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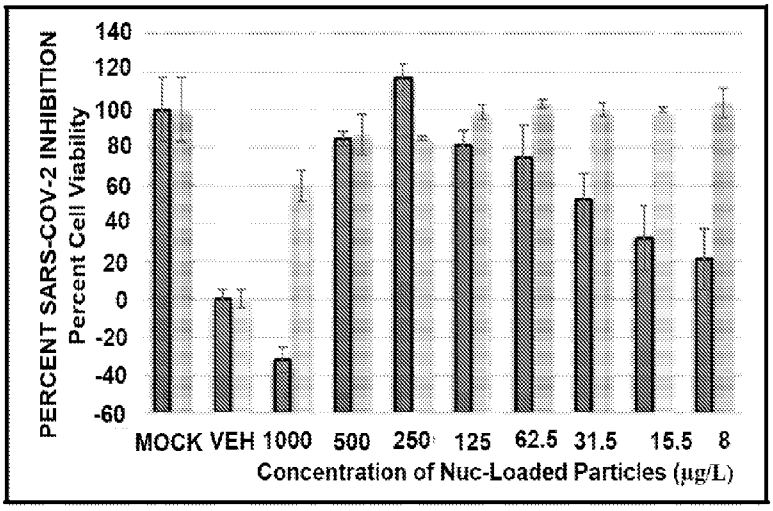


FIG. 1

(57) Abstract: Compositions of matter and pharmaceutical formulations and the delivery and manufacture thereof that contain an antiviral nucleotide 5'-phosphate, for example, a 5'-monophosphate, 5'- diphosphate, or 5'-triphosphate, in a cationic lipid nanoparticle or lipid microparticle for effective delivery of the nucleotide phosphate into an infected cell in a host such as a human in need thereof.

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LNP AND LMP DELIVERY OF ANTIVIRAL NUCLEOTIDE 5'-PHOSPHATES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/143,596 which was filed on January 29, 2021. The entirety of this application is hereby incorporated by reference for all purposes.

FIELD OF THE INVENTION

This application is in the area of compositions of matter and pharmaceutical formulations and the delivery and manufacture thereof that contain an antiviral nucleotide 5'-phosphate, for example, a 5'-monophosphate, 5'-diphosphate, or 5'-triphosphate, in a cationic lipid nanoparticle or lipid microparticle for effective delivery of the nucleotide phosphate into an infected cell in a host in need thereof.

BACKGROUND OF THE INVENTION

Derivatized nucleosides and nucleotides have historically served as a backbone of medical therapy for a broad range of viral diseases. The nucleo(s)tide (which refers to a nucleoside or nucleotide) must be delivered into the cell to exert therapeutic activity. The therapeutic nucleo(s)tide once in the cell is converted by selective kinases to a 5'-monophosphate, then diphosphate and then triphosphate for intracellular activation. 5'-Phosphates bear negative charges under *in vivo* conditions that prevent the molecule from passing through the human cell membrane and therefore cannot be delivered as already fully or partially activated molecules. On the other hand, a separate problem is that derivatized nucleo(s)tides can be derivatized in a manner that prevents or minimizes intracellular kinase enzymatic activation to the 5'-triphosphate once inside the cell. This can be especially problematic for derivatized nucleosides that are derivatized in the sugar moiety, which can particularly interfere with the kinase enzyme's ability to phosphorylate the 5'-hydroxyl group.

Therefore, there is a two-pronged challenge to provide active therapeutic nucleotides intracellularly to treat a viral infection in a host in need thereof. There have been a number of approaches to overcome these challenges.

The most successful approach that has been that discovered to overcome these challenges was developed by Professor Chris McGuigan at Cardiff University, who pioneered the successful "ProTide" technology that uses a 5'-phosphoramidate prodrug to deliver a lipophilic nucleotide through the human cell membrane which is then metabolized to the 5'-monophosphate that can be converted to the 5'-di- and then 5'-tri-phosphorylated compound via selective kinases. See generally US Patent Nos.: 8,933,053; 8,759,318; 8,658,616; 8,263,575; 8,119,779; 7,951,787 and 7,115,590. A number of antivirals have been developed using Professor McGuigan's ProTide technology that have been approved by the FDA, such as Sovaldi for HCV, or are under an Emergency Use Authorization, such as Remdesivir, or in clinical trials, such as AT-527 for treatment of COVID19.

While Professor McGuigan's ProTide technology has been by far the most successful way to administer derivatized antiviral therapeutic nucleotides, it has several drawbacks. The 5'-phosphoramidate is a complex prodrug with a chiral phosphorus atom. This leads to complexity of manufacturing and potential stereochemistry issues if one of the phosphorus enantiomers is desired over the other.

Other legacy methods to provide intracellular nucleotide phosphates have included amino acid esters, phosphate esters, cyclic nucleotides, phosphorothioate esters, and covalent lipid prodrugs. Alios BioPharma, acquired by Johnson & Johnson, disclosed thiophosphoramidates in US 8,895,723 and 8,871,737, and cyclic nucleotides in US Patent No. 8,772,474. Idenix Pharmaceuticals, acquired by Merck, Inc. disclosed cyclic phosphoramidates and phosphoramidate/SATE derivatives in WO 2013/177219, and substituted carbonyloxymethylphosphoramidate compounds in WO 2013/039920. Karl Hostetler at the University of California, San Diego, has disclosed a range of covalent lipid phosphate prodrugs, see, for example, US 7,517,858 and covalent lipid conjugates of phosphonate prodrugs, see, for example, US 8,889,658; 8,846,643; 8,710,030; 8,309,565; 8,008,308; and 7,790,703. Emory University has disclosed nucleotide sphingolipid and lipid derivatives in WO 2014/124430. HepDirect™ technology is disclosed in the article "Design, Synthesis, and Characterization of a Series of Cytochrome P(450) 3A-Activated Prodrugs (HepDirect Prodrugs) Useful for Targeting Phosph(on)ate-Based Drugs to the Liver," (J. Am. Chem. Soc. 126, 5154-5163 (2004). Additional phosphate prodrugs include, but are not limited to phosphate esters, 3',5'-cyclic phosphates including CycloSAL, SATE derivatives (S-acyl-2-thioesters) and DTE (dithiodiethyl) prodrugs.

