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, 4-11, 4-12, 4-13, and 4-14) and 1 equivalence of the acid counter-ion was added. The samples were then ground for 2 hours at 650 rpm using a Fritsch milling system with an Automaxion adapter. Most of the samples after grinding were found to be clear gums and were not analyzed further (Table 13). Those that were observed to contain solid were analyzed by XRPD and, in all cases, the patterns obtained were found to match those of the crystalline acid counter ion with no additional peaks (FIG. 13B).

Table 13. Observations and XRPD Results from LAG of Compounds 1

Sample ID	Acid	Observation after grinding	XRPD
4-1	Pamoic	Yellow gum/solid	Pamoic acid & amorphous halo
4-2	Malonic	Clear gum	-
4-3	D-Glucuronic	White gum/solid	D-Glucuronic acid & amorphous halo
4-4	DL-Mandelic	Clear gum	-
4-5	D-Gluconic	Clear gum	-
4-6	Glycolic	Clear gum	
4-7	L-Lactic	Clear gum	-
4-8	Oleic	Clear gum	-
4-9	L-Ascorbic	White gum/solid	L-Ascorbic acid & amorphous halo
4-10	Adipic	Clear gum	-
4-11	Caproic	Clear gum	-
4-12	Stearic	White gum/solid	Stearic acid & amorphous halo
4-13	Palmitic	White gum/solid	Palmitic acid & amorphous halo
4-4	Methanesulfonic	Clear gum	-

Example 9. Failure to Obtain Adequate Salt Formation using Methyl Ethyl Ketone (MEK)

[0139] Methyl ethyl ketone (MEK) was next utilized as a solvent to study appropriate salts other than the hemi-sulfate salt. Using the 14 acidic counter ions in Table 12, the study was performed by dissolving Compound 1 (50 mg) in MEK (20 vol) at room temperature. The solutions were treated with 1 equivalence of the selected counter-ions (Table 12). The samples were then cooled down to 5 °C at 0.1 °C/min and stirred at this temperature overnight. All samples were allowed to evaporate under ambient conditions and any solids observed were analyzed by XRPD. This evaporation mainly produced gums, with the exception of the samples with steric acid (sample 4-12) and palmitic acid (sample 5-13), which afforded glassy solvents. These solids were amorphous by XRPD, but no crystalline forms of the salt were obtained. Results are shown in Table 14. (FIG. 13A).

Table 14. Results from dissolving Compound 1 in MEK (20 volumes)

Sample ID	Acid	Solvent for acid at 1 M	Observation upon acid addition	Observation upon cooling	Observation upon evaporation
5-1	Pamoic	DMSO	Yellow solution	Yellow solution	Yellow gum
5-2	Malonic	THF	Solution	Solution	Clear gum
5-3	D-Glucuronic	Water	Solution	Solution	Clear gum
5-4	DL-Mandelic	THF	Solution	Solution	Clear gum
5-5	D-Gluconic	THF	White precipitate	Turbid solution	Clear gum
5-6	Glycolic	THF	Solution	Solution	Clear gum
5-7	L-Lactic	THF	Solution	Solution	Clear gum
5-8	Oleic	THF	Solution	Solution	Clear gum
5-9	L-Ascorbic	Water	Solution	Solution	Yellow gum
5-10	Adipic	THF (heat)	Solution	Solution	Clear gum
5-11	Caproic	THF	Solution	Solution	Clear gum
5-12	Stearic	THF	Solution	Turbid solution	Clear glassy solid*
5-13	Palmitic	THF	Solution	Solution	Clear glassy solid*
5-14	Methanesulfonic	THF	Solution	Solution	Clear gum
Stock solution prepared prior to acid addition *Samples were analyzed by XRPD and gave amorphous patterns plus peaks from the acid counter ion					

[0140] Since all samples were amorphous, all samples were redissolved in MEK (5 vol) and cyclohexane was added (20 vol antisolvent) at room temperature followed by 1 hour of stirring at 25 °C. The samples were then matured between 50 - 5 °C (1 °C/min between temperatures, 4 hours at each temperature) for 2 days before the cycle was changed to 50 - 25 °C for a further 4 days. The samples were observed by eye following maturation. Results are shown in Table 15. Following the maturation, all samples except 5-1 (with pamoic acid) were found to be gums. Sample 5-1, a yellow solid, was analyzed by XRPD, and the pattern was found to match the known form of pamoic acid (FIG. 14B), and therefore no crystalline forms of the salt were obtained.

Table 15. Results from redissolving Compound 1 in MEK (5 volumes) and antisolvent

Sample ID	Immediate Observation	Observation after 10 minutes	Observation after 60 minutes	Observation after Maturation
5-1	Precipitate	Gum	Gum	Yellow suspension**
5-2	Precipitate	Gum	Gum	Gum
5-3	Precipitate/gum	Gum	Gum	Gum
5-4	Precipitate	Gum	Gum	Gum
5-5	Precipitate/gum	Gum	Gum	Gum
5-6	Precipitate	Gum	Gum	Gum
5-7	Precipitate	Gum	Gum	Gum
5-8	Precipitate	Light suspension	Gum	Gum
5-9	Precipitate	Gum	Gum	Gum
5-10	Precipitate	Gum	Gum	Gum

(continued)

Sample ID	Immediate Observation	Observation after 10 minutes	Observation after 60 minutes	Observation after Maturation
5-11	Precipitate	Light suspension	Gum	Gum
5-12	Precipitate	Light suspension	Gum	Gum
5-13	Precipitate	Light suspension	Gum	Gum
5-14	Precipitate	Gum	Gum	Gum
**Sample analyzed by XRPD with pattern matching known form of pamoic acid (no additional peaks				

Example 10. Failure to Obtain Adequate Salt Formation using Ethyl Acetate

[0141] Ethyl acetate was next utilized to study appropriate salts other than hemi-sulfate salt. Utilizing the 14 acidic counter ions in Table 12, the study was performed by dissolving Compound 1 (50 mg) in ethyl acetate (20 vol) at 50 °C. The solutions were treated with 1 equivalent of the selected counter-ions (Table 12). The samples were then cooled down to 5 °C at 0.1 °C/min and stirred at this temperature for 4 days. The solutions were allowed to evaporate under ambient conditions while any solids were analyzed by XRPD. The results from the crystallizations using ethyl acetate are in Table 16. In contrast to Example 8 where MEK was the solvent, the majority of samples were observed to be suspensions following cooling of the acid:compound mixture (those that were solutions were allowed to evaporate under ambient conditions). However, the XRPD diffractograms were generally found to match crystalline Compound 1. Samples 6-2, 6-4, and 6-5 have some slight differences (FIG. 14A and FIG. 15A). No crystalline forms of the salt were obtained.

Table 16. Results from dissolving Compound 1 in EtOAc (20 volumes)

Sample ID	Acid	Solvent for acid at 1 M	Observation upon acid addition	Observation upon Cooling	XRPD	Observation upon Evaporation
6-1	Pamoic	DMSO	Yellow solution	Yellow solution*	-	Gum
6-2	Malonic	THF	Solution	White suspension	Slight differences to freebase	-
6-3	D-Glucuronic	Water	Solution	Solution*	-	Gum
6-4	DL-Mandelic	THF	Solution	White suspension	Slight differences to freebase	-
6-5	D-Gluconic	THF	White precipitate	Possible white gum	Slight differences to freebase	-
6-6	Glycolic	THF	Solution	White suspension	Freebase	-
6-7	L-Lactic	THF	Solution	White suspension	Freebase	-
6-8	Oleic	THF	Solution	White suspension	Freebase	-
6-9	L-Ascorbic	Water	Solution	Solution*	-	White solid on side / yellow gum - amorphous
6-10	Adipic	THF (heat)	Solution	White suspension	Freebase	-

(continued)

Sample ID	Acid	Solvent for acid at 1 M	Observation upon acid addition	Observation upon Cooling	XRPD	Observation upon Evaporation
6-11	Caproic	THF	Solution	White suspension	Freebase	-
6-12	Stearic	THF	Solution	White suspension	Freebase	-
6-13	Palmitic	THF	Solution	White suspension	Freebase	-
6-14	Methanesulfonic	THF	White precipitate	Solution / clear gum*	-	Clear gum

Example 11. Chemical Purity Determination by HPLC

[0142] Purity analysis in Example 2 and Example 4 was performed on an Agilent HP1100 series system equipped with a diode array detector and using ChemStation software vB.04.03 using the method shown in Table 17.

Table 17. HPLC method for chemical purity determinations

Parameter	Value		
Type of method	Reverse phase with gradient elution		
Sample Preparation	0.5 mg/ml in acetonitrile : water 1:1		
Column	Supelco Ascentis Express C18, 100 × 4.6 mm, 2.7 μm		
Column Temperature (°C)	25		
Injection (□1)	5		
Wavelength, Bandwidth (nm)	255, 90		
Flow Rate (ml/min)	2		
Phase A	0.1% TFA in water		
Phase B	0.085% TFA in acetonitrile		
Timetable	Time (min)	% Phase A	% Phase B
	0	95	5
	6	5	95
	6.2	95	5
	8	95	5

Example 12. X-Ray Powder Diffraction (XRPD) Techniques

[0143] The XRPD patterns in Examples 2, 3, 4, 5, 6, 7, 8, and 9 were collected on a PANalytical Empyrean diffractometer using Cu K□ radiation (45 kV, 40 mA) in transmission geometry. A 0.5° slit, 4 mm mask and 0.4 rad Soller slits with a focusing mirror were used on the incident beam. A PIXcel^{3D} detector, placed on the diffracted beam, was fitted with a receiving slit and 0.04 rad Soller slits. The instrument is performance checked using silicon powder on a weekly basis. The software used for data collection was X'Pert Data Collector v. 5.3 and the data were analyzed and presented using Diffrac *Plus* EVA v. 15.0.0.0 or Highscore Plus v. 4.5. Samples were prepared and analyzed in either a metal or Millipore 96 well-plate in transmission mode. X-ray transparent film was used between the metal sheets on the metal well-plate and powders (approximately 1-2 mg) were used as received. The Millipore plate was used to isolate and analyze solids from suspensions by adding a small amount of suspension directly to the plate before filtration under a light vacuum.

[0144] The scan mode for the metal plate used the gonio scan axis, whereas a 2θ scan was utilized for the Millipore

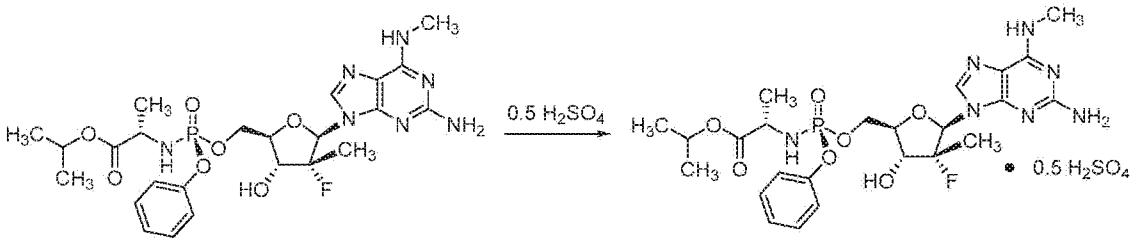
plate. A performance check was carried out using silicon powder (metal well-plate). The details of the data collection were an angular range of 2.5 to 32.0° 2θ, a step size of 0.0130° 2θ, and a total collection time of 2.07 minutes.

[0145] Samples were also collected on a Bruker D8 diffractometer using Cu K□ radiation (40 kV, 40 mA), θ - 2θ goniometer, and divergence of V4 and receiving slits, a Ge monochromator and a Lynxeye detector. The instrument is performance checked using a certified Corundum standard (NIST 1976). The software used for data collection was DiffracPlus XRD Commander v2.6.1 and the data were analyzed and presented using Diffrac *Plus* EVA v15.0.0.0.

[0146] Samples were run under ambient conditions as flat plate specimens using powder as received. The sample was gently packed into a cavity cut into polished, zero-background (510) silicon wafer. The sample was rotated in its own plane during analysis. The details of the data collection were an angular range of 2 to 42° 2θ, a step size of 0.05° 2θ, and collection time of 0.5 s/step.

Example 13. Synthesis of Amorphous Compound 2

[0147]



Compound 1 Compound 2

[0148] A 250 mL flask was charged with MeOH (151 mL) and the solution was cooled to 0-5 °C. A concentrated solution of H_2SO_4 was added dropwise over 10 minutes. A separate flask was charged with Compound **1** (151 g) and acetone (910 mL), and the H_2SO_4/MeOH solution was added dropwise at 25-30 °C over 2.5 hours. A large amount of solid was precipitated. After the solution was stirred for 12-15 hours at 25-30 °C, the mixture was filtered, washed with MeOH/acetone (25 mL/150 mL), and dried at 55-60 °C in vacuum to afford Compound **2** (121 g, 74%).

Analytic Method for Compound 2: The purity of Compound **2** was obtained using an Agilent 1100 HPLC system with a Waters XTerra Phenyl 5μm 4.6*250mm column with the following conditions: 1 mL/min flow rate, read at 254 nm, 30 °C column temperature, 10 μL injection volume, and a 30 minute run time. The sample was dissolved in ACN:water (90:10, v/v). The Gradient method for separation is shown below. Rt(min) of Compound **2** was approximately 12.0 minutes.

Time (min)	0.1% H_3PO_4 in Water (A)%	Acetonitrile (B)%
0	90	10
20	20	80
20.1	90	10
30	90	10

[0149] ^{1}HNMR: (400 MHz, DMSO-d_6): δ 8.41 (br, 1H), 7.97 (s, 1H), 7.36 (t, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.17 (t, *J* = 8.0 Hz, 1H), 6.73 (s, 2H), 6.07 (d, *J* = 8.0 Hz, 1H), 6.00 (dd, *J* = 12.0, 8.0 Hz, 1H), 5.81(br, 1H), 4.84-4.73 (m, 1H), 4.44-4.28 (m, 3H), 4.10 (t, *J* = 8.0 Hz, 2H), 3.85-3.74 (m, 1H), 2.95 (s, 3H), 1.21 (s, *J* = 4.0 Hz, 3H), 1.15-1.10 (m, 9H).

Example 14. Characterization of Compound 2

[0150] Compound **2** was further characterized by eye, ^{1}HNMR, ^{13}CNMR, ^{19}FNMR, MS, HPLC, and XRPD (FIG. 15B). Residual solvent was measured by GC. Water content was measured by Karl Fischer Titration, and the water content was only 0.70%. Data is summarized in Table 18.

Table 18. Summary of Additional Characterization Data of Compound 2

Test	Result
Appearance	White Solid
NMR	^{1}HNMR peaks are listed in Example 4
MS	MS(ESI+ve) $[M+H]^+$ = 582.3 - conforms to structure
HPLC	99.8% by AUC at 254 nm (average of two preparations)
Residual Solvent by GC	Methanol - 57 ppm Acetone - 752 ppm Dichloromethane - 50 ppm Ethyl Acetate - 176 ppm
Water Content	0.70%

Example 15. Solubility of Compound 1 and Compound 2

[0151] Compound **1** and Compound **2** were both tested for solubility in biorelevant test medias, including simulated gastric fluid (SGF), fasted-state simulated gastric fluid (FaSSIF), and fed-state gastric fluid (FeSSIF). Results for Compound **1** are shown in Table 19 and results for Compound **2** are shown in Table 20. Samples were stirred at room temperature (20 - 25 °C). Compound **2** was more than 40-fold more soluble than Compound **1** in water at 2 hours and more than 25-fold more soluble at 24 hours. In SGF conditions, Compound **2** had a solubility of 84.2 mg/mL at 24 hours compared to the solubility of 15.6 mg/mL of Compound **1** at the same time point. Compound **2** was also more soluble at 2 hours in the SGF conditions than Compound **1,** and soluble enough to allow for testing even after 48 hours while testing at 48 hours was not done with Compound **1.**

Table 19. Compound 1 solubility testing results

Test Media	Solubility (in mg/mL)		Appearance	Descriptive term
	2 hours	24 hours		
Water	1.5	2.5	Clear Solution*	Slightly Soluble
SGF	13.8	15.6	Clear Solution with gum at the bottom	Sparingly Soluble
FaSSIF	1.7	1.7	Turbid	Slightly Soluble
FeSSIF	2.8	2.9	Turbid	Slightly Soluble
*Sample appeared to be clear, yet a solubility of only 1.5 mg/mL was achieved. Upon further investigation, it was noted that a gummy film formed on the stir bar. The compound 1 active pharmaceutical ingredient formed a gummy ball in diluent (90% water/10% acetonitrile) during standard preparation which required a long sonication time to dissolve completely.				

Table 20. Compound 2 solubility testing results

Test Media	Solubility (in mg/mL salt base)			Appearance	Descriptive term
	2 hours	24 hours	48 hours		
Water	65.3	68.0	N/A	Turbid	Soluble
SGF	89.0	84.2	81.3	Turbid	Soluble
FaSSIF	1.9	2.0	N/A	Turbid	Slightly Soluble
FeSSIF	3.3	3.4	N/A	Turbid	Slightly Soluble

Example 16. Chemical Stability of Compound 2

[0152] Compound **2** was tested for chemical stability at 25 and 40 °C over a 6 month time period by monitoring organic

purity, water content, ^{1}HNMR, DSC, and Ramen IR. The container closure system for the study was a combination medicinal valve bag with a pharmaceutical laminated film over the pouch and desiccant silica gel between the two layers. Compound **2** (1 g) was measured into each container. Bags were then stored at 25°C/60%RH (relative humidity) and 40°C/75%RH (relative humidity). Organic purity, water content, ^{1}HNMR, DSC and Raman were measured at Time 0, Month 1, Month 2, Month 3 and Month 6.

[0153] The purity of Compound **2** was obtained using a Shimadzu LC-20AD system with a Waters XTerra Phenyl, 5μm, 4.6x250mm column with the following conditions: 1 mL/min flow rate, read at 254 nm, 35 °C column temperature, and 10 μL injection volume. The sample was dissolved in acetonitrile - water (90:10) (v/v). The gradient method is shown below.

Time (min)	A% (ACN)	B% (water)
0	90	10
20	20	80
20.1	90	10
30	90	10

[0154] The water content of Compound **2** (250 mg) was determined by a water titration apparatus using the Karl Fischer titration method.

[0155] Results are shown in Table 21 and Table 22. When Compound **2** was stored for 6 months at 25 and 40 °C, the rate of degradation was minimal. At 3 months, Compound **2** was 99.75% percent pure at the 25 °C conditions and 99.58% pure at the 40 °C conditions. At 6 months, Compound **2** was still 99.74% pure at the 25 °C conditions and 99.30% pure at the 40 °C conditions. At 25 °C, the percent of degradation product increased from 0.03% at Day 0 to 0.08% after 6 months. At 40 °C, the percent of degradation product increased from 0.03% to 0.39%. Over the course of 6 months, the percent of water increased approximately 0.6% at 25 °C and increased approximately 0.7% at 40 °C.

[0156] Characterization by ^{1}HNMR, Raman, and DSC of Compound **2** at 1, 2, 3, and 6 months was the same as the characterization of Compound **2** on day 0 at both temperature conditions (Table 22), highlighting the long-term stability of Compound **2.**

Table 21. Compound 2 rate of degradation over 6 months at 25 and 40 °C

	Time Tested	Percent Water	Percent Purity	Percent of Degradation Product	Maximum Impurity Percent
25 °C	Day 0	1.2	99.82	0.03	0.12
	Month 1	1.9	99.77	0.04	0.12
	Month 2	1.8	99.75	0.06	0.12
	Month 3	1.8	99.75	0.06	0.12
	Month 6	1.8	99.74	0.08	0.13
40 °C	Day 0	1.2	99.82	0.03	0.12
	Month 1	2.0	99.71	0.09	0.12
	Month 2	1.9	99.63	0.15	0.12
	Month 3	1.9	99.58	0.20	0.12
	Month 6	1.9	99.30	0.39	0.14

Table 22. Characterization of Compound 2 during degradation study

	Time Tested	^{1}HNMR	Raman	DSC
25 °C	Day 0	Initial Test	Initial Test	Initial Test
	Month 1	The same as Day 0	The same as Day 0	The same as Day 0
	Month 2	The same as Day 0	The same as Day 0	The same as Day 0
	Month 3	The same as Day 0	The same as Day 0	The same as Day 0
	Month 6	The same as Day 0	The same as Day 0	The same as Day 0
40 °C	Day 0	Initial Test	Initial Test	Initial Test
	Month 1	The same as Day 0	The same as Day 0	The same as Day 0
	Month 2	The same as Day 0	The same as Day 0	The same as Day 0
	Month 3	The same as Day 0	The same as Day 0	The same as Day 0
	Month 6	The same as Day 0	The same as Day 0	The same as Day 0

[0157] Additional chemical stability studies of Compound **2** were measured to determine the impurity and water levels. Three conditions were tested: accelerated stability (40 + 2°C / 75 + 5% RH) over a 6-month time period, ambient stability (25 + 2°C / 60 + 5% RH) over a 9-month period, and stability under refrigerator conditions (5 + 3°C) over a 9-month time period. The results for accelerated stability, ambient stability, and refrigerator conditions are shown in Table 23, Table 24, and Table 25, respectively. Based on the results of these studies, Compound **2** is very chemically stable.

[0158] In the accelerated stability study (Table 23), at each time point (1st month, 3rd month, and 6th month) where Compound **2** was measured, the appearance of Compound **2** was always a white solid and the IR matched the reference standard. After six months, the total related substance 1 impurities was only 0.08% and there was no detection of related substance 2 and isomers.

Table 23. Accelerated Stability (40 ± 2°C / 75 ± 5% RH) of Compound 2

Items		Specification	Testing time point			
			0 month	1st month	3rd month	6th month
Appearance		White or off-white solid	White solid	White solid	White solid	White solid
IR		correspond with reference standard	correspond with reference standard	/	correspond with reference standard	correspond with reference standard
Water		≤2.0%	0.45%	0.21%	0.36%	0.41%
Related Substance 1	Impurity A	≤0.15%	N.D.	N.D.	N.D.	N.D.
	Impurity B	≤0.15%	N.D.	N.D.	N.D.	N.D.
	Impurity F	≤0.15%	N.D.	N.D.	N.D.	0.01%
	Impurity H	≤0.15%	N.D.	N.D.	N.D.	N.D.
	Any other single impurity	≤0.10%	0.01%	0.02%	0.01%	0.05%
	Total Impurities	≤0.2%	0.01%	0.02%	0.02%	0.08%
Related Substance 2	Impurity G	≤0.15%	N.D.	N.D.	N.D.	N.D.

(continued)

Items		Specification	Testing time point			
			0 month	1st month	3rd month	6th month
Isomer	Impurity C	≤0.15%	N.D.	/	N.D.	N.D.
	Impurity D	≤0.15%	N.D.	/	N.D.	N.D.
	Impurity E	≤0.15%	N.D.	/	N.D.	N.D.
Assay		98.0%~102.0%	98.8%	101.5%	99.6%	99.5%
Microbial Testing	TAMC	≤1000cfu/g	<1cfu/g	/	/	/
	Mold and Yeast	≤100cfu/g	<1cfu/g	/	/	/
	E.Coli	Not Detected	N.D.	/	/	/
N.D.: Not Detected						

[0159] In the ambient stability study where the appearance, IR, water and impurity levels were measured for nine months, the appearance of Compound **2** was always a white solid and the IR always corresponded with the reference sample. The results (Table 24) highlight how chemically stable Compound **2** is. After 9 months, the percentage of water in the sample was only 0.20% and the total related substance 1 impurities was only 0.02%. Similarly to the accelerated stability studies, related substance 2 and any isomers of Compound **2** were not detected.

Table 24. Ambient stability (25 ± 2°C / 60 ± 5% RH) of Compound 2

Item		Specification	Testing time point				
			0 month	1st month	3rd month	6th month	9th month
Appearance		White or off-white solid	White solid	White solid	White solid	White solid	Off-white solid
IR		correspond with reference standard	correspond with reference standard	/	correspond with reference standard	correspond with reference standard	correspond with reference standard
Water		≤2.0%	0.45%	0.19%	0.29%	0.46%	0.20%
Related Substance 1	Impurity A	≤0.15%	N.D.	N.D.	N.D.	N.D.	N.D.
	Impurity B	≤0.15%	N.D.	N.D.	0.03%	N.D.	N.D.
	Impurity F	≤0.15%	N.D.	N.D.	0.02%	0.01%	N.D.
	Impurity H	≤0.15%	N.D.	N.D.	N.D.	N.D.	N.D.
	Any other single impurity	≤0.10%	0.01%	0.01%	0.03%	0.02%	0.02%
	Total Impurities	≤0.2%	0.01%	0.02%	0.11%	0.05%	0.02%
Related Substance 2	Impurity G	≤0.15%	N.D.	N.D.	N.D.	N.D.	N.D.

(continued)

Item		Specification	Testing time point				
			0 month	1st month	3rd month	6th month	9th month
Isomer	Impurity C	≤0.15%	N.D.	/	N.D.	N.D.	N.D.
	Impurity D	≤0.15%	N.D.	/	N.D.	N.D.	N.D.
	Impurity E	≤0.15%	N.D.	/	N.D.	N.D.	N.D.
Assay		98.0%~102.0 %	98.8%	101.1%	99.6%	99.7%	100.9%
Microbial Testing	TAMC	≤1000cfu/g	<1cfu/g	/	/	/	/
	Mold and Yeast	≤100cfu/g	<lcfu/g	/	/	/	/
	E.Coli	Not Detected	N.D.	/	/	/	/
N.D.: Not Detected							

[0160] The results of measuring the stability under refrigerator conditions are shown in Table 25. The only impurities detected even after 9 months were those from related substance 1 and water. The water content after 9 months was 0.32% and the total impurities of related substance 1 were only 0.01% of the sample. Compound 2 is very chemically stable under refrigerator conditions.

Table 25. Stability under refrigerator conditions (5 ± 3°C) of Compound 2

Item		Specification	Testing time point				
			0 month	1st month	3rd month	6th month	9th month
Appearance		White or off-white solid	White solid	White solid	White solid	White solid	Off-white solid
IR		correspond with reference standard	correspond with reference standard	/	correspond with reference standard	correspond with reference standard	correspond with reference standard
Water		<2.0%	0.45%	0.19%	0.32%	0.42%	0.32%
Related Substance 1	Impurity A	≤0.15%	N.D.	N.D.	N.D.	N.D.	N.D.
	Impurity B	≤0.15%	N.D.	N.D.	0.01%	N.D.	N.D.
	Impurity F	≤0.15%	N.D.	N.D.	N.D.	N.D.	N.D.
	Impurity H	≤0.15%	N.D.	N.D.	N.D.	N.D.	N.D.
	Any other single impurity	≤0.10%	0.01%	0.01%	0.01%	0.01%	0.01%
	Total Impurities	≤0.2%	0.01%	0.01%	0.03%	0.03%	0.01%
Related Substance 2	Impurity G	≤0.15%	N.D.	N.D.	N.D.	N.D.	N.D.

(continued)

Item		Specification	Testing time point				
			0 month	1st month	3rd month	6th month	9th month
Isomer	Impurity C	≤0.15%	N.D.	/	N.D.	N.D.	N.D.
	Impurity D	≤0.15%	N.D.	/	N.D.	N.D.	N.D.
	Impurity E	≤0.15%	N.D.	/	N.D.	N.D.	N.D.
Assay		98.0%~102.0 %	98.8%	101.1%	100.2%	98.6%	101.4%
Microbial Testing	TAMC	≤1000cfu/g	<1cfu/g	/	/	/	/
	Mold and Yeast	≤100cfu/g	<1cfu/g	/	/	/	/
	E.Coli	Not Detected	N.D.	/	/	/	/
N.D.: Not Detected							

Example 17. Plasma Levels of Metabolites following single oral doses of Compound 2

[0161] A single oral dose of Compound **2** was administered to rats, dogs, and monkeys, and the plasma levels of certain metabolites shown in Scheme 1 were measured.

[0162] The conversion of Compound **2** to Compound **1** and metabolite **1-7** are shown in Table 26 and the results for metabolite 1-8 and metabolite 1-2 are shown in Table 27. In rats, low levels of Compound **1** exposure were observed, but high levels of metabolite **1-7,** the nucleoside metabolite of the active triphosphate (metabolite **1-6),** were observed. In monkeys, roughly dose-proportional exposures of Compound 1 were measured. In dogs, supra-proportional Compound **1** exposures, indicative of first-pass metabolic clearance in the liver, were measured. Throughout the study, significantly more vomiting in dogs (5/5 in high dose group) than in monkeys (1/5 in high dose group) was observed.

Table 26. Plasma levels of Compound 1 and metabolite 1-7 after single oral doses of Compound 2

Species	Dose* (mg/kg)	Compound 1			Metabolite 1-7	
		C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0\text{-last}}$ (hr*ng/mL)	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (hr*ng/mL)
Rat[a]	500	70.5	0.25	60.9	748	12000
Dog[b]	30	1530	0.25-1	1300	783	9270
	100	8120	0.5-1	10200	2030	24200
	300	21300	204	44300	4260	60800
Monkey[b]	30	63.5	0.5-2	176	42.5	1620
	100	783	1-2	1100	131	3030
	300	501	204	1600	93.6	3660
3 males per dose per species; *dose formulations: [a]0.5% CMC, 0.5% Tween 80 in water, [b]powder in capsules						

Table 27. Plasma levels of metabolites 1-8 and 1-2 after single oral dose of Compound 2

Species	Dose* (mg/kg)	Metabolite 1-8		Metabolite 1-2	
		C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (hr*ng/mL)	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (hr*ng/mL)
Raf	500	5060	35100	9650	20300

(continued)

Species	Dose* (mg/kg)	Metabolite 1-8		Metabolite 1-2	
		C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (hr*ng/mL)	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (hr*ng/mL)
Dog[b]	30	291	905	196	610
	100	1230	4370	886	2830
	300	5380	35300	2380	8710
Monkey[b]	30	209	5690	300	1730
	100	406	12300	1350	8160
	300	518	16800	1420	11400
3 males per dose per species; *dose formulations: [a]0.5% CMC, 0.5% Tween 80 in water; [b]powder in capsules					

Example 18. Tissue Exposure of Active Triphosphate following Compound 2 Oral Dose

[0163] Heart and liver tissue levels of the active triphosphate (TP) of Compound **2** (metabolite **1-6**) were measured 4 hours after oral doses of Compound **2.** Samples of liver and heart were obtained at 4 hours after a single dose of Compound **2,** flash-frozen, homogenized and analyzed by LC-MS/MS for intracellular levels of the active TP. Tissue levels were measured in rats, dogs, and monkeys as shown in FIG. 16A. High levels of the active TP were measured in the liver of all species tested. Relatively low levels of the active TP were measured in the hearts of dogs due to saturation of first-pass hepatic metabolism, and unquantifiable levels of TP were measured in rat and monkey hearts, indicative of liver-specific formation of the active TP. While not shown, compared to Compound **1** dosing, Compound **2** dosing improved TP distribution.

Example 19. Pharmacological Comparison of Compound 1 and Compound 2 in Dogs

[0164] A head-to-head comparison of dogs dosed with Compound **1** and Compound **2** was conducted. The study measured plasma levels of Compound **1** and metabolite **1-7** (from Scheme 1) out to 4 hours after dosing with Compound **1** (25 mg/kg) and Compound **2** (30 mg/kg) (Table 28), and the $AUC_{(0\text{-}4hr)}$ of metabolite **1-7** was twice as great with Compound **2** compared to Compound **1.** Dose-normalized exposures to Compound **1** and metabolite **1-7** are shown in Table 28. Values for $AUC_{(0\text{-}4hr)}$ for Compound **1,** metabolite **1-7,** and the sum of Compound **1** + metabolite **1-7** were greater after dosing with Compound **2.**

Table 28. Comparison of Plasma Levels following dosing with Compound 1 and Compound 2

Dosed Compound	Mean Dose-normalized $AUC_{(0\text{-}4hr)}$[a] (μM*hr) for:		
	Compound **1**	Metabolite **1-7**	Compound **1** + Metabolite **1-7**
Compound **1** (25 mg/kg)	0.2	1.9	2.1
Compound **2** (30 mg/kg)	1.0	4.1	5.1
*$AUC_{(0\text{-}4hr)}$ values normalized to a dose of 25 mg/kg			

[0165] Liver/heart ratio triphosphate concentrations indicate that dosing with Compound **2,** as compared to Compound **1,** increases the selective delivery of the triphosphate to the liver, as shown in Table 29. The $AUC_{(0\text{-}4hr)}$ of the active guanine metabolite **(1-6)** after administration of Compound **1** measured in the heart was 174 pM*hr, while the $AUC_{(0\text{-}4hr)}$ of the active guanine metabolite **(1-6)** after administration of Compound **2** measured in the heart was 28 pM*hr. The liver/heart ratio for Compound **2** was 20 compared to a liver/heart ratio of 3.1 for Compound **1.**

Table 29. Comparison of Liver and Heart Exposure following dosing with Compound 1 and Compound 2

Dosed Compound	Mean Dose-normalized $AUC_{(0\text{-}4hr)}$[a] (μM*hr) for:		
	Liver	Heart	Liver / Heart
Compound 2	565	28[b]	20

(continued)

Dosed Compound	Mean Dose-normalized $AUC_{(0-4hr)}$[a] (μM*hr) for:		
	Liver	Heart	Liver / Heart
Compound 1	537	174	3.1
[a]Active TP concentrations (**1-6;** Scheme 1) normalized to a dose of 25 mg/kg [b]Extrapolated below the lower limit of quantitation of the calibration curve			

[0166] The effect of increased selectivity for the liver over the heart when Compound **2** was administered compared to Compound **1** is also shown in FIG. 16B. The heart and liver tissue levels of the active triphosphate following a dosage of Compound **2** (30 mg/kg) were compared to the tissue levels of the active triphosphate following a dosage of Compound **1** (25 mg/kg). The concentration of the active TP was higher in the liver than the heart for both Compound **1** and Compound **2,** but the active TP was more selective for the liver over the heart when Compound **2** was dosed compared to Compound **1.**

Example 20. Plasma Profiles of Compound 2 Metabolites in Rats and Monkeys

[0167] Male Sprague-Dawley rats and cynomolgus monkeys (3 animals per dose group) were given single oral doses of Compound **2.** Aliquots of plasma prepared from blood samples treated with Dichlorvos were analyzed by LC-MS/MS for concentrations of Compound **1** and metabolite **1-7** (the nucleoside metabolite of the active triphosphate of Compound **2** shown in Scheme 1), and pharmacokinetic parameters were determined using WinNonlin. The results for a single 500 mg/kg dose in rats is shown in FIG. 17 and the results for a single 30, 100, or 300 mg/kg dose in monkeys is shown in FIG. 18. The results are also summarized in Table 30.

[0168] High plasma levels of metabolite **1-7,** the nucleoside metabolite of the active triphosphate (TP) of Compound **2,** are indicative of formation of high levels of the TP, even in rats where very low plasma levels of parent nucleotide prodrug are observed due to the short half-life of Compound **1** in rat blood (<2 min). Persistent plasma levels of metabolite **1-7** reflect the long half-life of the TP.

[0169] In monkeys, plasma exposures (AUC) of Compound **1** were roughly dose-proportional, while metabolite **1-7** exposures were somewhat less than dose-proportional, although AUC values for both parent drug and the nucleoside metabolite of the active TP continue to increase up to the highest dose tested (300 mg/kg).

[0170] Oral administration of Compound **2** in rats and monkeys produced high and dose-dependent plasma exposures to metabolite **1-7** (the nucleoside metabolite of the intracellular active triphosphate of Compound **2**); metabolite **1-7** exposures continued to increase up to the highest dose tested, reflecting substantial formation of the active **TP** in these species.

Table 30. Plasma levels of Compounds 1 and 1-7 after single oral dose of Compound 2

	Species	**Rat[a]**	**Monkey[b]**		
	Dose (mg/kg)	**500**	**30**	**100**	**300**
Compound 1	**C_{max} (ng/mL)**	60.8	63.5	783	501
	T_{max} (hr)	0.25	0.5-2	1-2	204
	AUC_{0-last} (hr*ng/mL)	78.2	176	1100	1600
Metabolite 1-7	**C_{max} (ng/mL)**	541	42.5	131	93.6
	AUC_{0-last} (hr*ng/mL)	9640	1620	3030	3660
	T_{max} (hr)	6-8	12-24	4	4-24
	$T_{1/2}$ (hr)	15.3	11.5	15.0	18.8
dose formulations: [a]0.5% CMC, 0.5% Tween 80 in water, [b]powder in capsules					

Example 21. The Effect of the Active Triphosphate of Compound 1 and Compound 2 on Mitochondrial Integrity

[0171] The relative efficiency of incorporation of the active triphosphate (TP) of Compound **1** and Compound **2,** me-

tabolite **1-6** (Scheme 1), by human mitochondrial RNA polymerase was compared to the relative efficiency of the active TP of sofosbuvir and the active TP of INX-189. Compound **1** and Compound **2** are not likely to affect mitochondrial integrity since their active triphosphate is poorly incorporated by human mitochondrial RNA polymerase with an efficiency similar to that of the triphosphate of sofosbuvir; the relative efficiency of incorporation of the triphosphate of INX-189 was up to 55-fold greater. Results are shown in Table 31. The incorporation of these analogs by human mitochondrial RNA-dependent RNA polymerase (POLRMT) were determined according to Arnold et al. (Sensitivity of Mitochondrial Transcription and Resistance of RNA Polymerase II Dependent Nuclear Transcription to Antiviral Ribonucleotides. PLoS Pathog., 2012, 8, e1003030).

Table 31. Kinetic Parameters for Nucleotide Analogs Evaluated with Human Mitochondrial RNA Polymerase

Nucleotide Analog	K_{pol} (s^{-1})	$K_{d,app}$ (μM)	$K_{pol/Kd,app}$ ($\mu M^{-1}s^{-1}$)	Relative Efficiency*
2'-deoxy-2'-F-2'-C-methyl UTP (active TP of sofosbuvir)	0.00034 ± 0.00005	590 ± 250	$5.8 \times 10^{-7} \pm 2.6 \times 10^{-7}$	1.0×10^{-6}
2'-C-methyl GTP (active TP of INX-189)	0.051 ± 0.002	240 ± 26	$2.1 \times 10^{-4} \pm 0.2 \times 10^{-4}$	5.5×10^{-5}
Active TP of Compound 1 and Compound 2 (metabolite 1-6)	0.0017 ± 0.0002	204 ± 94	$8.3 \times 10^{-6} \pm 4.0 \times 10^{-6}$	2.2×10^{-6}
*Relative efficiency = $(K_{pol}/K_{d,app})_{analog\ nicleotide}$ / $(K_{pol}/K_{d,app})_{natural\ nucleotide}$				

Example 22. Activity of Compound 1 against Replicons Containing the NS5B Sequence

[0172] A panel of replicons containing the NS5B sequences from various HCV genotypes derived from 6 laboratory reference strains (GT1a, 1b, 2a, 3a, 4a and 5a) (FIG. 19) and from 8 HCV patient plasma samples (GT1a, 1b, 2a, 2b, 3a-1, 3a-2, 4a and 4d) (FIG. 20) were used to determine the potency of Compound **1** and sofosbuvir.

[0173] Compound **1** was more potent than sofosbuvir against clinical and laboratory strains of HCV. Compound **1** showed potent pan-genotypic antiviral activity *in vitro* against wild-type clinical isolates with EC_{95} < 80 nM, which is 4- to 14-fold more potent than sofosbuvir. As shown in FIG. 20, EC_{95} values for Compound **1** were 7-33 times lower than sofosbuvir against clinical isolates of all HCV genotypes tested. EC_{50} values for Compound **1** were 6-11 times lower than sofosbuvir against laboratory strains of HCV Genotypes 1-5 (FIG. 19).