For literature reviews that disclose non-limiting examples see: A. Ray and K. Hostetler, "Application of kinase bypass strategies to nucleoside antivirals," Antiviral Research (2011) 277-291; M. Sofia, "Nucleotide prodrugs for HCV therapy," Antiviral Chemistry and Chemotherapy 2011; 22-23-49; R. Geraghty et al. "Broad-Spectrum Antiviral Strategies and Nucleoside Analogues," Viruses 2021 13, 667; and S. Peyrottes et al., "SATE Pronucleotide Approaches: An Overview," Mini Reviews in Medicinal Chemistry 2004, 4, 395;

While the prodrug strategy to delivery active antiviral nucleosides into the cell for 5'-triphosphate activation by the relevant kinases has provided improvements, these prodrug strategies present the different challenges of increased cost and size of the molecule, manufacturing complexity, potential additional stereochemistry considerations as well as the effect of the prodrug itself on the safety and efficacy of the active nucleoside as well as the effect on pharmacokinetics.

Given the critical need for effective and less expensive antiviral agents to treat the broad range of viruses that are treatable with derivatized nucleo(s)tides, it would be beneficial to provide compositions of matter and pharmaceutical formulations and the delivery and manufacture thereof that can pass through the cell membrane and wherein the derivatized nucleo(s)tide can become effectively 5'-phosphorylated for *in vivo* for therapeutic applications that do not rely, or rely less, on complex prodrug strategies.

Therefore, it is an object of the present invention to provide compositions of matter, methods of treatment of viral diseases and processes of preparation for the delivery of active nucleotides that do not require complex prodrug strategies.

SUMMARY OF THE INVENTION

It has been discovered that anti-viral 5'-phosphorylated nucleotides that are negatively charged under in vivo conditions can be delivered effectively through a cell membrane in cationic lipid nano- or micro- particles. In certain embodiments, the 5-nucleotide is a 5'-monophosphate, 5'-diphosphate or 5'-triphosphate. In the present invention the nucleotide is in a monomeric form, and is not an oligomer, RNA, DNA, or a multi-nucleotide component of RNA or DNA. It was surprising that this simple approach can be used to bypass the need for a complex nucleotide prodrug strategy and can provide an intracellular 5'-triphosphate in the cell for the intended

therapeutic purpose. It is especially surprising that this simple approach can work given the many years of investment in complex nucleotide prodrug strategies.

The invention herein does not pertain to the anti-viral nucleo(s)tides themselves, and thus any antiviral nucleo(s)tide can be used in the present invention if it is known to treat a viral disease and must be activated into its 5'-phosphate form in the cell to achieve its therapeutic effect.

In certain embodiments the compositions of the present invention can deliver 5'-monophosphate compounds that in the absence of the lipid nano or micro particle of the present invention cannot enter the cell (see Example 4 showing that sofosbuvir monophosphate Formulation 3 and sofosbuvir monophosphate Formulation 4 is active in an HCV cellular assay while sofosbuvir monophosphate in the absence of a lipid nanoparticle of the present invention is not active). This invention provides a more efficient and cost-effective means to deliver therapeutic antiviral nucleos(t)ides to treat or prevent the viral infection targeted by the active antiviral nucleoside.

As indicated, 5'-monophosphates, -diphosphates, and -triphosphate of active antiviral nucleosides typically cannot penetrate the cell membrane due to their negative charge. 5'-Monophosphates, -diphosphates, and -triphosphate of nucleosides can also be unstable due to the susceptibility to hydrolysis of the phosphate bonds. The compositions of matter and formulations of the present invention address these problems by delivering the 5'-monophosphates, -diphosphates, or -triphosphate of the active antiviral nucleoside in a manner that the negative charge is ionically stabilized by the cationic lipid nanoparticle.

In certain embodiments, lipid nano or micro particles can be selected to target the antiviral compound to specific tissues in the body, provide delayed release, delayed onset, or sustained therapeutic benefit as described further herein.

In certain embodiments, the 5'-monophosphate, 5'-diphosphate or 5'-triphosphate of an antiviral nucleoside or nucleotide is delivered into a cell for a therapeutic or prophylactic use in an effective amount to a host, including a human, in need thereof encased in an ionizable or permanently ionized cationic lipid nano- or micro- particle. While the cationic lipid particle can be a liposomal particle (that typically has some amount of aqueous solvent in the core); a particle with a solid core that does not include a significant amount of water or other solvent; or a particle that has some degree of solvent, such as water, but wherein the lipid carrier is not organized as

normally found in a liposome, as used herein, the term cationic lipid nano or micro particle refers to a solid or primarily solid form and does not have a liposomal construction.

In certain embodiments the cationic lipid nano or micro particle comprises a cationic lipid (in a non-limiting illustrative embodiment, for example 1,2-dioleoyl-3-dimethylammonium-propane (DODAP)), a phospholipid (in a non-limiting illustrative embodiment, for example distearoylphosphatidylcholine (DSPC)), and an antiviral 5'-phosphate nucleotide. In other embodiments the cationic lipid nano or micro particle comprises a cationic lipid (in a non-limiting illustrative embodiment, for example DODAP), a phospholipid (in a non-limiting illustrative embodiment, for example DSPC), cholesterol or other sterol or modified steroid, and an antiviral 5'-phosphate nucleotide

In an alternative embodiment, the cationic lipid nano or micro particle can be a cationic liposome. The cationic lipid nanoparticle carrier, for example, can be composed of one or more amphipatic lipids that form a sphere surrounding a hydrophilic core. The lipid nanoparticle generally, regardless of structure, is typically of a size, for example, including but not limited to at least about 40 to up to about 1,000 nm or more in diameter (including but not limited to up to about 50, 75, 100, 150, 200, 400, 500, 750 or 1,000 nm or more).

The cationic lipid nano or micro particle can be ionizable as a function of pH, or can be permanently ionized. As nonlimiting examples, lipid amines are ionizable as a function of pH, however, lipid quaternary amines are permanently ionized. The counterion to the quaternary amine of the lipid can be any counterion that achieves the desired purpose, as further described herein.

Numerous examples of cationic lipids that can be used in the cationic lipid nanoparticles are generally known and are described herein. General categories of cationic lipids include but are not limited to long chain amino ethers, long chain carbamates, long chain amino or carbamate esters or diesters, pegylated amines or carbamates that may also have an ester, long chain alkyl, alkenyl or alkynyl esters or ethers generally of an amine or carbamate or other ionizable nitrogen moiety. Any lipid carbonyl(s) can be protected, for example, as a ketal, acetal, acylal, or dithiane.

The lipid amine can be in the form of a primary, secondary, tertiary or quaternary amine. The amine can be protected, as non-limiting examples, with/as a carbobenzyloxy group, a methoxybenzyl carbonyl, a t-butyloxycarbonyl, acyl, acetyl, benzoyl, benzyl, carbamate, methoxybenzyl, phenyl, tosyl, as a sulfonamide or amide.