Example 23. Single Ascending Dose (SAD) Study of Compound 2 in Healthy Volunteers (Part A) and GT1-HCV Infected Patients (Part B)

[0174] Compound **2** was tested in a single ascending dose (SAD) study to measure its safety, tolerability, and pharmacokinetic in healthy subjects (Part A). Part A was a randomized, double-blind, placebo-controlled SAD study. Healthy subjects in Part A received a single dose of Compound **2** or placebo in the fasting state. Subjects were confined to the clinic from Day -1 to Day 6.

[0175] Dosing in each cohort was staggered such that 2 subjects (1 active: 1 placebo) were evaluated for 48 hours after dosing before the remainder of the cohort was dosed. Each cohort received Compound **2** in ascending order. Dosing of sequential cohorts occurred based on review of available safety data (through Day 5) and plasma pharmacokinetic data (through 24 h) of the prior cohort.

[0176] Dose escalation proceeded following satisfactory review of these data. As pharmacokinetic and safety data emerged from prior cohorts, doses evaluated in Cohorts 3a-4a were adjusted by increments no more than 100 mg. The total maximum dose evaluated in Part A did not exceed 800 mg. The dosing regimen for Part A is shown in Table 32.

Table 32. Dosing Regimen for Compound 2 Administration Part A of Study

Cohort	Population	N (active: placebo)	Compound 2 (Compound 1)*
1a	Healthy	6:2	50 (45) mg × 1 day
2a	Healthy	6:2	100 (90) mg × 1 day
3a	Healthy	6:2	200 (180) mg × 1 day

(continued)

Cohort	Population	N (active: placebo)	Compound 2 (Compound 1)*
4a	Healthy	6:2	400 (360) mg × 1 day
*Clinical doses are expressed in terms of Compound **2,** with the approximate Compound 1 base equivalent in parenthesis			

[0177] Healthy volunteers in the Part A portion of the study were male and female subjects between the ages of 18 and 65. Active and placebo recipients were pooled within each Part A cohort to preserve the study blind.

[0178] Compound **2** was also tested in a single ascending dose (SAD) study to measure its safety, tolerability, pharmacokinetic, and antiviral activity in GT1-HCV infected patients (Part B). Subjects in Part B received a single dose of Compound **2** in the fasting state. Patients were confined to the clinic from Day -1 to Day 6.

[0179] Part B was initiated after the safety (through Day 5) and plasma pharmacokinetic (through 24 h) data review from Cohort 3a in Part A. Available safety data (through Day 5) and pharmacokinetic data (through 24 h) was reviewed for the first cohort in Part B (Cohort 1b) before enrolling subsequent Part B cohorts. Subsequent Part B cohorts were only dosed following review of available safety and pharmacokinetic data from the respective doses in Part A as well as available safety (through Day 5) from the prior Part B cohorts.

[0180] Dose escalation up to 600 mg in HCV-infected patients proceeded following satisfactory review of these data. The dosing regimen for Part B is shown in Table 33.

Table 33. Dosing Regimen for Compound 2 in Part B of Study

Cohort	Population	N (active)	Compound 2 (Compound 1)*
1b	GT1 HCV-Infected	3	100 (90) mg × 1 day
2b	GT1 HCV-Infected	3	300 (270) mg × 1 day
3b	GT1 HCV-Infected	3	400 (360) mg × 1 day
4b	GT1 HCV-Infected	3	600 (540) mg × 1 day
*Clinical doses are expressed in terms of Compound 2, with the approximate Compound 1 base equivalent in parenthesis.			

[0181] Patients infected with HCV were treatment-naive, non-cirrhotic GT1-infected subjects with a viral load of ≥ 5 $\log_{10}$ IU/mL.

[0182] No serious adverse events were recorded and no premature discontinuations were required in either Part A or Part B. All adverse effects were mild to moderate in intensity and no dose-related patterns, including laboratory parameters, vital signs, and ECGs were evident.

Example 24. Results of the Single Ascending Dose (SAD) Study of Compound 2

[0183] Pharmacokinetic of Compound **1** and nucleoside metabolite **1-7** were measured following the single dose of Compound **2.** The C_{24} trough plasma concentrations (C_{24h}) of metabolite **1-7** in HCV-infected patients following a 600 mg dose of Compound **2** was 25.8 ng/mL, which is more than double the plasma concentration dose following a 300 mg dose of Compound **2.** Metabolite **1-7** (shown in Scheme 1) can only be generated via dephosphorylation of the intracellular phosphate metabolite **1-4,** metabolite **1-5,** and metabolite **1-6,** which is the active species. Therefore, metabolite **1-7** can be considered a surrogate of the active species. The pharmacokinetic data for all cohorts is shown in Table 34 and Table 35. Values are reported as mean ± SD, except tor T_{max} where median (range) is reported. Pharmacokinetic parameters were comparable in healthy and HCV-infected patients.

Table 34. Human Pharmacokinetic of Compound 1 and Metabolite 1-7 after Administration of a single dose of Compound 2 in Healthy Volunteers

	Dose (mg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{tot} (ng*h/mL)	$T_{1/2}$ (h)	C_{24h} (ng/mL)
Part A, Healthy Subjects						
Compd 1	50	46.4 ±17.6	0.5 (0.5-0.5)	36.4 ± 12.3	0.32 ± 0.02	--
	100	156 ± 96.3	0.5 (0.5-1.0)	167 ± 110	0.53 ± 0.24	--
	200	818 ± 443	0.5 (0.5-3.0)	656 ± 255	0.71 ± 0.16	--
	400	1194 ± 401	0.5 (0.5-1.0)	1108 ± 326	0.86 ± 0.15	--
Metabolite 1-7	50	27.9 ± 5.62	3.5 (3.0-4.0)	285 ± 69.4	7.07 ± 4.59	2.28 ± 0.95
	100	56.6 ± 14.0	4.0 (3.0-6.0)	663 ± 242	17.7 ± 14.7	4.45 ± 1.87
	200	111 ± 38.8	5.0 (3.0-6.0)	1524 ± 497	15.9 ± 7.95	13.7 ± 5.09
	400	153 ± 49.4	6.0 (4.0-8.0)	2342 ± 598	15.6 ± 6.37	23.5 ± 6.31
*Based on 24-hr profile.						

Table 35. Human Pharmacokinetic of Compound 1 and Metabolite 1-7 after Administration of Compound 2 in GT1-HCV Infected Patients

	Dose (mg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{tot} (ng*h/mL)	$T_{1/2}$ (h)	C_{24h} (ng/mL)
Compd 1	100	212 ± 32.0	0.5 (0.5-1.0)	179 ± 54.4	0.54 ± 0.12	--
	300	871 ± 590	0.5 (0.5-1.0)	818 ± 475	0.64 ± 0.20	--
	300	2277 ± 893	0.5 (0.5-1.0)	1856 ± 1025	0.84 ± 0.18	--
	400	2675 ± 2114	1.0 (1.0-2.0)	2408 ± 1013	0.86 ± 0.18	--
	600	3543 ± 1649	1.0 (0.5-1.0)	4132 ± 1127	0.70 ± 0.13	--

(continued)

	Dose (mg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{tot} (ng*h/mL)	$T_{1/2}$ (h)	C_{24h} (ng/mL)
Metabolite 1-7	100	50.2 ± 15.4	6.0 (4.0-6.0)	538 ± 103*	8.4 ± 4.3*	3.60 ± 0.40
	300	96.9 ± 38.9	6.0 (3.0-6.0)	1131 ± 273*	8.1 ± 2.4*	10.9 ± 3.51
	300	123 ± 16.6	4.0 (3.0-6.0)	1420 ± 221	--	18.0 ± 8.83
	400	160 ± 36.7	4.0 (4.0-4.0)	2132 ± 120	11.6 ± 1.21	22.5 ± 3.29
	600	198 ± 19.3	4.0 (4.0-6.0)	2176 ± 116	--	25.8 ± 4.08
*Based on 24-hr profile.						

[0184] The mean plasma concentration-time profiles of Compound **1** and metabolite **1-7** were also calculated for all cohorts of Part A and Part B of the study. FIG. 21 is the mean plasma-concentration of Compound **1** following a single dose of Compound **2** and FIG. 22 is the mean plasma-concentration of metabolite **1-7** following a single dose of Compound **2.** As shown in FIG. 21, Compound **1** was quickly absorbed and rapidly/extensively metabolized in all cohorts from Part B. As shown in FIG. 22, metabolite **1-7** was a major metabolite and exhibited sustained plasma concentrations. Plasma exposure of Compound **1** was dose-related while exposure of metabolite **1-7** was dose-proportional.

[0185] For the HCV-infected subjects of Part B, measurements of HCV RNA quantitation were performed before, during, and after administration of Compound **2.** Plasma HCV RNA determinations were performed through the use of a validated commercial assay. Baseline was defined as the mean of Day -1 and Day 1 (pre-dose). A single 300 mg dose of Compound **2** (equivalent to 270 mg of Compound **1**) resulted in significant antiviral activity in GT1b-HCV infected subjects. The mean maximum HCV RNA reduction 24 hours post-dose following a single 300 mg dose was 1.7 $\log_{10}$ IU/mL and this compares to a -2 log10 IU/mL reduction after 1 day of 400 mg of sofosbuvir monotherapy in GT1a HCV-infected subjects. The mean maximum HCV RNA reduction 24 hours post-dose following a single 100 mg dose was 0.8 $\log_{10}$ IU/mL. The mean maximum HCV RNA reduction was 2.2 $\log_{10}$ IU/mL following a single 400 mg dose. Individual pharmacokinetic/pharmacodynamic analyses for the individual subjects from Part B of the study are shown in FIGS. 23A-23F. Metabolite **1-7** concentration is plotted against HCV RNA reduction concentration, and as shown in FIGS. 23A-23F, plasma HCV RNA reduction correlates with plasma metabolite **1-7** exposure. Viral response is sustained with metabolite **1-7** plasma concentrations that are greater than the EC_{95} value against GT1b. The correlation between plasma concentration and HCV RNA reduction levels indicates that a more profound response will be achievable with higher doses of Compound **2.**

Example 25. Predicted Steady-State Trough Levels of Metabolite 1-7 exceed Compound 1 EC_{95} Values against Clinical Isolates of HCV GT 1-4

[0186] As shown in FIG. 24, the steady-state trough plasma levels ($C_{24,ss}$) of metabolite **1-7** following Compound 2 dosing in humans (600 mg QD (550 mg free base equivalent) and 450 mg QD (400 mg free base equivalent)) was predicted and compared to the EC_{95} of Compound **1** *in vitro* across all tested clinical isolates to determine if the steady state plasma concentration is consistently higher than the EC_{95}, which would result in high efficacy against any or all tested clinical isolates *in vivo*. The EC_{95} for Compound **1** is the same as the EC_{95} of Compound **2.** For Compound **2** to be effective, the steady-state trough plasma level of metabolite **1-7** should exceed the EC_{95}.

[0187] As shown in FIG. 24, the EC_{95} of Compound **2** against all tested clinical isolates ranged from approximately 18 to 24 nM.

[0188] As shown in FIG. 24, Compound **2** at a dose of 450 mg QD (400 mg free base equivalent) in humans of provides a predicted steady state trough plasma concentration ($C_{24,ss}$) of approximately 40 ng/mL. Compound **2** at a dose of 600 mg QD (550 mg free base equivalent) in humans of provides a predicted steady state trough plasma concentration ($C_{24,ss}$) of approximately 50ng/mL.

[0189] Therefore, the predicted steady state plasma concentration of surrogate metabolite **1-7** is almost double the EC_{95} against all tested clinical isolates (even the hard to treat GT3a), which indicates superior performance.

[0190] In contrast, the EC_{95} of the standard of care nucleotide sofosbuvir ranges from 50 to 265 nM across all tested HCV clinical isolates, with an EC_{95} less than the predicted steady state concentration at the commercial dosage of 400

mg for only two isolates, GT2a and GT2b. The EC_{95} for the commercial dosage of 400 mg of sofosbuvir is greater than the predicted steady state concentration for other clinical isolates, GT1a, GT1b, GT3a, GT4a, and GT4d.

[0191] The Compound **2** 450 mg steady state trough plasma concentration ($C_{24,ss}$) was predicted using the 300 mg steady state trough plasma concentration ($C_{24,ss}$). The mean steady state trough plasma concentration ($C_{24,ss}$) at 300 mg was 26.4 ng/mL, and therefore the calculation was 26.4*450/300=39.6 ng/mL.

[0192] The 600 mg steady state trough plasma concentration ($C_{24,ss}$) was predicted using three approaches: 1) the 600 mg Day 1 C_{24} mean was 25.8 ng/mL and a 60% increase was assumed for reaching steady state. Therefore the calculation was 25.8*1.6=41.3 ng/mL; 2) the 400 mg day 1 C_{24} mean was 22.5 ng/mL and a 60% increase was assumed for reaching steady state. Taking dose proportional PK into account, the calculation was 22.5*1.6*600/400=54 ng/mL; and 3) the 300 mg steady state trough plasma concentration ($C_{24,ss}$) was 26.4 ng/mL and a proportional PK was assumed. Therefore the calculation was 26.4*2=52.8 ng/mL. The 600 mg steady state trough plasma concentration ($C_{24,ss}$) is the average of the 3 data points ((41.3+54+52.8)/3=49.3 ng/mL). There is generally about a 60% increase in C_{24} at steady state compared to C_{24} following a single dose.

[0193] The data comparing the efficacy and pharmacokinetic steady state parameters in FIG. 24 clearly demonstrates the unexpected therapeutic importance of Compound **2** for the treatment of hepatitis C. In fact, the predicted steady-state plasma level after administration of Compound **2** is predicted to be at least 2-fold higher than the EC_{95} for all genotypes tested, and is 3- to 5-fold more potent against GT2. This data indicates that Compound **2** has potent pan-genotypic antiviral activity in humans. As shown in FIG. 24, the EC_{95} of sofosbuvir at GT1, GT3, and GT4 is greater than 100 ng/mL. Thus surprisingly, Compound **2** is active against HCV at a dosage form that delivers a lower steady-state trough concentration (40-50 ng/mL) than the steady-state tough concentration (approximately 100 ng/mL) achieved by a similar dosage form of sofosbuvir.

Example 26. Formulation Description and Manufacturing of Compound 2

[0194] A representative non-limiting batch formula for Compound **2** tablets (50 mg and 100 mg) is presented in Table 36. The tablets were produced from a common blend using a direct compression process as shown in FIG. 25. The active pharmaceutical ingredient (API) is adjusted based on the as-is assay, with the adjustment made in the percentage of microcrystalline cellulose. The API and excipients (microcrystalline cellulose, lactose monohydrate, and croscarmellose sodium) were screened, placed into a V-blender (PK Blendmaster, 0.5L bowl) and mixed for 5 minutes at 25 rpm. Magnesium Stearate was then screened, added and the blend was mixed for an additional 2 minutes. The common blend was divided for use in producing 50 mg and 100 mg tablets. The lubricated blend was then compressed at a speed of 10 tablets/minutes using a single punch research tablet press (Korsch XP1) and a gravity powder feeder. The 50 tablets were produced using round standard concave 6 mm tooling and 3.5 kN forces. The 100 mg tablets were produced using 8 mm round standard concave tooling and 3.9-4.2 kN forces.

Table 36. Formulation of 50 mg and 100 mg Compound 2 Tablets

Raw Material	% w/w	g/batch	Mg/unit	
			50 mg Tablet	100 mg Tablet
Compound **2**	50.0	180.0	50.0	100.0
Microcrystalline Cellulose, USP/NF, EP	20.0	72.0	20.0	40.0
Lactose Monohydrate, USP/NF, BP, EP, JP	24.0	86.4	24.0	48.0
Croscarmellose Sodium, USP/NF, EP	5.0	18.0	5.0	10.0
Magnesium Stearate, USP/NF, BP, EP JP	1.0	3.6	1.0	2.0
Total			100.0	200.0

[0195] Compound **2** was adjusted based on the as-is assay, with the adjustment made in the percentage of microcrystalline cellulose. Compound **2** and excipients (microcrystalline cellulose, lactose monohydrate, and croscarmellose sodium) were screened, placed into a V-blender (PK Blendmaster, 0.5L bowl) and mixed for 5 minutes at 25 rpm. Magnesium stearate was then screened, added and the blend was mixed for an additional 2 minutes. The common blend was divided for use in producing 50 mg and 100 mg tablets. The lubricated blend was then compressed at a speed of 10 tablets/minutes using a single punch research tablet press (Korsch XP1) and a gravity powder feeder. The 50 mg tablets were produced using round standard concave 6 mm tooling and 3.5 kN forces. The 100 mg tablets were produced using 8 mm round standard concave tooling and 3.9-4.2 kN forces. The specifications of the 50 mg and 100 mg tablets are shown in Table 37.

Table 37. Specifications of 50 mg and 100 mg Tablets of Compound 2

	50 mg Tablets	100 mg Tablets
Average Weight (n=10)	100 ± 5 mg	200 ± 10 mg
Individual Weight	100 ± 10 mg	200 ± 20 mg
Hardness	5.3 kp	8.3 kp
Disintegration	< 15 minutes	< 15 minutes
Friability	NMT 0.5%	NMT 0.5%

[0196] The 50 mg and 100 mg tablets produced as described above were subjected to 6 month stability studies under three conditions: 5°C (refrigeration), 25°C/60% RH (ambient), and 40°C/75% RH (accelerated). Both the 50 mg and 100 mg tablets were chemically stable under all three conditions tested.

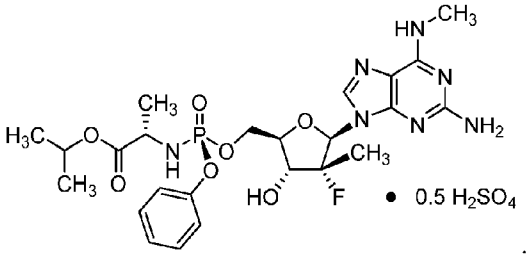
[0197] Under refrigeration conditions (5 °C), both the 50 mg and 100 mg tablets remained white solids that did not change in appearance from T=0 to T=6 months. Throughout the 6-month study, no impurities were reported that were greater than 0.05% for either the 50 mg tablets or the 100 mg tablets. The water content after 6 months was also less than 3.0 % w/w for both tablets. Similar results were reported when the tablets were subjected to ambient conditions (25 °C/60% RH); no impurities that were greater than 0.05% were reported throughout the 6 months for both tablets and the water content did not exceed 3.0 % w/w at the 6-month mark. When the tablets were subjected to accelerated conditions (40 °C/75% RH), the appearance of the 50 mg and 100 mg tablets did not change from a white, round tablet. One impurity was reported after 3 months, but the impurity was only 0.09%. A second impurity was reported after 6 months, but the total impurity percentage was only 0.21% for both the 50 mg and 100 mg tablets. Water content was 3.4 % w/w at 6 months for the 50 mg tablets and 3.2 % w/w for the 100 mg tablets.

[0198] In a separate study, the stability of 50 mg and 100 mg tablets of Compound 2 at ambient conditions (25 °C/60% RH) was measured over 9 months. The appearance of the 50 mg and 100 mg tablet did not change from a white round tablet over the course of 9 months. Impurities in the 50 mg tablet were less than 0.10% after 9 months and impurities in the 100 mg tablet were less than 0.05%. The water content of the 50 mg tablet and the 100 mg tablet after 9 months was only 2.7 % w/w and 2.6 % w/w, respectively.

[0199] This specification has been described with reference to embodiments of the invention. However, one of ordinary skill in the art appreciates that various modifications and changes can be made without departing from the scope of the invention as set forth in the claims below. Accordingly, the specification is to be regarded in an illustrative rather than a restrictive sense, and all such modifications are intended to be included within the scope of invention.

Krav:

1. Forbindelse med formlen:



2. Forbindelse ifølge krav 1, hvori forbindelsen er mindst 90 % fri for den modsatte phosphor-R-enantiomer.

3. Forbindelse ifølge krav 1, hvori forbindelsen er mindst 98 % fri for den modsatte phosphor-R-enantiomer.

4. Forbindelse ifølge krav 1, hvori forbindelsen er mindst 99 % fri for den modsatte phosphor-R-enantiomer.

5. Farmaceutisk sammensætning omfattende forbindelsen ifølge ethvert af kravene 1 – 4, valgfrit i en farmaceutisk acceptabel bærer.

6. Farmaceutisk sammensætning ifølge krav 5 i en oral doseringsform.

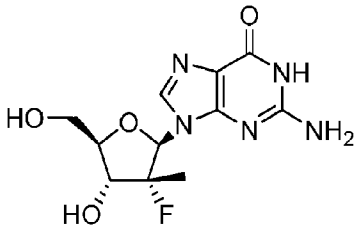
7. Farmaceutisk sammensætning ifølge krav 6, hvori den orale doseringsform er en fast doseringsform.

8. Farmaceutisk sammensætning ifølge krav 7, hvori den faste doseringsform er en tablet eller en kapsel.

9. Farmaceutisk sammensætning ifølge krav 6, hvori den orale doseringsform er en flydende doseringsform.

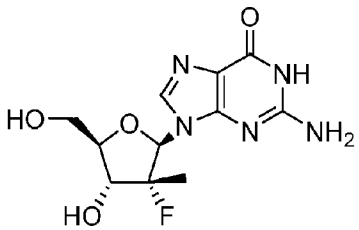
10. Farmaceutisk sammensætning ifølge krav 9, hvori den flydende doseringsform er en suspension eller opløsning.

11. Farmaceutisk sammensætning ifølge ethvert af kravene 5 – 10, som tilvejebringer et steady-state plasmabundniveau ($C_{24,ss}$) af metabolitten



på mellem ca. 20 – 60 ng/ml.

12. Farmaceutisk sammensætning ifølge krav 11, hvori arealet under kurven for metabolitten



er på mellem ca. 1.500 ng*t/mL og 3.000 ng*t/mL.

13. Forbindelse ifølge ethvert af kravene 1 – 4, valgfrit i en farmaceutisk acceptabel bærer, til anvendelse i behandlingen af en hepatitis C infektion hos et menneske med behov derfor.

14. Forbindelse til anvendelse ifølge krav 13, hvori forbindelsen indgives oralt.

15. Forbindelse til anvendelse ifølge krav 13, hvori forbindelsen indgives intravenøst.

16. Forbindelse til anvendelse ifølge ethvert af kravene 13 – 15, hvori der indgives en doseringsform, som afgiver mindst 500 mg af hemisulfatsaltformen.

17. Forbindelse til anvendelse ifølge ethvert af kravene 13 – 15, hvori der indgives en doseringsform, som afgiver mindst 600 mg af hemisulfatsaltformen.

18. Farmaceutisk sammensætning ifølge krav 5 i en intravenøs formulering.

19. Farmaceutisk sammensætning ifølge krav 5 i en parenteral formulering.

DRAWINGS

Drawing

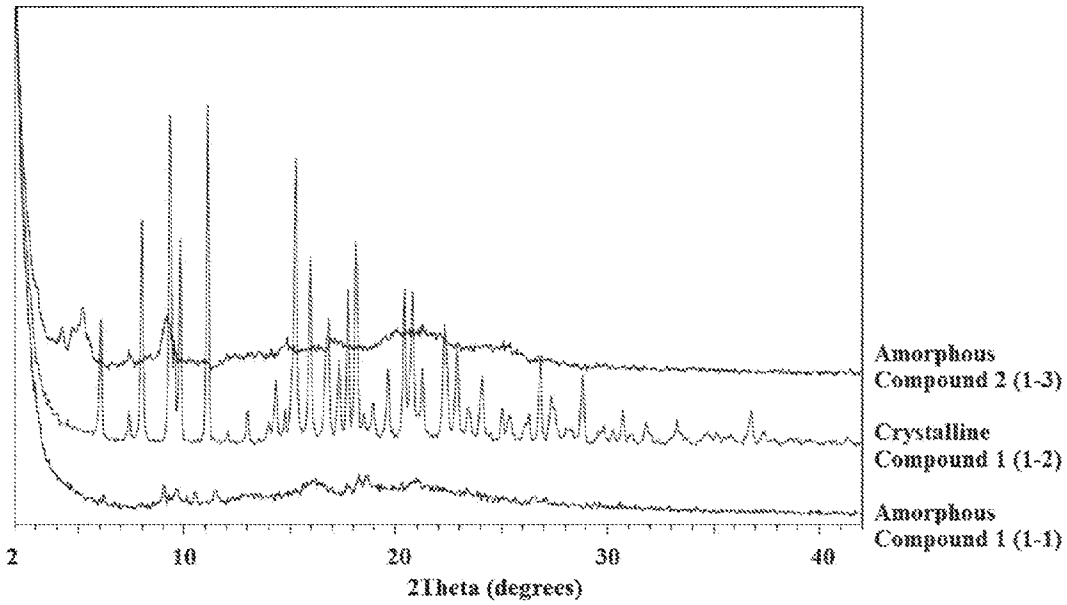


FIG. 1A

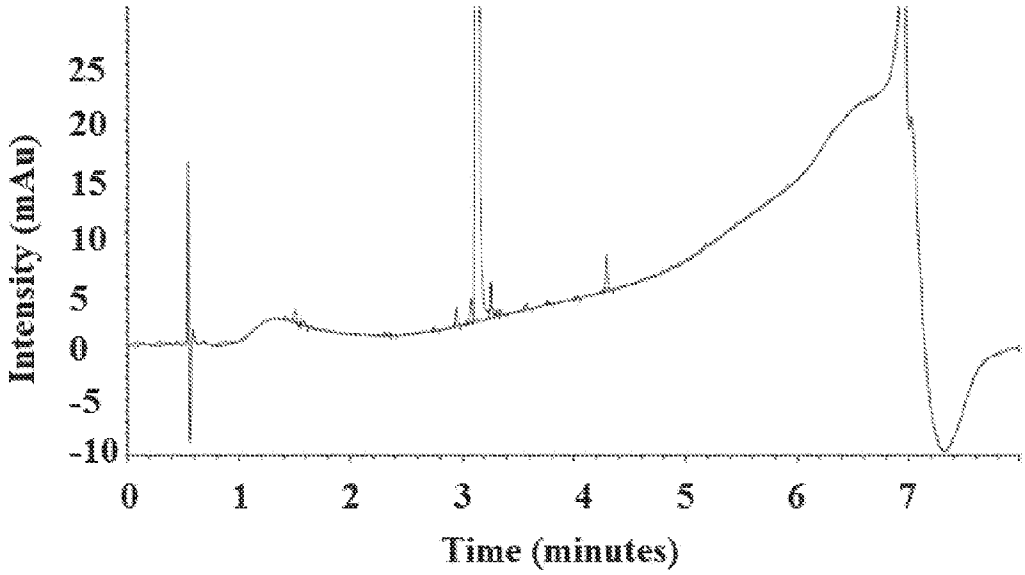


FIG. 1B

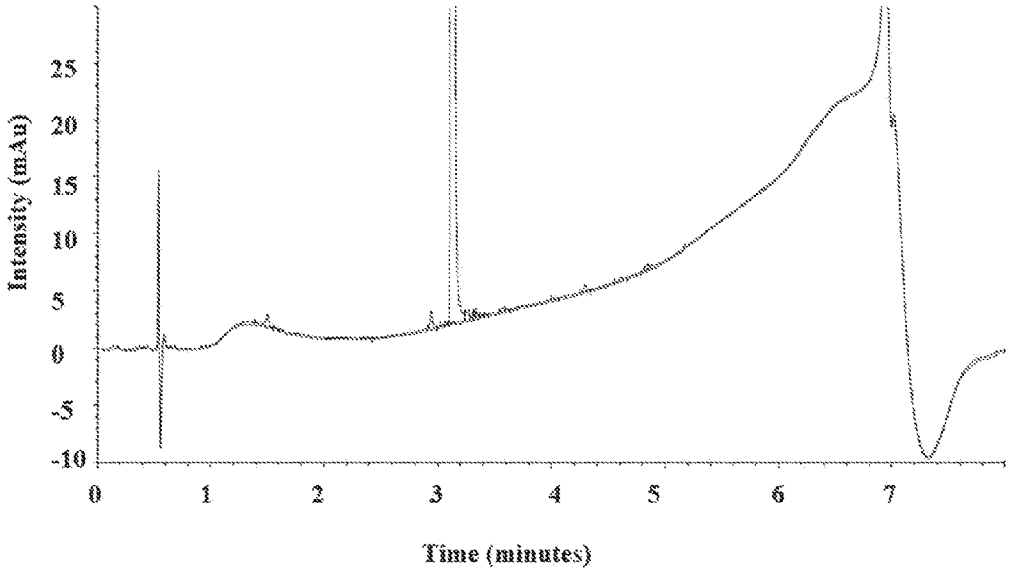

Intensity (mAu)
25
20
15
10
5
0
-5
-10
0
1
2
3
4
5
6
7
Time (minutes)

FIG. 2A

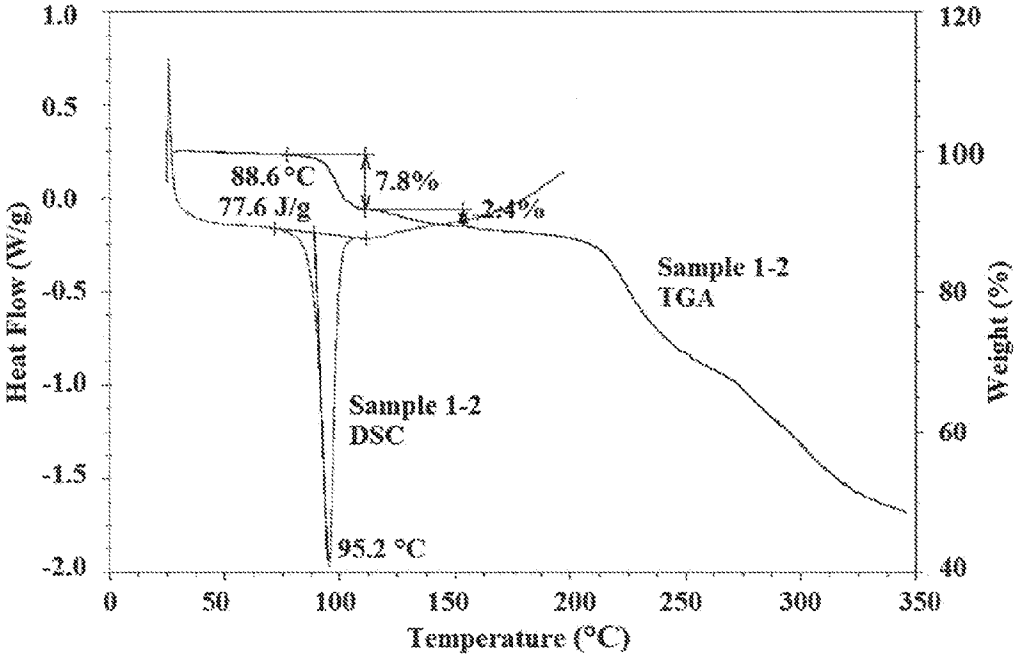

Heat Flow (W/g)
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Weight (%)
120
100
80
60
40
88.6 °C
77.6 J/g
7.8%
2.4%
Sample 1-2
TGA
Sample 1-2
DSC
95.2 °C
0
50
100
150
200
250
300
350
Temperature (°C)

FIG. 2B

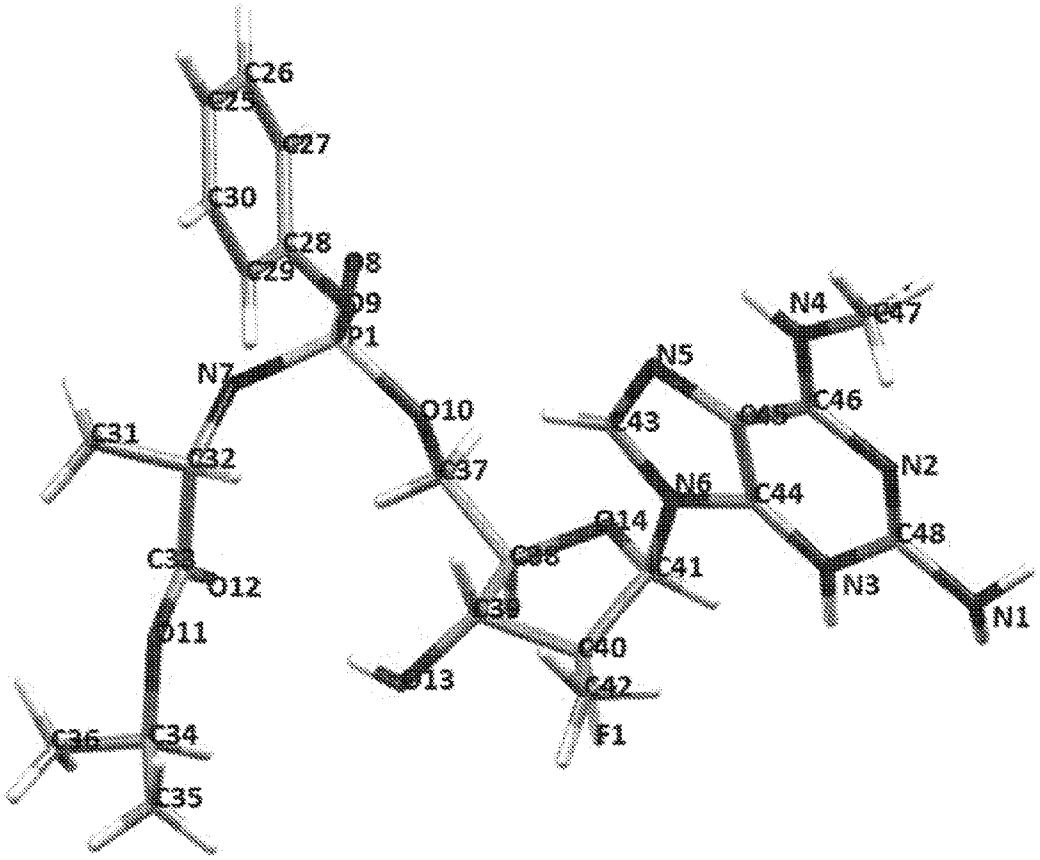

C26
C25
C27
C30
C28
C29
O8
O9
P1
N7
O10
C31
C32
C37
C38
O14
C41
C39
C40
C42
O13
F1
C33
O12
O11
C36
C34
C35
N5
C43
C45
C46
N4
C47
N6
C44
N2
C48
N3
N1

FIG. 3

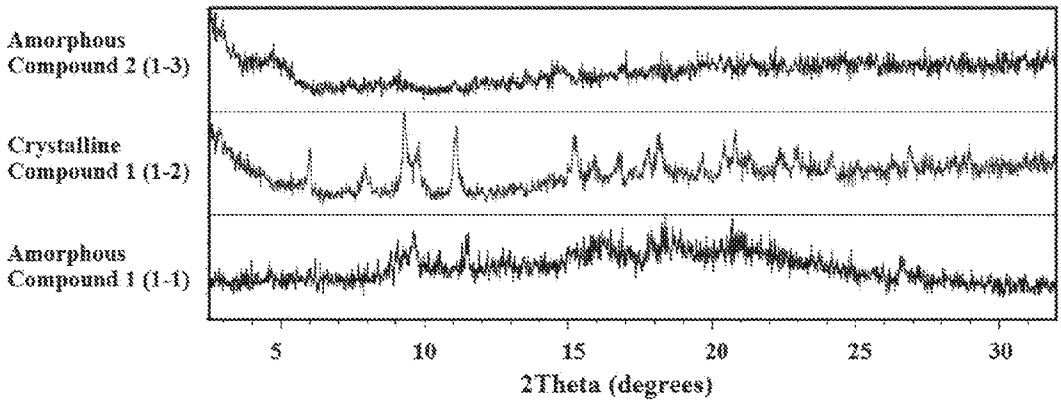

Amorphous
Compound 2 (1-3)
Crystalline
Compound 1 (1-2)
Amorphous
Compound 1 (1-1)
5
10
15
20
25
30
2Theta (degrees)

FIG. 4A

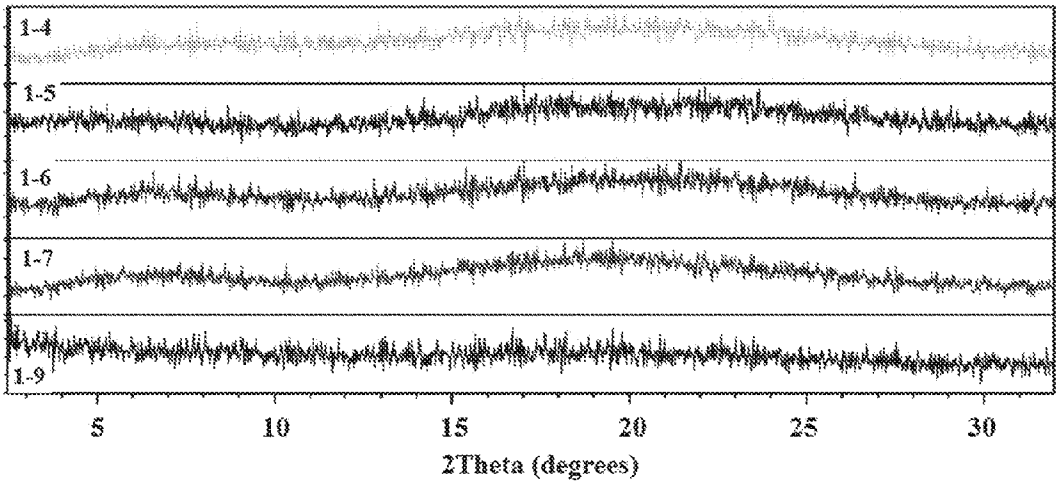

1-4
1-5
1-6
1-7
1-9
5
10
15
20
25
30
2Theta (degrees)

FIG. 4B

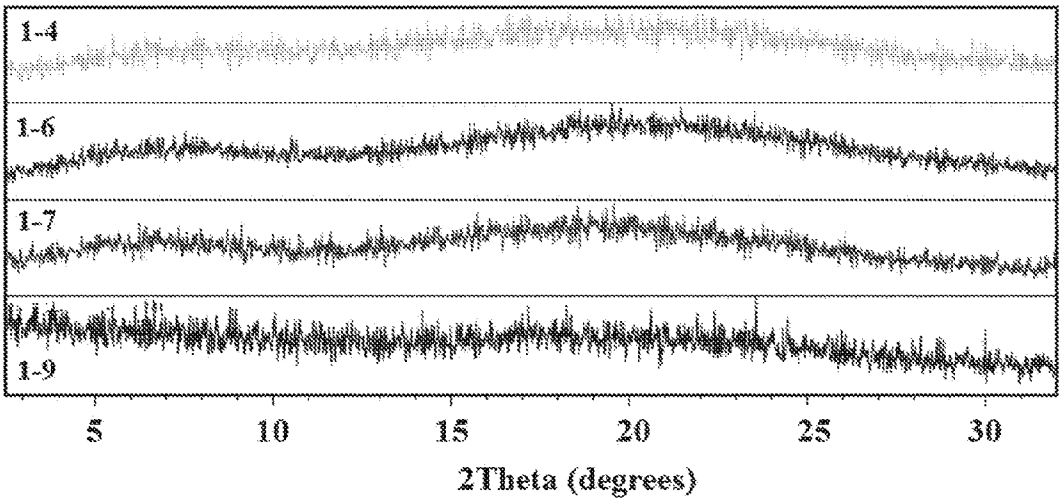

1-4
1-6
1-7
1-9
5
10
15
20
25
30
2Theta (degrees)