Specific examples of cationic lipids are described in more detail below and include, as nonlimiting illustrative examples, PEG-DMG, PEG-DSG, DODMA, DODAP, DOTMA, DOTAP, KC2 and MC3.

In certain aspects, the delivery of the 5'-monophosphate, 5'-diphosphate or 5'-triphosphate is accomplished in a composition that includes several components as described in more detail herein, which may include, for example, two or more lipid materials, at least one of which is ionizable or ionized, a sterol such as cholesterol, and one or more other components to achieve the desired properties.

In alternative embodiments, the antiviral nucleotide is delivered into the cell as a negatively charged 5'-phosphate derivative other than 5'-monophosphate, 5'-diphosphate or 5'-triphosphate, that is compatible with the cationic LNP or LMP and metabolizes to the 5'-monophosphate, 5'-diphosphate or 5'-triphosphate in the cell.

BRIEF DESCRIPTIONS OF FIGURES

FIG. 1 is a graph of **Formulation 1** in SARS-CoV-2 Cytopathic Effect (CPE) Assay in Vero Cells. The percent SARS-CoV-2 inhibition is shown with the black bars and the percent cell viability is shown in the gray bars. The x-axis is the concentration of particles measured in ug/L and the y-axis is SARS-CoV-2 inhibition measured in percent cell viability.

FIG. 2 is a graph of **Formulation 2** in SARS-CoV-2 Cytopathic Effect (CPE) Assay in Vero Cells. The percent SARS-CoV-2 inhibition is shown with the black bars and the percent cell viability is shown in the gray bars. The x-axis is the concentration of particles measured in ug/L and the y-axis is SARS-CoV-2 inhibition measured in percent cell viability.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

The term "active antiviral nucleoside" or "active antiviral nucleotide" as used herein refers to a nucleoside or nucleotide that has been shown to exhibit inhibitory activity against the growth or replication of a disease-causing or implicated virus. The nucleoside or nucleotide is typically activated by conversion to its 5'-triphosphate form in the cell, however, according to this invention, is delivered into the cell in the form of a 5'-phosphate which is, or is then anabolized to, the 5'-triphosphate form in the cell.

The term "alkyl" refers to a linear, branched or cyclic fully saturated hydrocarbon radical or alkyl group which can be optionally substituted (for example, with halogen, including, F). For example, an alkyl group can have 1, 2, 3, 4, 5, 6, 7 or 8 carbon atoms (i.e., C_1 -C_8 alkyl), 1, 2, 3, 4, 5 or 6 carbon atoms (i.e., C_1-C_6 alkyl) or 1 to 4 carbon atoms (i.e., C_1-C_4 alkyl). Examples of suitable alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, tert-pentyl, neopentyl, hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl and 2,3-dimethylbutyl.

The term "alkenyl" refers to a non-aromatic hydrocarbon group which contains at least one double bond between adjacent carbon atoms and a similar structure to an alkyl group as otherwise described herein. For example, an alkenyl group can have 2 to 8 carbon atoms (i.e., C_2-C_8 alkenyl), or 2 to 4 carbon atoms (i.e., C_2-C_4 alkenyl). Examples of suitable alkenyl groups include, but are not limited to, ethenyl or vinyl (-CH=CH_2), allyl (-CH_2CH=CH_2), 1-butenyl (-C=CH-CH_2CH_3) and 2-butenyl (-CH_2CH=$CHCH_2$). The alkenyl group can be optionally substituted as described herein.

The term "alkynyl" refers to a non-aromatic hydrocarbon group containing at least one triple bond between adjacent carbon atoms and a similar structure to an alkyl group as otherwise described herein. For example, an alkynyl group can have 2 to 8 carbon atoms (i.e., C_2-C_8 alkyne,), or 2 to 4 carbon atoms (i.e., C_2-C_4 alkynyl). Examples of alkynyl groups include, but are not limited to, acetylenic or ethynyl and propargyl. The alkynyl group can be optionally substituted as described herein.

The term "acyl" refers to the moiety –C(O)R' in which the carbonyl moiety is bonded to R', for example, -C(O)alkyl. R' can be selected from alkoxy, alkyl, cycloalkyl, lower alkyl (i.e., C_1-C_4); alkoxyalkyl, including methoxymethyl; aralkyl- including benzyl, aryloxyalkyl- such as phenoxymethyl; aryl including phenyl optionally substituted with halogen, C_1 to C_4 alkyl or C_1 to C_4 alkoxy.

The term "alkoxy" refers to the group –OR'' where –OR'' is -O-alkyl, -O-alkenyl, -O-alkynyl, -O-(C_0-C_2)(cycloalkyl), -O-(C_0-C_2)(heterocyclo), -O-(C_0-C_2)(aryl), or -O-(C_0-C_2)(heteroaryl), each of which can be optionally substituted.

The term "amino" refers to a primary, secondary or tertiary amine, or in context may refer to a quaternary amine.

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

The term "azido" refers to the group $-N_3$, which may be derivatized.

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

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

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

A heteroaryl ring system is a saturated or unsaturated ring with one or more nitrogen, oxygen, or sulfur atoms in the ring (monocyclic) including but not limited to imidazole, furyl, pyrrole, furanyl, thiene, thiazole, pyridine, pyrimidine, purine, pyrazine, triazole, oxazole, or fused ring systems such as indole, quinoline, etc., among others, which may be optionally substituted as described above. Heteroaryl groups include nitrogen-containing heteroaryl groups such as pyrrole, pyridine, pyridone, pyridazine, pyrimidine, pyrazine, pyrazole, imidazole, triazole, triazine, tetrazole, indole, isoindole, indolizine, purine, indazole, quinoline, isoquinoline, quinolizine, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, imidazopyridine, imidazotriazine, pyrazinopyridazine, acridine, phenanthridine, carbazole, carbazoline, perimidine, phenanthroline, phenacene, oxadiazole, benzimidazole, pyrrolopyridine, pyrrolopyrimidine and pyridopyrimidine; sulfur-containing aromatic heterocycles such as thiophene and benzothiophene;

oxygen-containing aromatic heterocycles such as furan, pyran, cyclopentapyran, benzofuran and isobenzofuran; and aromatic heterocycles comprising two or more hetero atoms selected from among nitrogen, sulfur and oxygen, such as thiazole, thiadizole, isothiazole, benzoxazole, benzothiazole, benzothiadiazole, phenothiazine, isoxazole, furazan, phenoxazine, pyrazoloxazole, imidazothiazole, thienofuran, furopyrrole, pyridoxazine, furopyridine, furopyrimidine, thienopyrimidine and oxazole, among others, all of which may be optionally substituted.