FIG. 5A

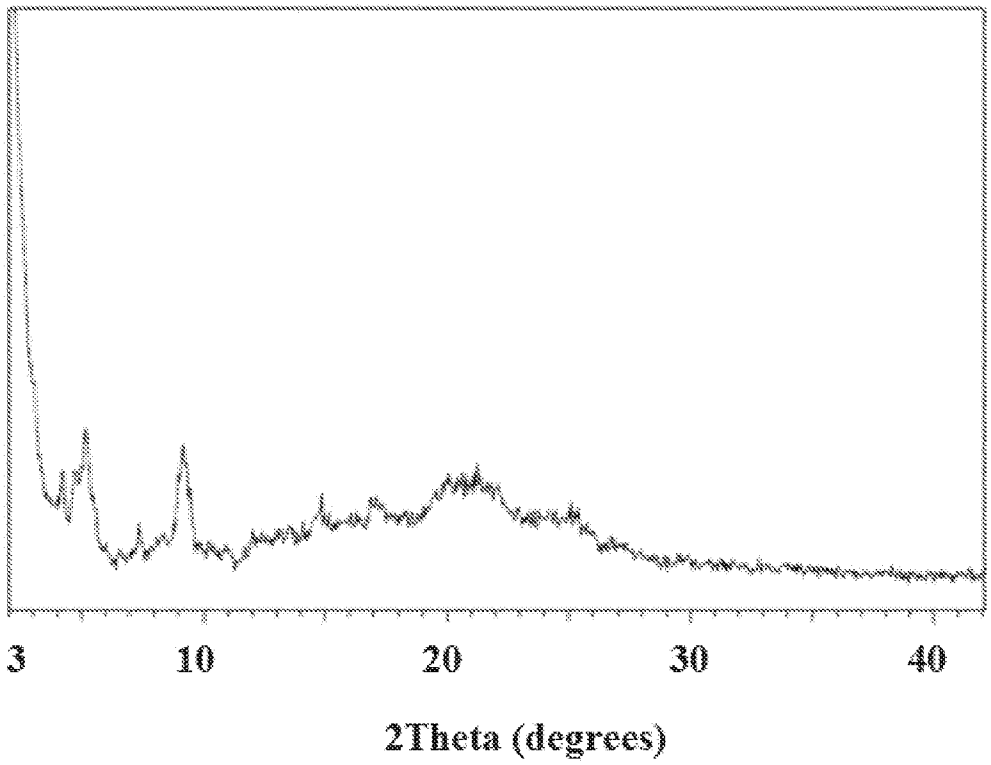

3
10
20
30
40
2Theta (degrees)

FIG. 5B

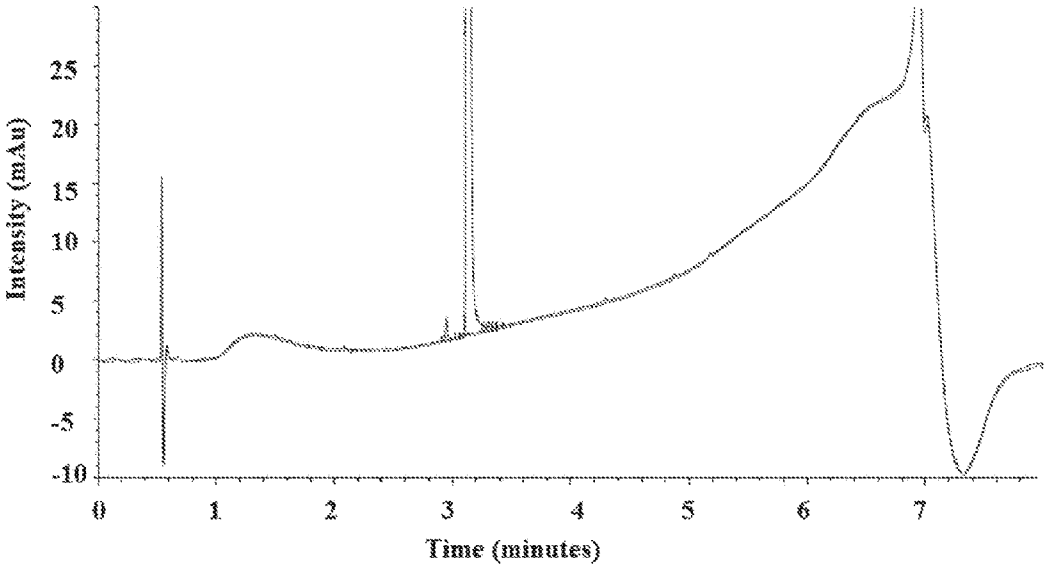

Intensity (mAu)
25
20
15
10
5
0
-5
-10
0
1
2
3
4
5
6
7
Time (minutes)

FIG. 6A

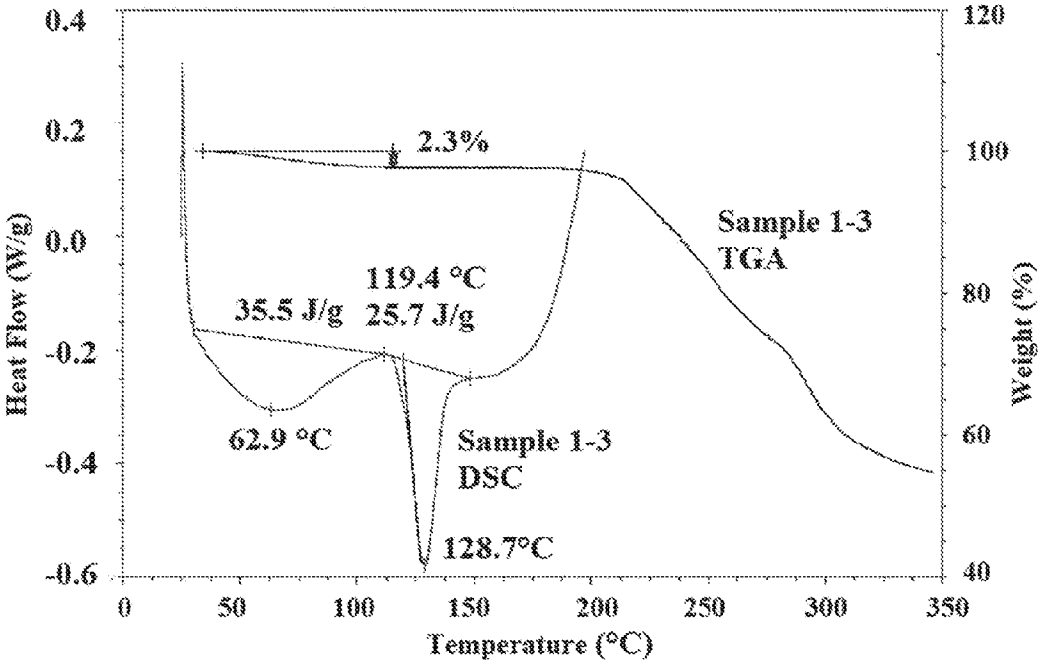

Heat Flow (W/g)
0.4
0.2
0.0
-0.2
-0.4
-0.6
Weight (%)
120
100
80
60
40
2.3%
Sample 1-3
TGA
119.4 °C
35.5 J/g
25.7 J/g
62.9 °C
Sample 1-3
DSC
128.7°C
0
50
100
150
200
250
300
350
Temperature (°C)

FIG. 6B

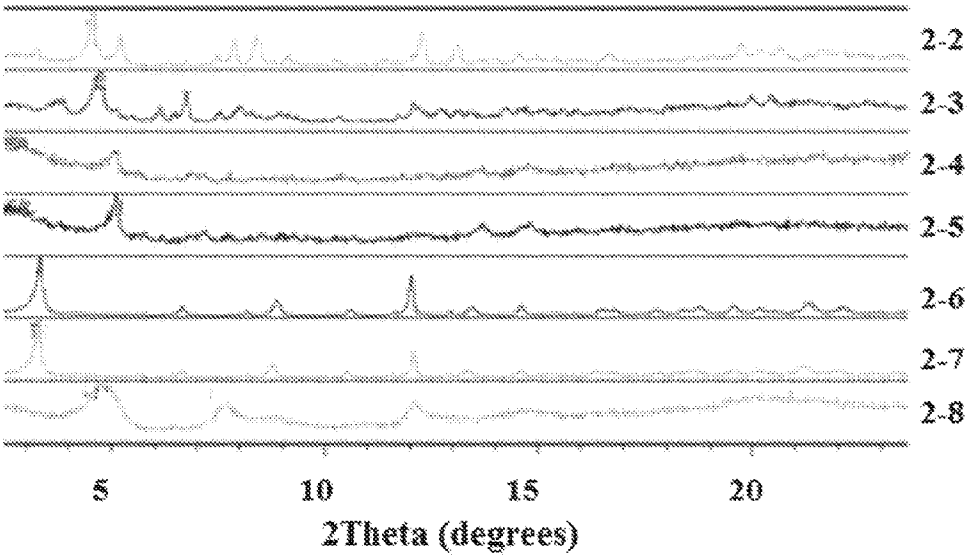

2-2
2-3
2-4
2-5
2-6
2-7
2-8
5
10
15
20
2Theta (degrees)

FIG. 7A

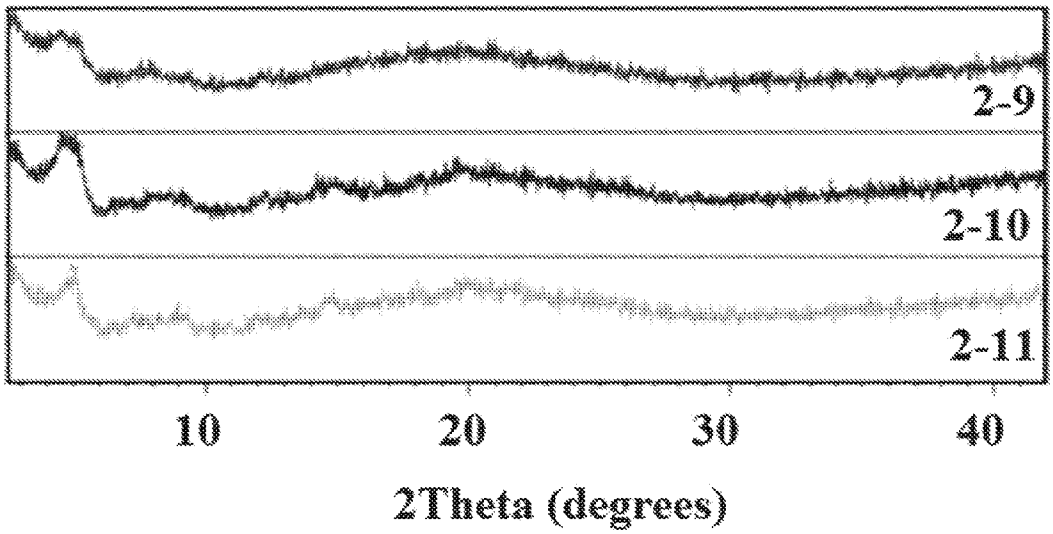

2-9
2-10
2-11
10
20
30
40
2Theta (degrees)

FIG. 7B

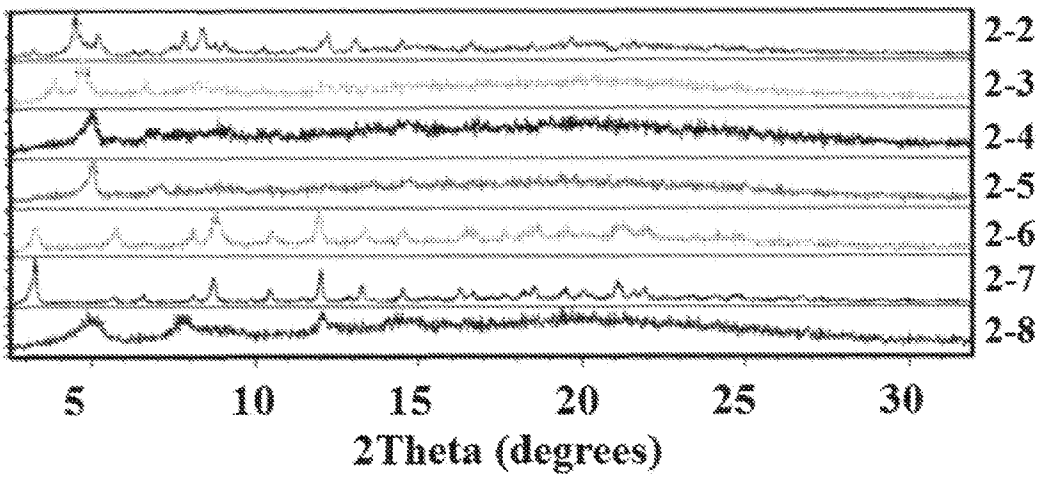

2-2
2-3
2-4
2-5
2-6
2-7
2-8
5
10
15
20
25
30
2Theta (degrees)

FIG. 8A

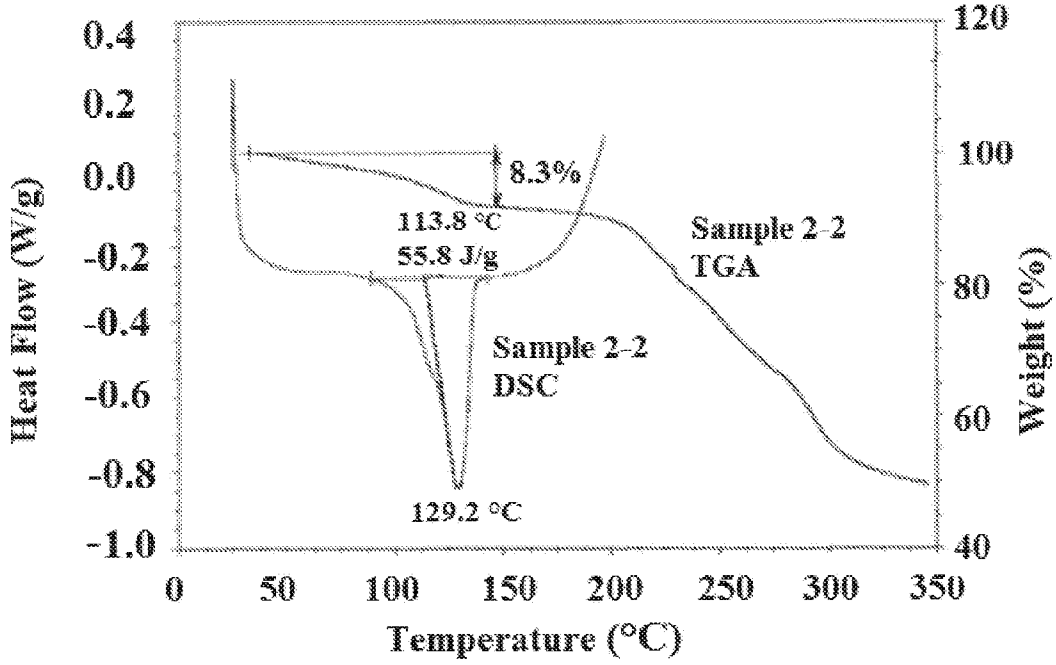

0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Heat Flow (W/g)
120
100
80
60
40
Weight (%)
8.3%
113.8 °C
55.8 J/g
Sample 2-2
TGA
Sample 2-2
DSC
129.2 °C
0
50
100
150
200
250
300
350
Temperature (°C)

FIG. 8B

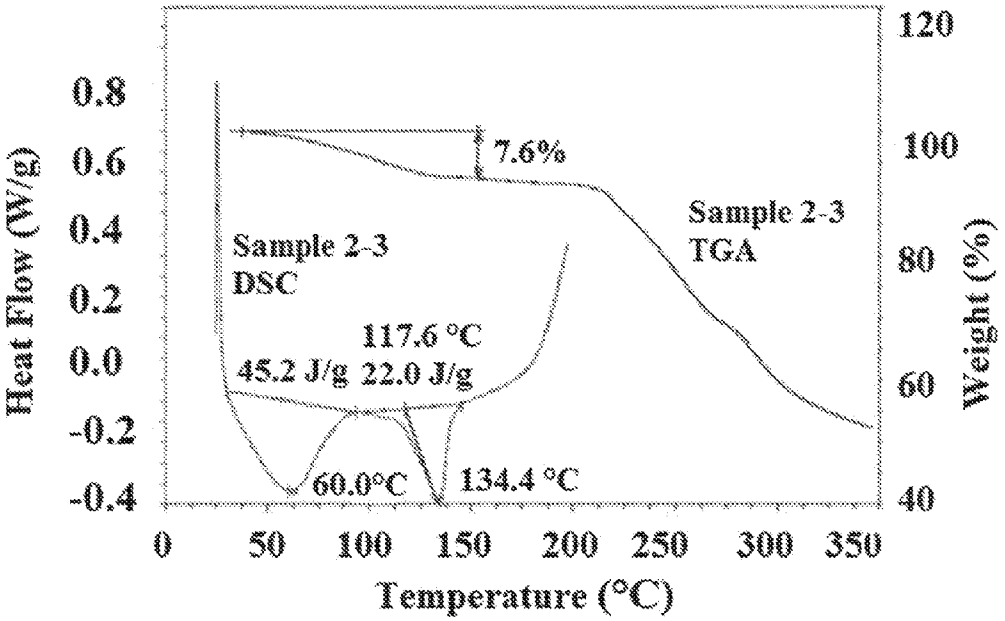

Heat Flow (W/g)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
120
100
80
60
40
Weight (%)
7.6%
Sample 2-3
TGA
Sample 2-3
DSC
117.6 °C
45.2 J/g 22.0 J/g
60.0°C
134.4 °C
0
50
100
150
200
250
300
350
Temperature (°C)

FIG. 9A

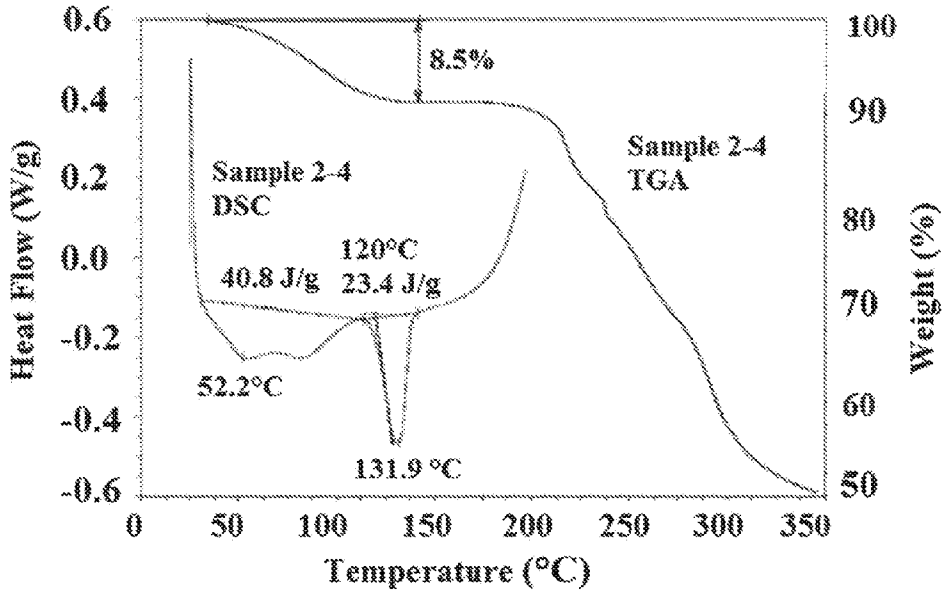

Heat Flow (W/g)
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
100
90
80
70
60
50
Weight (%)
8.5%
Sample 2-4
TGA
Sample 2-4
DSC
120°C
40.8 J/g 23.4 J/g
52.2°C
131.9 °C
0
50
100
150
200
250
300
350
Temperature (°C)

FIG. 9B

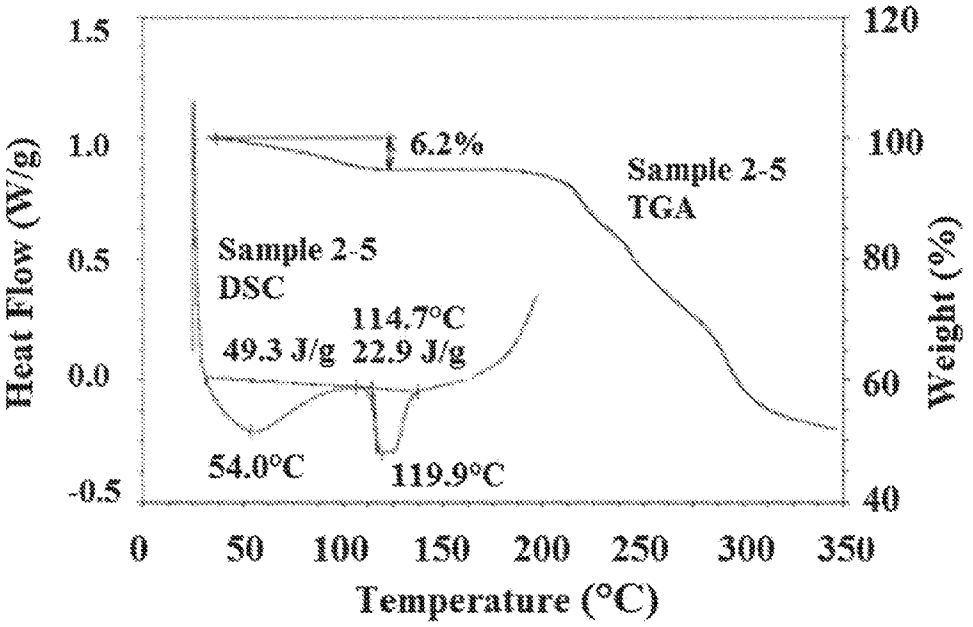


FIG. 10A

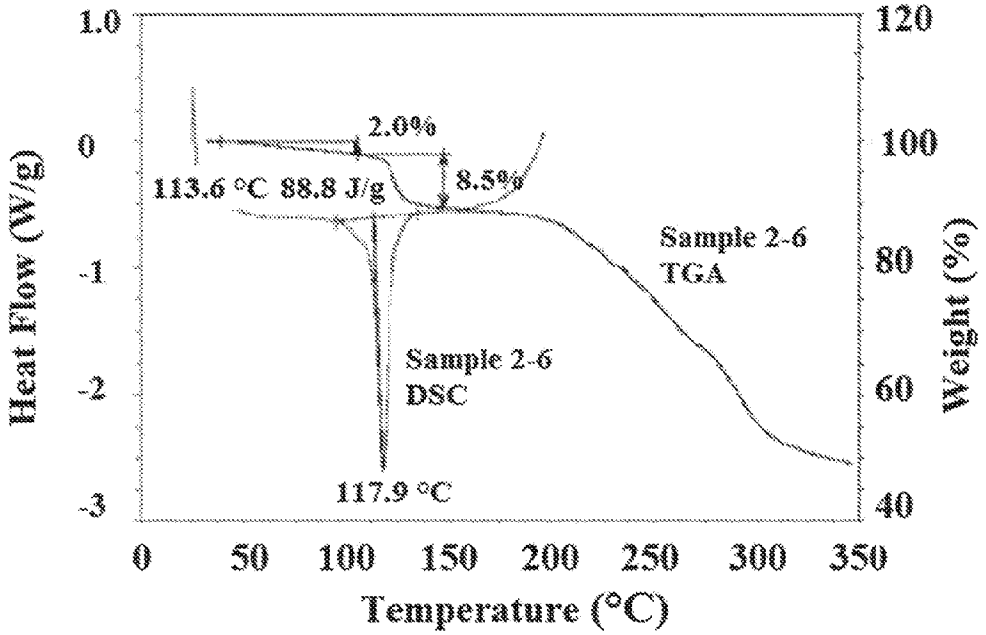


FIG. 10B

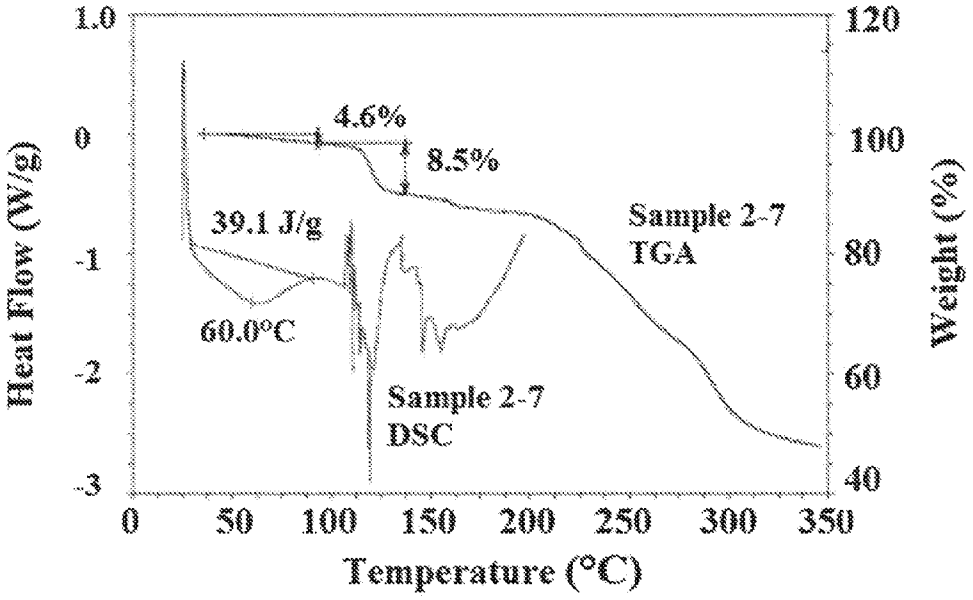


FIG. 11A

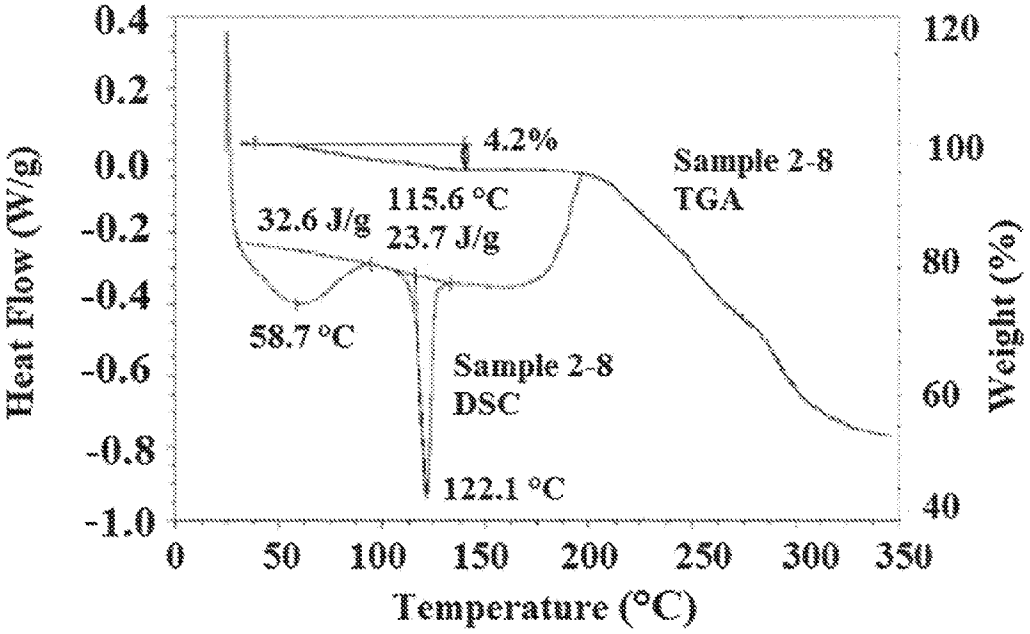


FIG. 11B

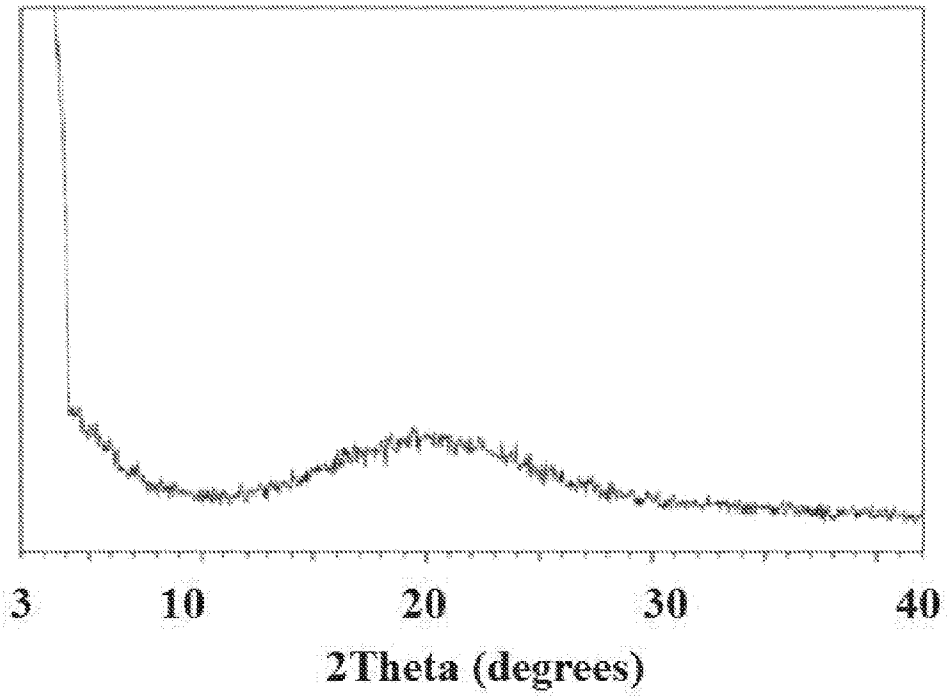


FIG. 12A

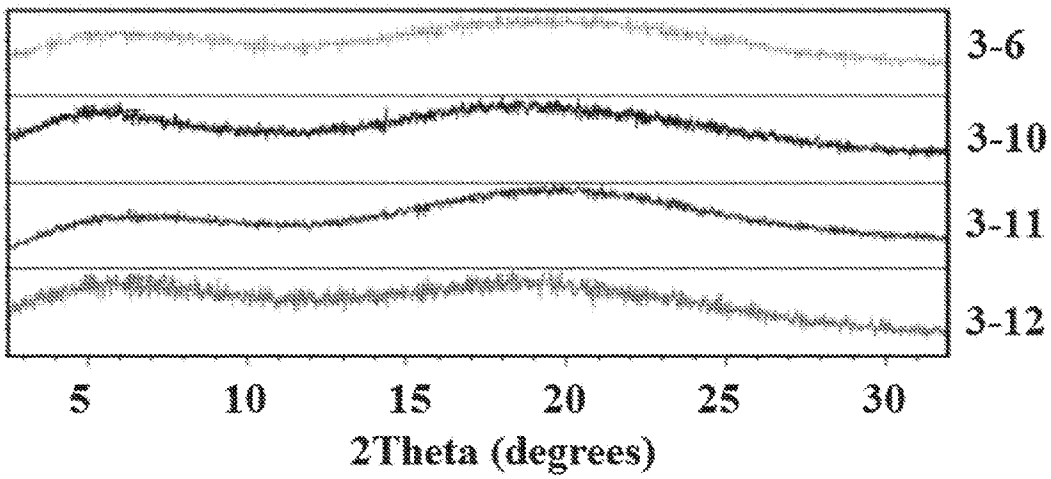


FIG. 12B

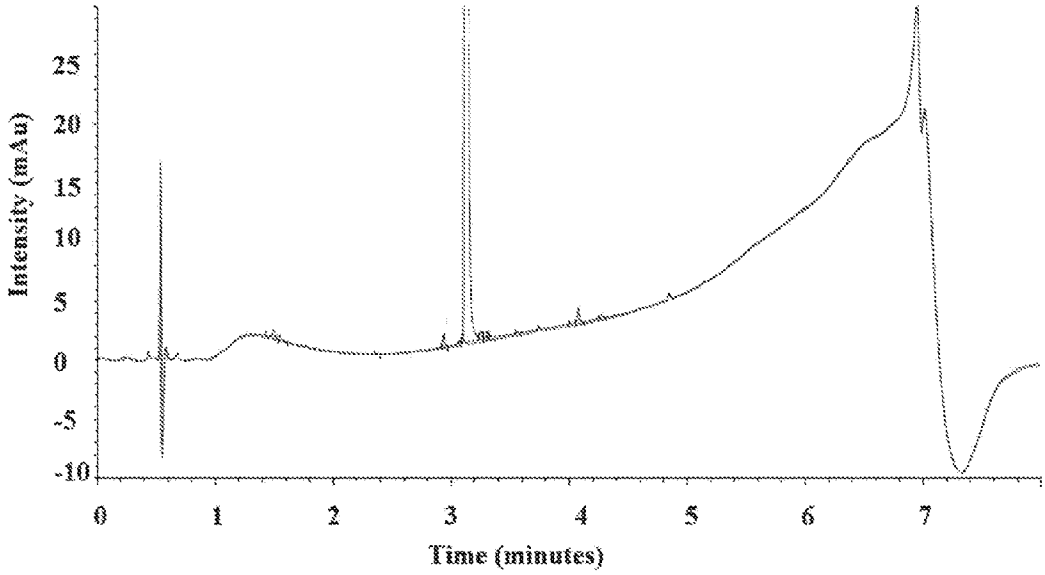


FIG. 13A

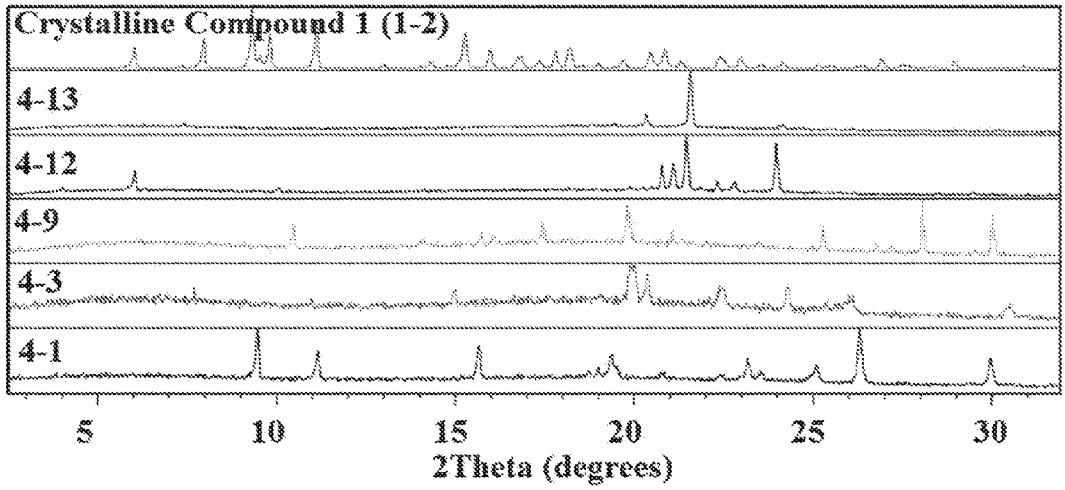


FIG. 13B

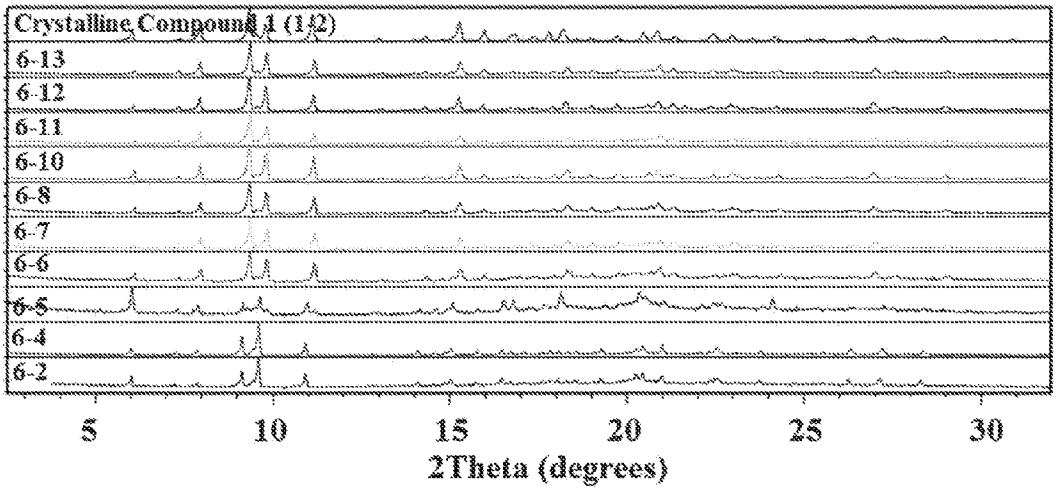


FIG. 14A

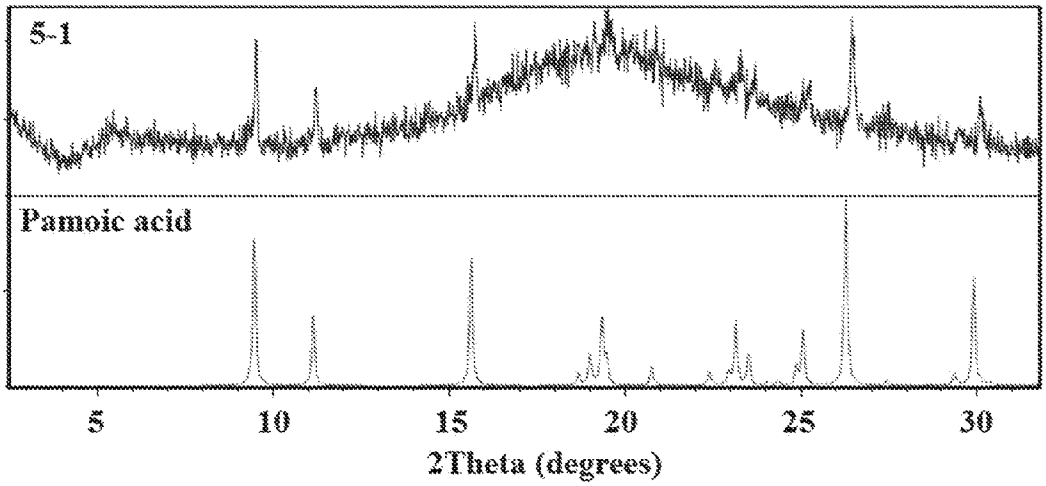


FIG. 14B

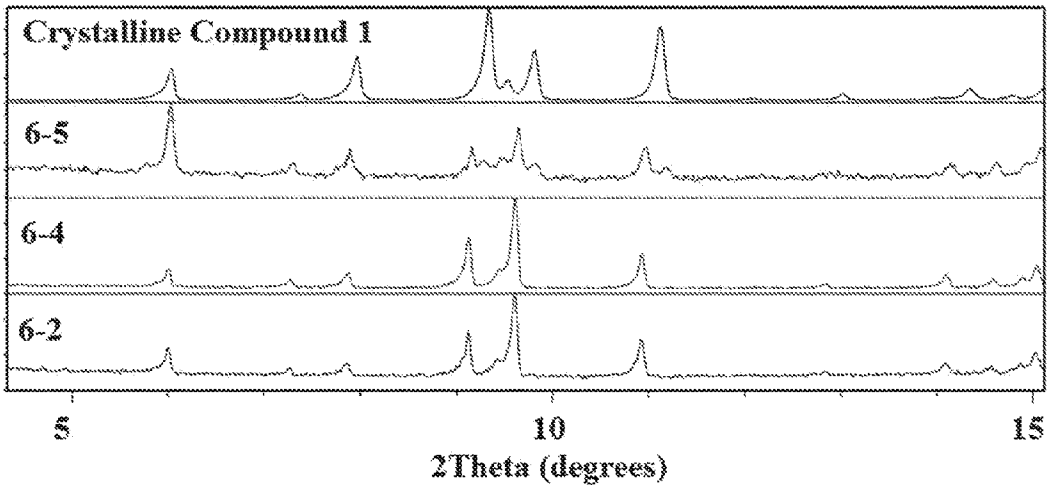


FIG. 15A

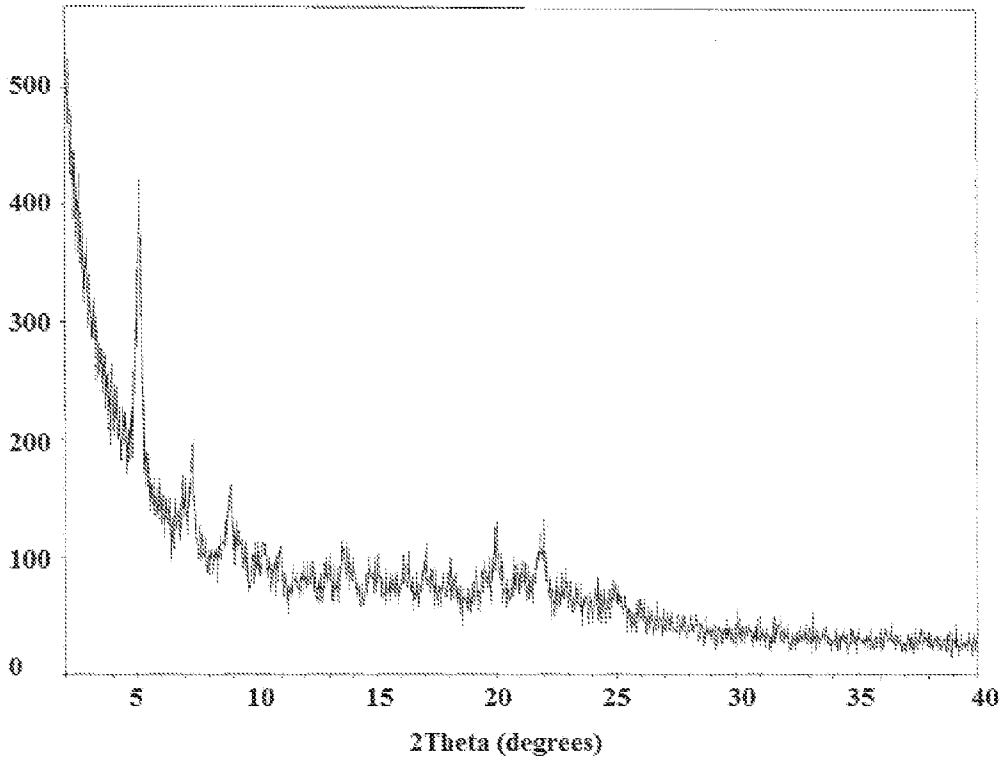


FIG. 15B

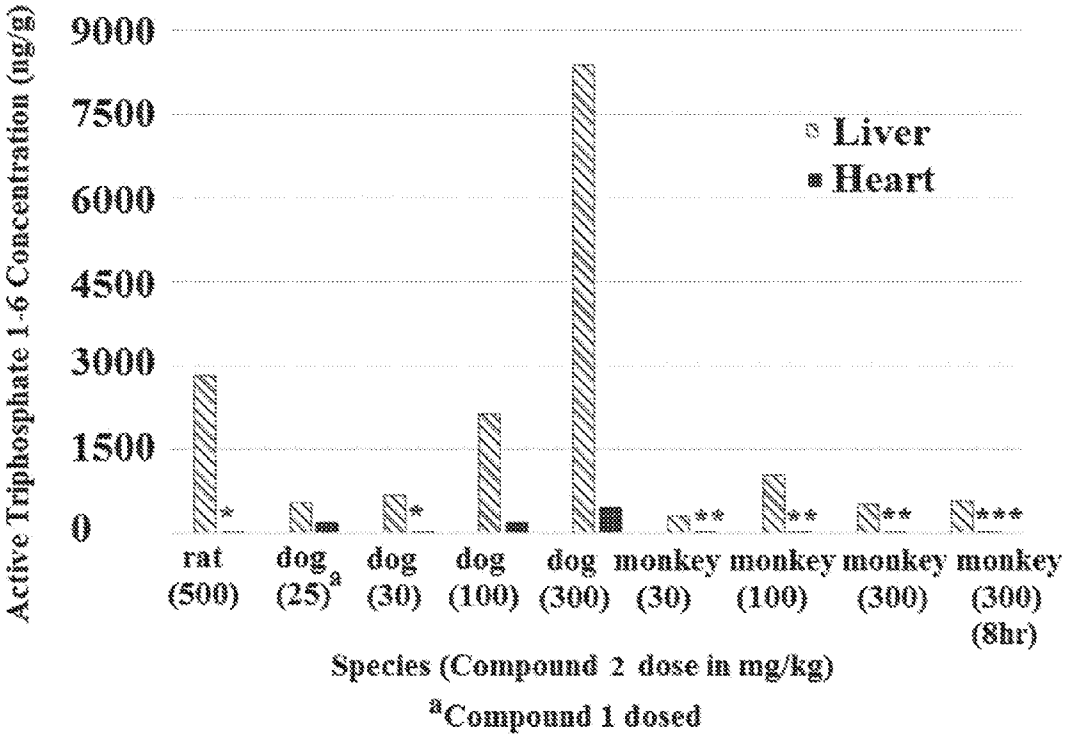

Active Triphosphate 1-6 Concentration (ng/g)
9000
7500
6000
4500
3000
1500
0
Liver
Heart
*
*
*
**
**
**
***
rat (500)
dog (25)a
dog (30)
dog (100)
dog (300)
monkey (30)
monkey (100)
monkey (300)
monkey (300) (8hr)
Species (Compound 2 dose in mg/kg)
aCompound 1 dosed

FIG. 16A

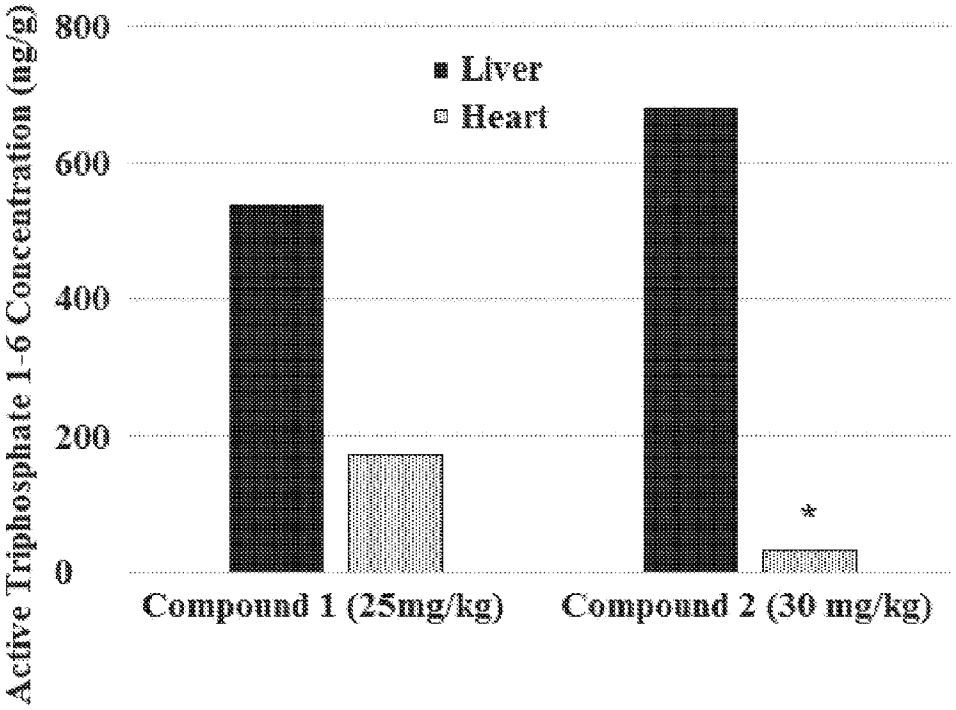