The term "heterocycle" or "heterocyclo" refers to a cyclic group which contains at least one heteroatom, i.e., O, N, or S, and may be aromatic (heteroaryl) or non-aromatic. Exemplary non-aromatic heterocyclic groups for use in the present invention include, for example, pyrrolidinyl, piperidinyl, piperazinyl, N-methylpiperazinyl, imidazolinyl, pyrazolidinyl, imidazolidinyl, morpholinyl, tetrahydropyranyl, azetidinyl, oxetanyl, oxathiolanyl, pyridone, 2-pyrrolidone, ethyleneurea, 1,3-dioxolane, 1,3-dioxane, 1,4-dioxane, phthalimide, and succinimide, among others, all of which may be optionally substituted.

The term “5’-phosphate” of a nucleoside refers to a 5’-monphosphate, 5’-diphosphate or 5’-triphosphate of the nucleoside or another negatively charged 5’-phosphate that is capable of anabolism into the 5’-triphophate form in the cell.

The term “long-chain” or “long chain aliphatic” refers to a long chain alkyl, alkenyl, or alkynyl group that is about 6-22 carbons in length (i.e., $C_{6\text{-}22}$alkyl, $C_{6\text{-}22}$alkenyl, or $C_{6\text{-}22}$alkynyl), for example 6-8, 6-10, 6-12, 6-14, 6-16, 6-18, 6-20, or 6-22 carbons in length, and specifically, 6, 7, 8, 9, 10,11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 or more carbon atoms in chain length.

The term “nanoparticle” refers to a particle that has a diameter of at least about 0.01, 0.1, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, or 950 or 999 nm. In certain embodiments the nanoparticle has a diameter between about 0.1 and about 300 nm. For example, the nanoparticle may have a diameter of at least about 10, 50, 100, 150, 200, or 250 nm. In certain embodiments the nanoparticle may have a diameter of at least about 200 nm. In certain embodiments the lipid antiviral formulation described herein is a nanoparticle.

The term “microparticle” refers to a particle that has a diameter of at least about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, or 950 or 999 μm. In certain embodiments the microparticle has a diameter between about 1 and about 10 μm. For example, the microparticle

may have a diameter of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 μm. In certain embodiments the microparticle may have a diameter of at least about 1, 2, or 3 μm. In certain embodiments the lipid antiviral formulation described herein is a microparticle.

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

The term "substituted" or "optionally substituted" indicates that the moiety can have at least one additional substituent including, but not limited to, halogen (F, Cl, Br, I), OH, phenyl, benzyl, N_3, CN, acyl, alkyl, including methyl; alkenyl, alkynyl, alkoxy, haloalkyl; including CHF_2, CH_2F and CF_3; etc. In certain embodiments, the term "substituted" or "optionally substituted" indicates that the moiety can have at least one additional substituent including, but not limited to, azido, cyano, halogen (fluoro, chloro, bromo, or iodo), alkyl, alkenyl, alkynyl, cycloalkyl, heterocycle, aryl, heteroaryl, haloalkyl, hydroxyl, alkoxy, amino, -NH(C_1-C_6 unsubstituted alkyl), -NH(C_1-C_6 substituted alkyl), -NH-(C_0-C_2alkyl)(C_3-C_8cycloalkyl), -NH-(C_0-C_2alkyl)(C_3-C_8heterocycle),-NH-(C_0-C_2alkyl)(aryl), -N(C_1-C_6 unsubstituted alkyl)$_2$, -N(C_1-C_6 unsubstituted alkyl)(C_1-C_6 substituted alkyl), -N(C_1-C_6 substituted alkyl)$_2$,-NH-(C_0-C_2alkyl)(C_3-C_8cycloalkyl), -NH-(C_0-C_2alkyl)(C_3-C_8heterocycle),-NH-(C_0-C_2alkyl)(aryl), acyl, nitro, sulfonic acid, sulfate, phosphonic acid, phosphate, phosphonate, or thiol. The compound with the substituent must lead to a stable molecule with correct valency and suitable for the intended purpose.

The terms "coadminister" and "coadministration" or combination therapy are used to describe the administration of at least one of the 5'-phosphate nucleo(s)tides delivered according to the present invention in combination with at least one other active agent.

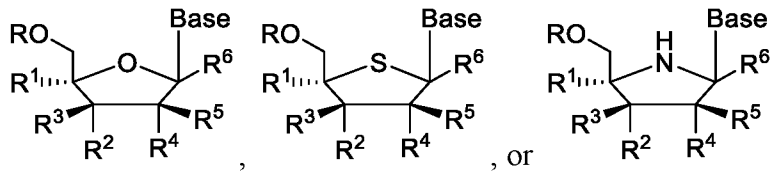
The term "host", as used herein, refers to an animal that is infected with or may be exposed to a disease-causing virus. It is typically a human. The term includes primates (including chimpanzees) and humans. Veterinary applications, in certain indications, however, are clearly anticipated by the present invention (such as chimpanzees). The host can be for example, bovine, equine, avian, canine, feline, etc.

Viruses that can be treated according to the present invention are any that can be treated with an antiviral nucleo(s)tide. The Baltimore classification system sorts viruses into Groups, labeled I-VII, according to their genome. DNA viruses belong to Groups I, II, and VII, while RNA viruses belong to Groups III-VI. RNA viruses use ribonucleic acid as their genetic material. An RNA virus can have double-stranded (ds) RNA or single stranded RNA and can also be positive-stranded or negative-stranded. Group III viruses are double-stranded RNA viruses. Groups IV and V are both single-stranded RNA viruses, but Groups IV viruses are positive-sense and Groups V are negative-sense. Group VI are positive-sense single-stranded RNA viruses that replicate through a DNA intermediate. The term “virus” refers to any virus classified in the Baltimore classification system. Any of these viruses that have an identified antiviral nucleoside that can be delivered as described herein can be treated according to the present invention.

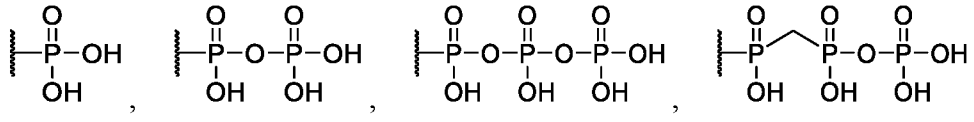
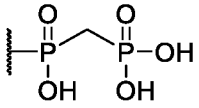
II. Active Antiviral Nucleos(t)ides

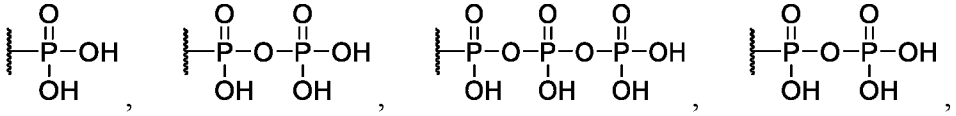
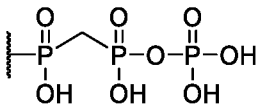
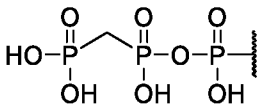
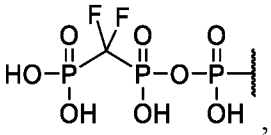
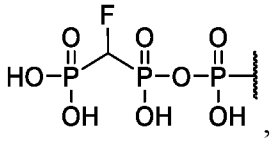
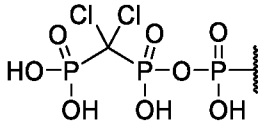
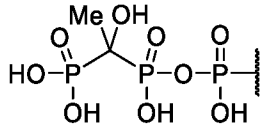
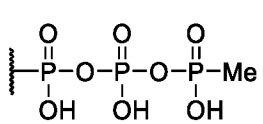
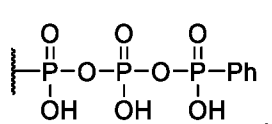
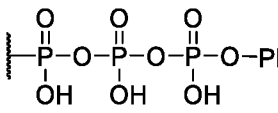
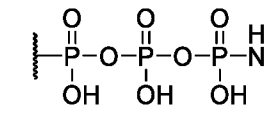
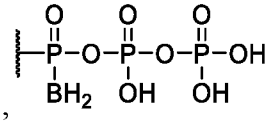
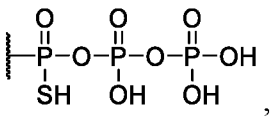
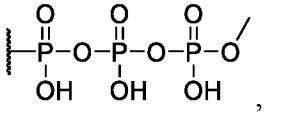
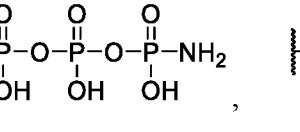
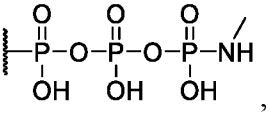
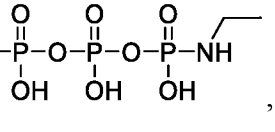
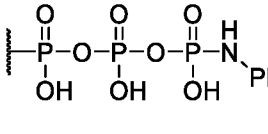
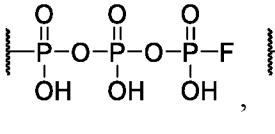
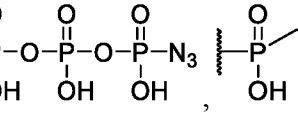
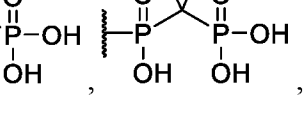
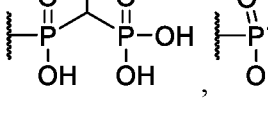
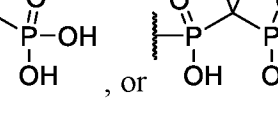
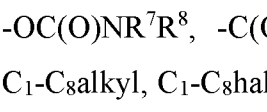
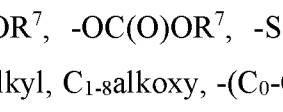
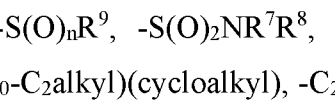
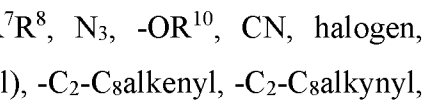
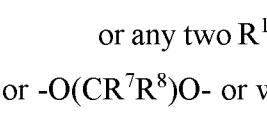
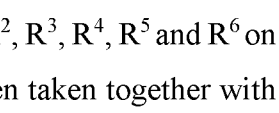
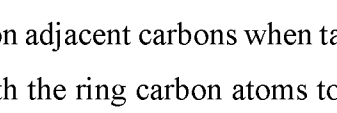
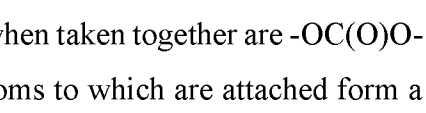
Any nucleos(t)ide that has antiviral activity can be used in the present invention. Where the nucleotides are sometimes depicted as 5’-monophosphates, it should be understood that the corresponding 5’-diphosphate or 5’-triphosphate can be used in its place, or another negatively charged 5’-phosphate that can be anabolized to the 5’-triphosphate in vivo.

In one aspect, the active compound of the present invention is a compound of Formula I, which can be provided as a pharmaceutically acceptable salt thereof wherein Formula I is:



, , or

R is , or, ;

or R is , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , or ;

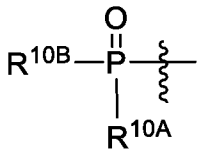
R^1, R^2, R^3, R^4, R^5 and R^6 is independently selected from H, NR^7R^8, $-C(O)NR^7R^8$, $-OC(O)NR^7R^8$, $-C(O)OR^7$, $-OC(O)OR^7$, $-S(O)_nR^9$, $-S(O)_2NR^7R^8$, N_3, $-OR^{10}$, CN, halogen, C_1-C_8alkyl, C_1-C_8haloalkyl, $C_{1\text{-}8}$alkoxy, -(C_0-C_2alkyl)(cycloalkyl), -C_2-C_8alkenyl, -C_2-C_8alkynyl, and aryl(C_1-C_8alkyl)-;

or any two R^1, R^2, R^3, R^4, R^5 and R^6 on adjacent carbons when taken together are -OC(O)O- or $-O(CR^7R^8)O$- or when taken together with the ring carbon atoms to which are attached form a double bond;