Active Triphosphate 1-6 Concentration (ng/g)
800
600
400
200
0
Liver
Heart
*
Compound 1 (25mg/kg)
Compound 2 (30 mg/kg)

FIG. 16B

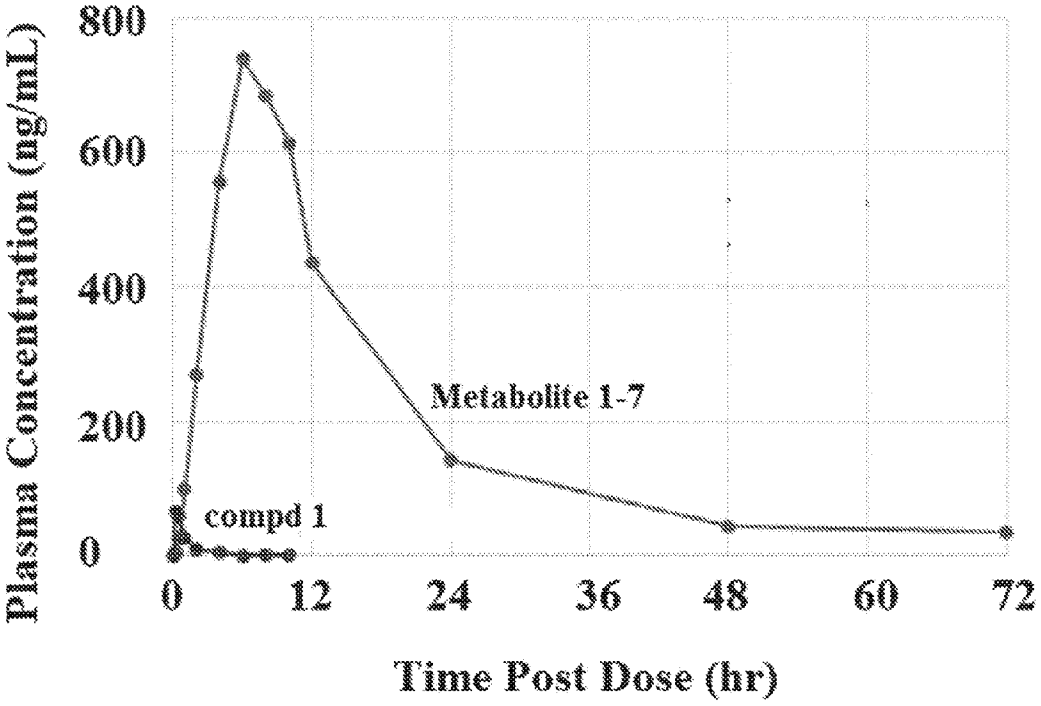

Plasma Concentration (ng/mL)
800
600
400
200
0
Metabolite 1-7
compd 1
0
12
24
36
48
60
72
Time Post Dose (hr)

FIG. 17

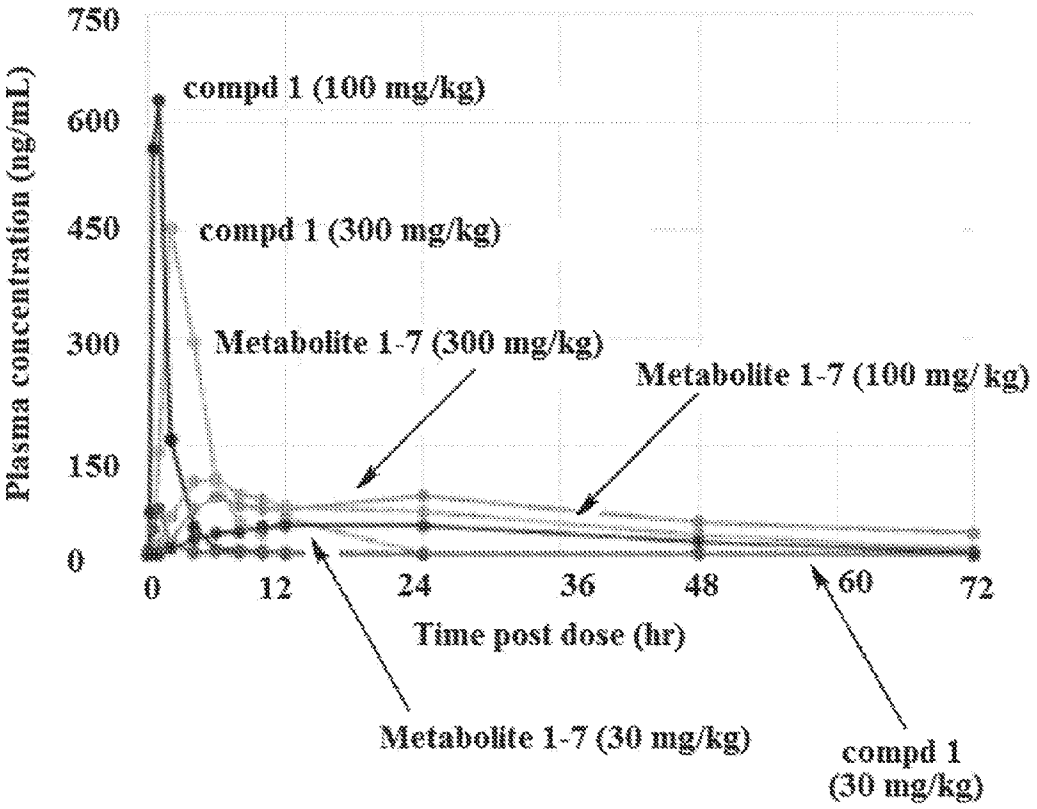

750
600
450
300
150
0
Plasma concentration (ng/mL)
compd 1 (100 mg/kg)
compd 1 (300 mg/kg)
Metabolite 1-7 (300 mg/kg)
Metabolite 1-7 (100 mg/kg)
0
12
24
36
48
60
72
Time post dose (hr)
Metabolite 1-7 (30 mg/kg)
compd 1
(30 mg/kg)

FIG. 18

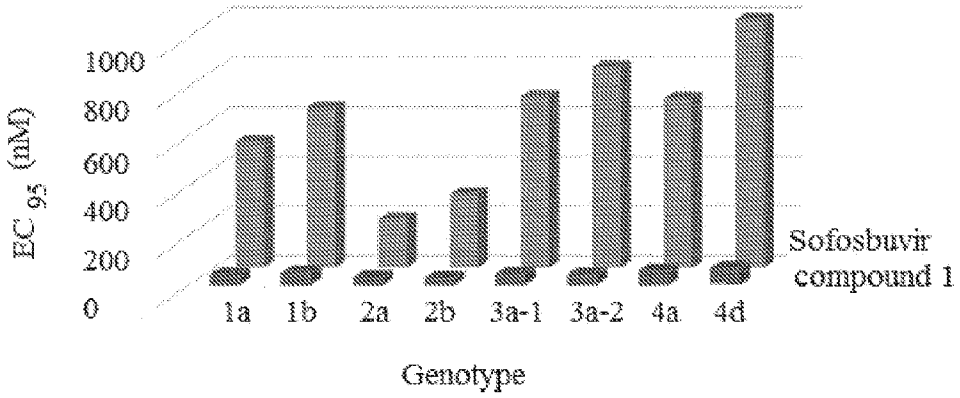

1000
800
600
400
200
0
EC 95 (nM)
1a
1b
2a
2b
3a-1
3a-2
4a
4d
Sofosbuvir
compound 1
Genotype

FIG. 19

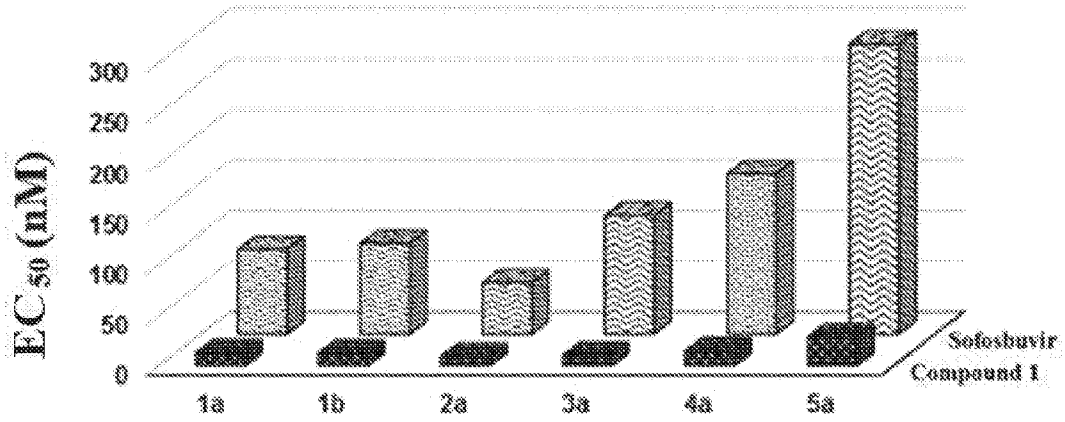

300
250
200
150
100
50
0
EC50 (nM)
1a
1b
2a
3a
4a
5a
Sofosbuvir
Compound 1

FIG. 20

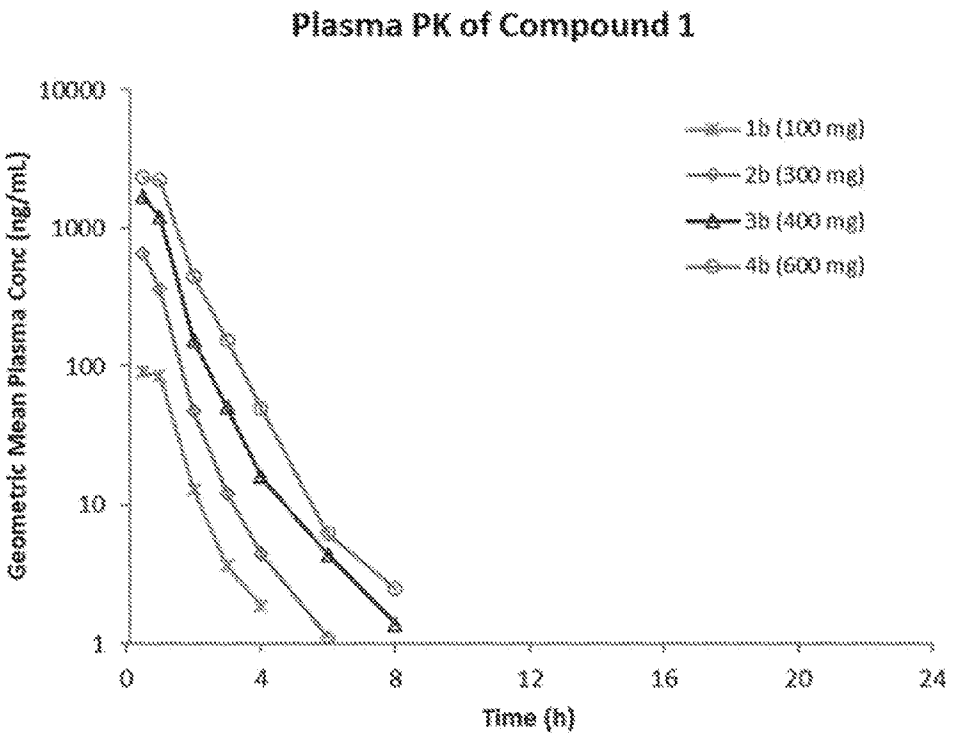

Plasma PK of Compound 1
Geometric Mean Plasma Conc (ng/mL)
10000
1000
100
10
1
0
4
8
12
16
20
24
Time (h)
1b (100 mg)
2b (300 mg)
3b (400 mg)
4b (600 mg)