R^7 and R^8 are independently selected from H, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, -(C_0-C_2alkyl)(cycloalkyl), aryl(C_1-C_8alkyl)-, -(C_0-C_2alkyl)(heterocyclo), aryl, heteroaryl, -C(O)R^{10C}, and -S(O)$_n$(C_1-C_8)alkyl or R^{11} and R^{12} taken together with a nitrogen to which they are both attached form a 3 to 7 membered heterocyclic ring wherein any one carbon atom of said heterocyclic ring can optionally be replaced with —O—, —S(O)$_n$— or —NR^a—;

R^9 is selected from C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, -(C_0-C_2alkyl)(cycloalkyl), aryl(C_1-C_8alkyl)-, -(C_0-C_2alkyl)(heterocyclo), aryl, and heteroaryl;

R^{10} is selected from H, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, -(C_0-C_2alkyl)(cycloalkyl), aryl(C_1-C_8alkyl)-, -(C_0-C_2alkyl)(heterocyclo), aryl, heteroaryl, -C(O)R^{10C}, -S(O)$_n$(C_1-C_8)alkyl,



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

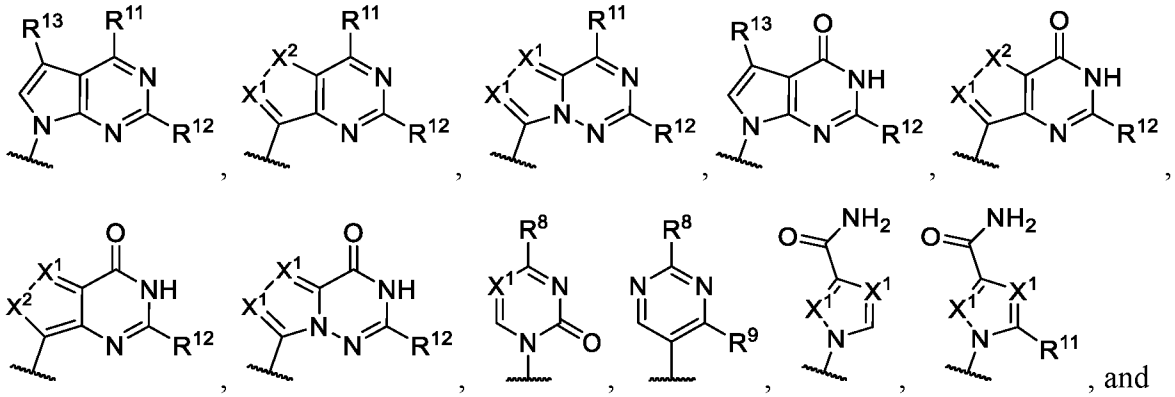
R^{10A} is selected from O^-, OH, an –O-optionally substituted aryl, an –O-optionally substituted heteroaryl, and an optionally substituted heterocyclyl;

R^{10B} is selected from O^-, OH, an optionally substituted N-linked amino acid, and an optionally substituted N-linked amino acid ester; and

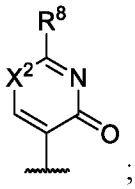
R^{10C} is alkyl, alkenyl, alkynyl, -(C_0-C_2alkyl)(cycloalkyl), -(C_0-C_2alkyl)(heterocyclo), -(C_0-C_2alkyl)(aryl), -(C_0-C_2alkyl)(heteroaryl), -O-alkyl, -O-alkenyl, -O-alkynyl, -O-(C_0-C_2alkyl)(cycloalkyl), -O-(C_0-C_2alkyl)(heterocyclo), -O-(C_0-C_2alkyl)(aryl), or -O-(C_0-C_2alkyl)(heteroaryl);

n is independently selected at each instance from 0, 1, 2, and 3;

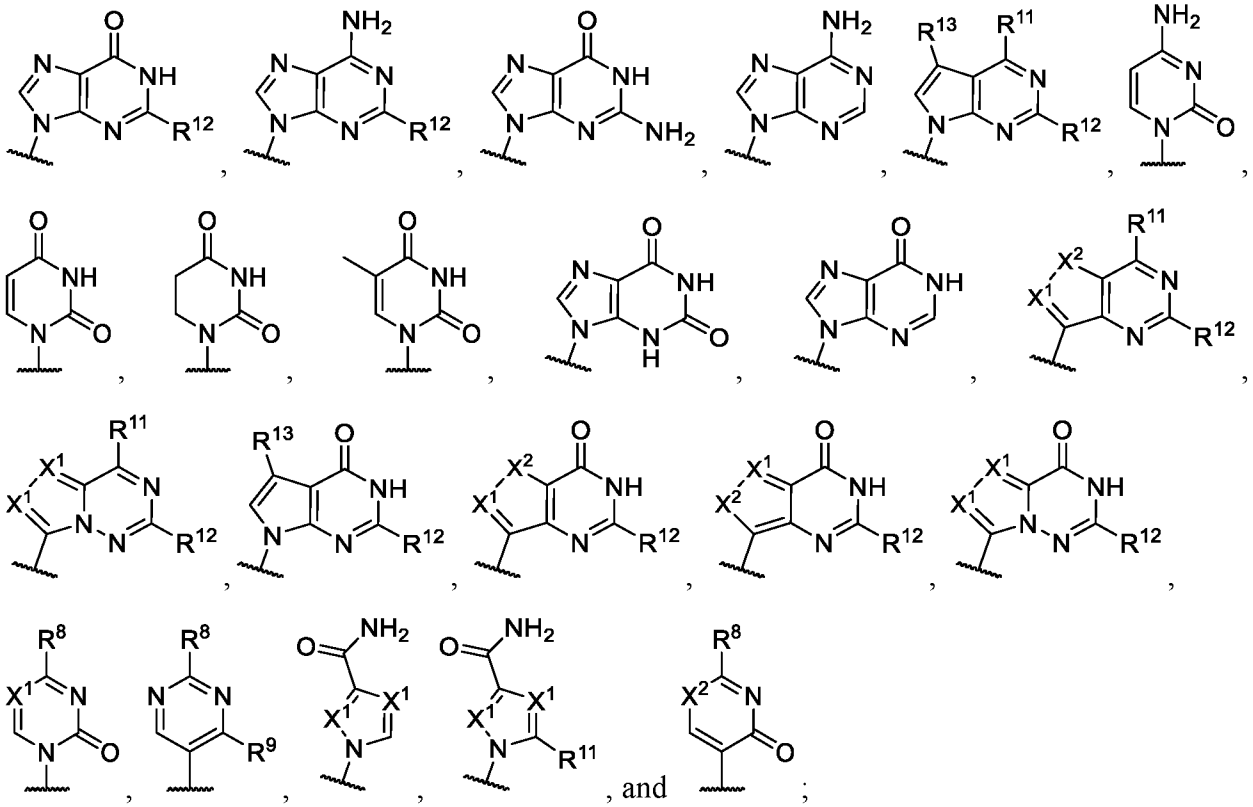
Base is selected from:



, and


;

or Base is selected from:


, and ;

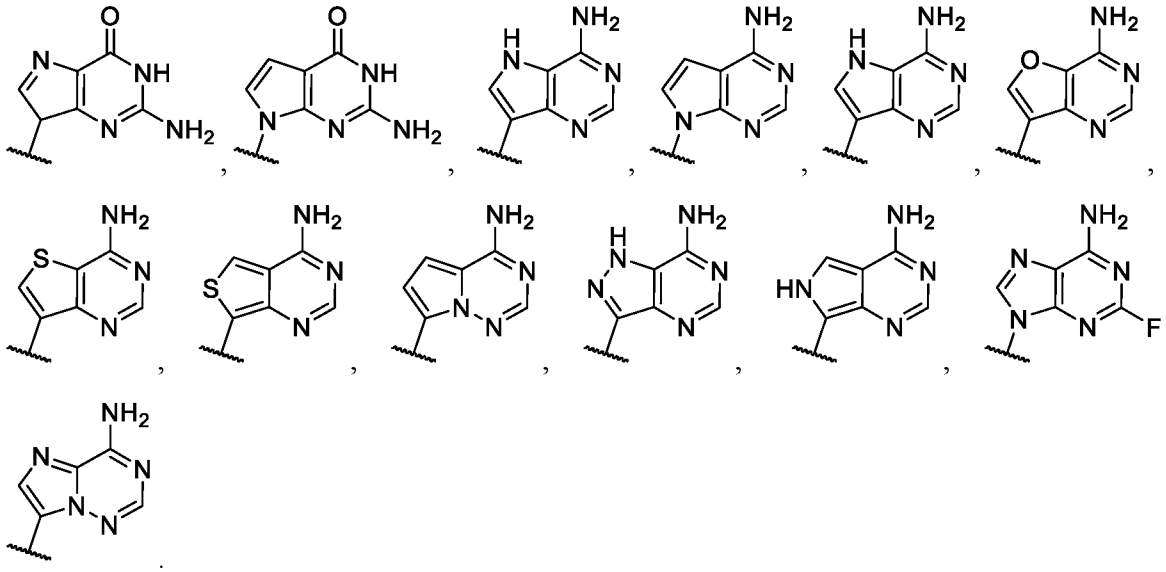
X^1 is independently N or CR^{13};

X^2 is independently NR^7, O, or $S(O)_n$;

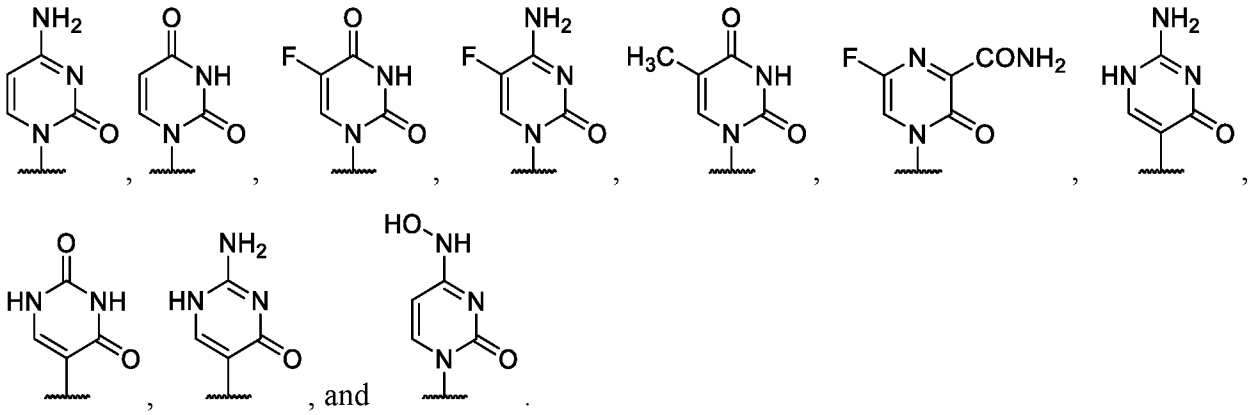
R^{11} is independently halogen, $-NR^7R^8$, $-N(R^7)OR^7$, $-NR^7NR^7R^8$, N_3, NO, NO_2, $C(O)R^{10C}$, CN, —CH(=NR^7), —CH=$NNHR^7$, —CH=$N(OR^7)$, —$CH(OR^7)_2$, —C(=O)NR^7R^8, $-C(=S)NR^7R^8$, $-C(=O)OR^7$, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, -(C_0-C_2alkyl)(cycloalkyl), aryl, -(C_0-C_2alkyl)(heterocyclo), heteroaryl, —$C(O)R^{10C}$, —$S(O)_n$(C_1-C_8)alkyl, aryl(C_1-C_8alkyl)-, or $OR^{10;}$ and

R^{12} or R^{13} are independently H, halogen, $-NR^7R^8$, $-N(R^7)OR^7$, $-NR^7NR^7R^8$, N_3, NO, NO_2, $C(O)R^{10C}$, CN, —CH(=NR^7), —CH=$NHNR^7$, —CH=$N(OR^7)$, —$CH(OR^7)_2$, —C(=O)NR^7R^8, $-C(=S)NR^7R^8$, —$C(=O)OR^7$, R^7, OR^{10}, or SR^8.

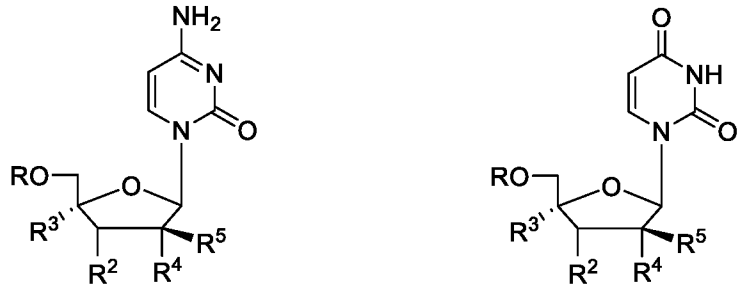
In certain embodiments, Base is selected from:



In certain embodiments, Base is selected from:



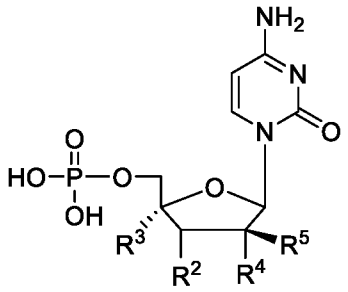
In one aspect, the active compound of the present invention is a compound of Formula Ia or Formula Ib, which can be provided as a pharmaceutically acceptable salt:



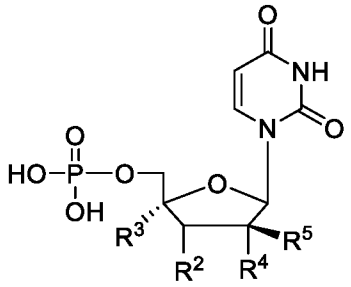
Formula Ia Formula Ib

wherein R, R^1, R^2, R^4, and R^5 are as defined herein.