FIG. 21

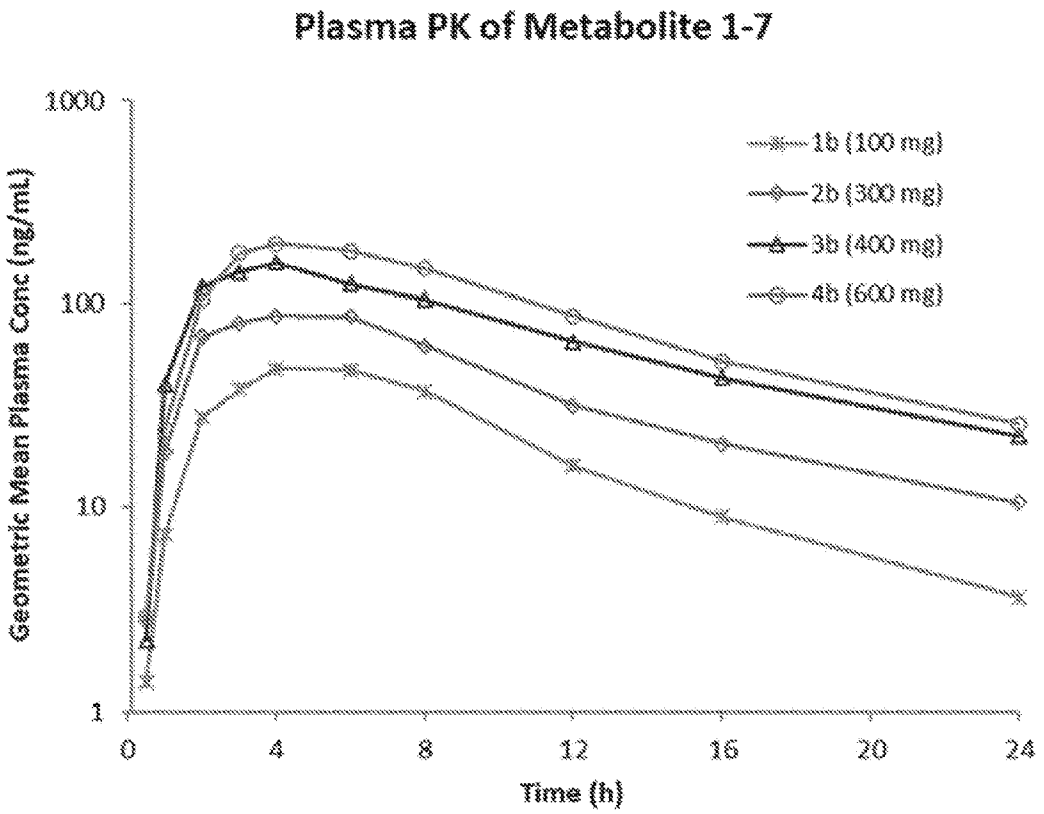

Plasma PK of Metabolite 1-7
Geometric Mean Plasma Conc (ng/mL)
1000
100
10
1
0
4
8
12
16
20
24
Time (h)
1b (100 mg)
2b (300 mg)
3b (400 mg)
4b (600 mg)

FIG. 22

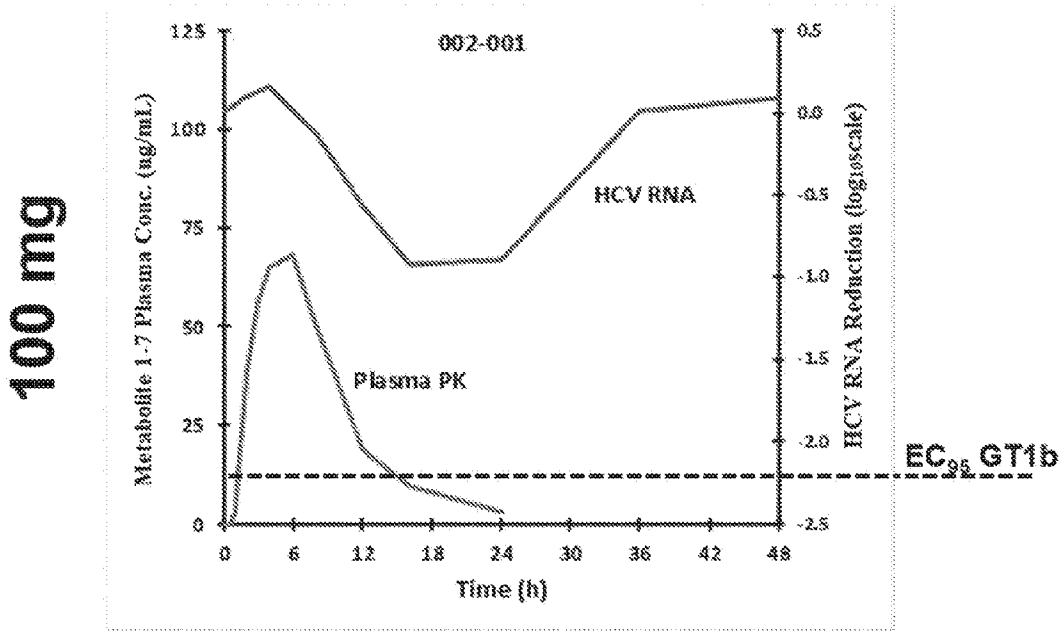


FIG. 23A

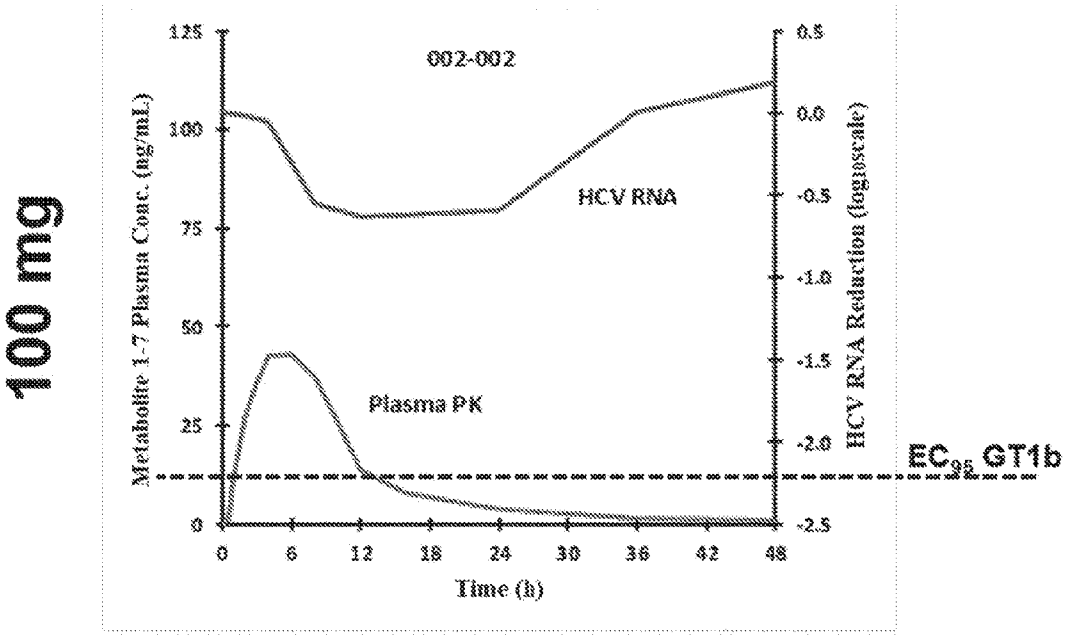


FIG. 23B

100 mg

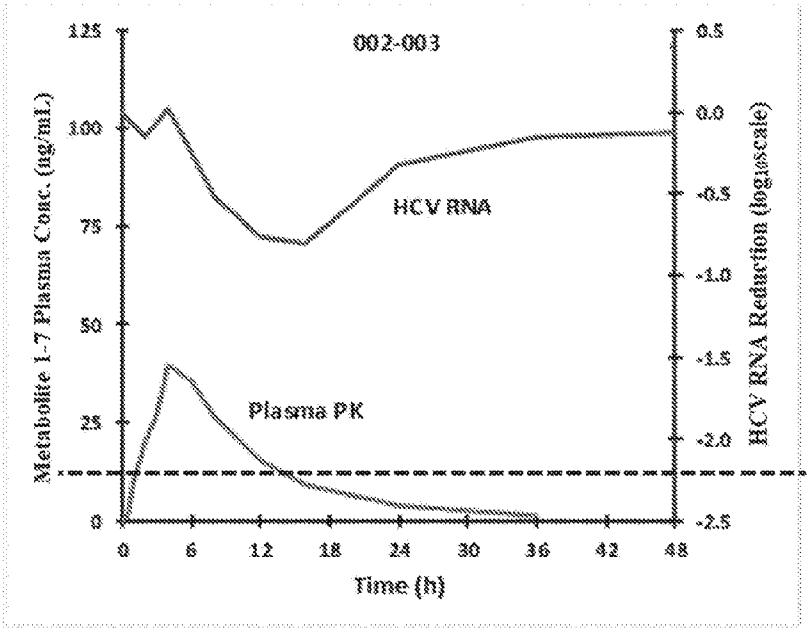


FIG. 23C

300 mg

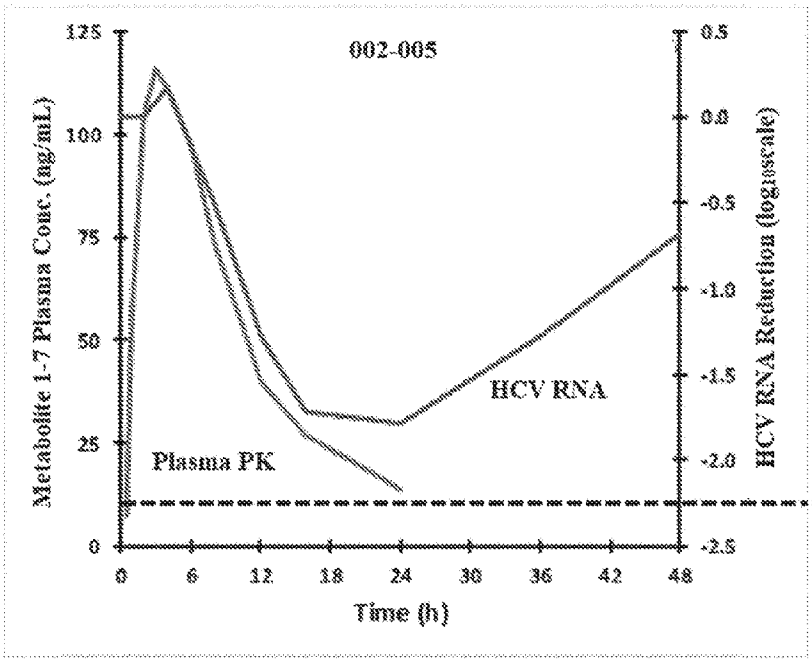


FIG. 23D

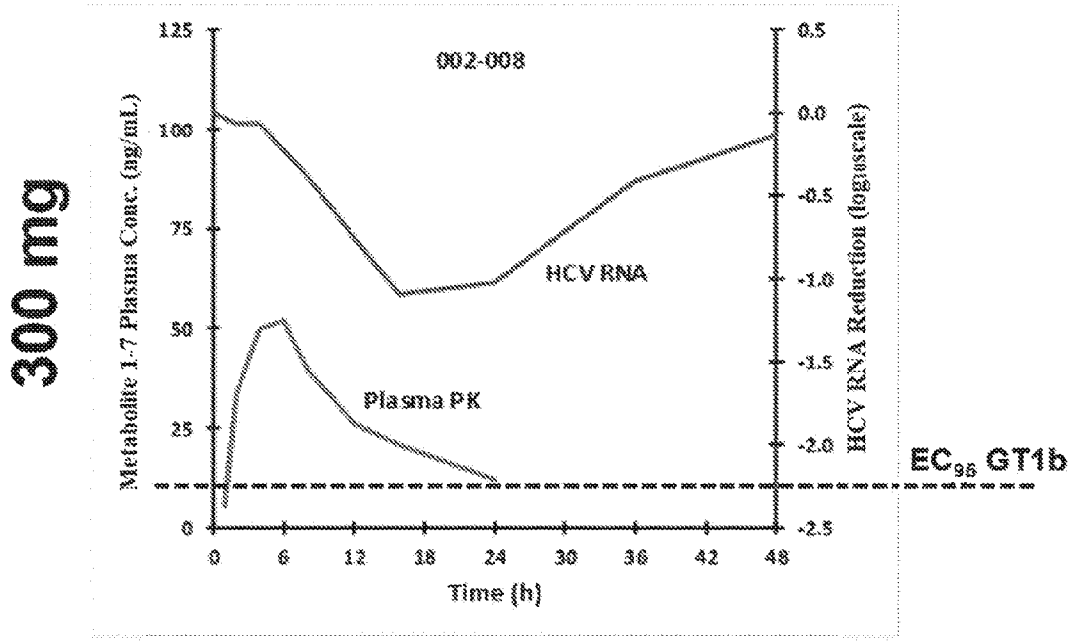


FIG. 23E

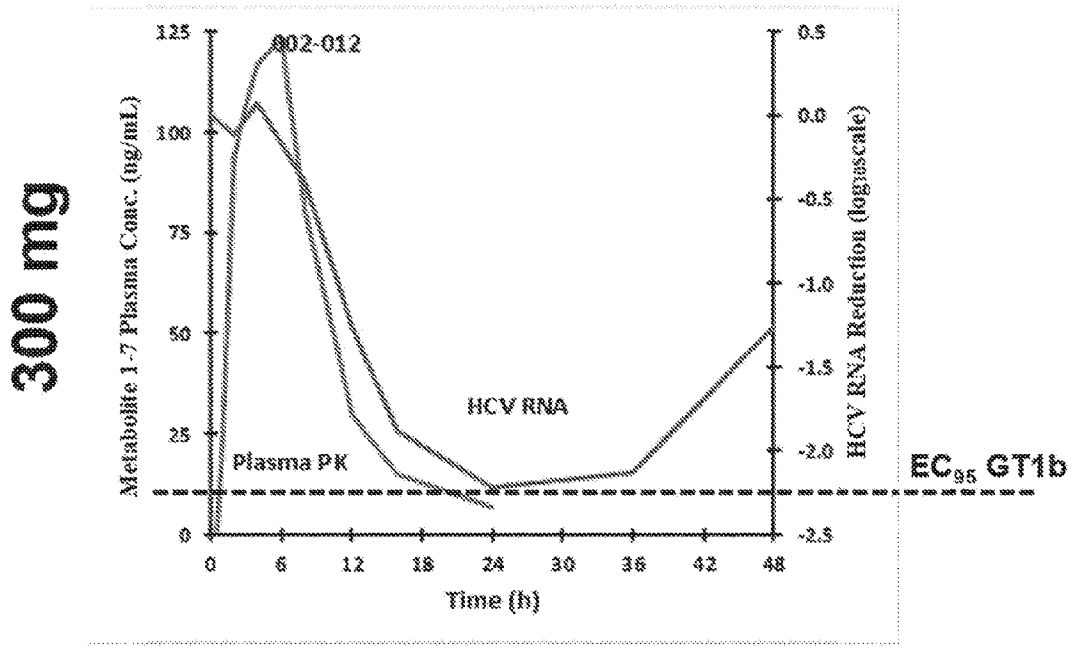


FIG. 23F

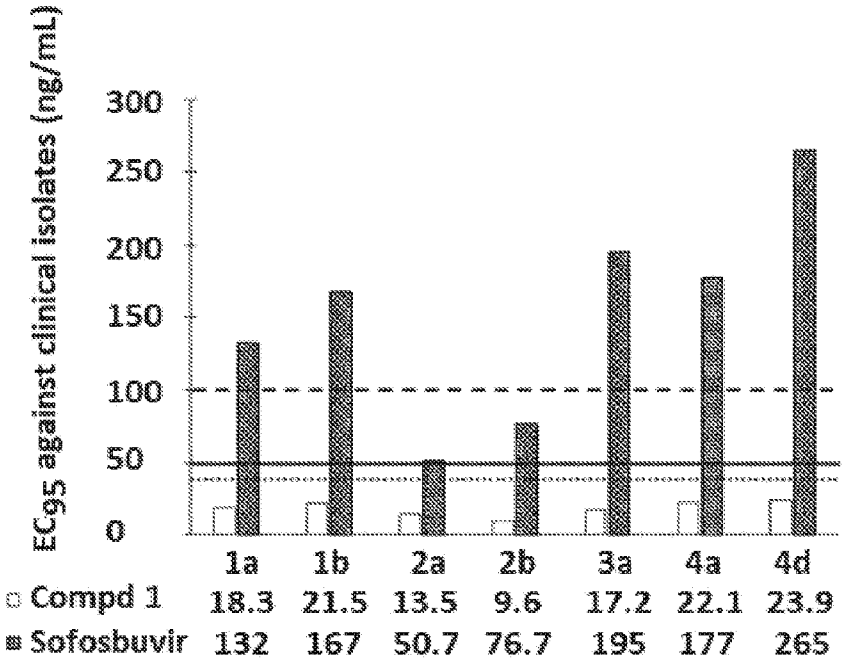

EC95 against clinical isolates (ng/mL)
300
250
200
150
100
50
0
1a
1b
2a
2b
3a
4a
4d
Compd 1
18.3
21.5
13.5
9.6
17.2
22.1
23.9
Sofosbuvir
132
167
50.7
76.7
195
177
265

FIG. 24

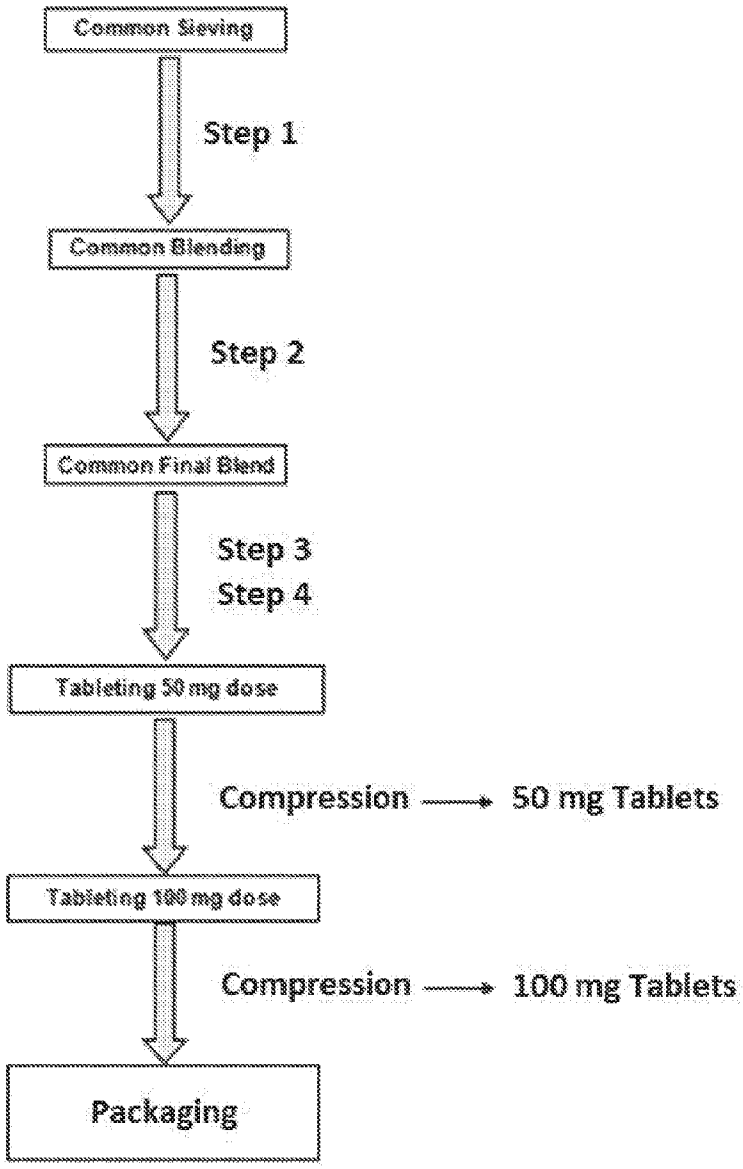

Common Sieving
Step 1
Common Blending
Step 2
Common Final Blend
Step 3
Step 4
Tableting 50 mg dose
Compression
50 mg Tablets
Tableting 100 mg dose
Compression
100 mg Tablets
Packaging

FIG. 25

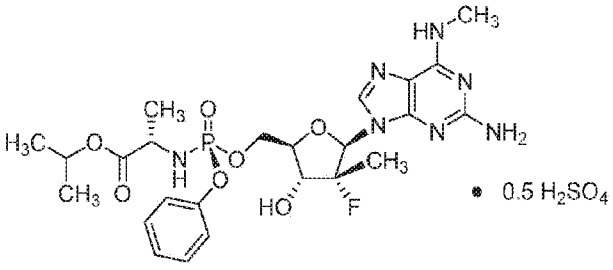


FIG. 26