In another aspect, the active compound of the present invention is a compound of Formula Ia' or Formula Ib', which can be provided as a pharmaceutically acceptable salt:



Formula Ia'



Formula Ib'

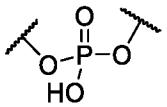
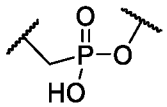
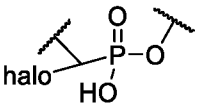
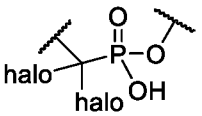
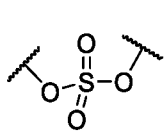
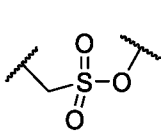
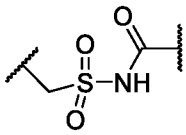
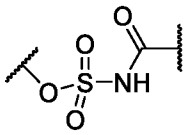
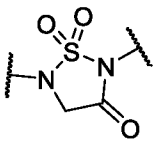
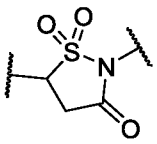
wherein R, R^1, R^2, R^4, and R^5 are as defined herein.

In certain embodiments of Formula Ia or Formula Ib, R^3 is selected from C_{1-8}alkyl, C_{1-8}haloalkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, C_{1-8}alkoxy, OR^{10}, and cyano. In a further embodiment, R^{10} is selected from C_1-C_8alkyl, C_2-C_8alkenyl, and -(C_0-C_2alkyl)(cycloalkyl).

In certain embodiments of Formula Ia or Formula Ib, R^5 is hydrogen, halogen (including F, Cl, or Br), C_{1-6}alkyl (including, but not limited to methyl).

In certain embodiments of Formula Ia or Formula Ib, R^4 is hydrogen, halogen (including F, Cl, or Br), OR^2 (including, but not limited to, OH);

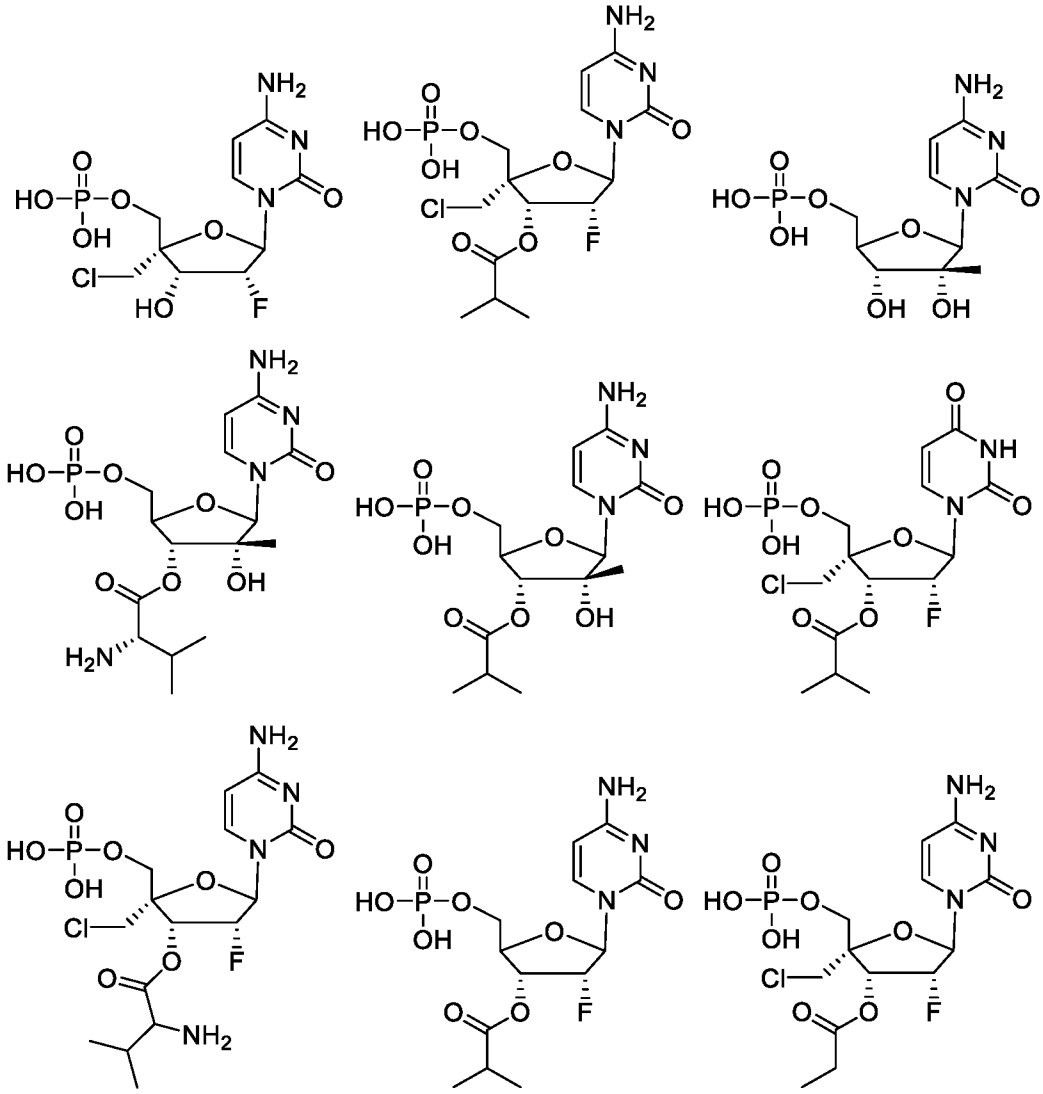
In certain embodiments of Formula Ia or Formula Ib, R^2 is hydrogen.

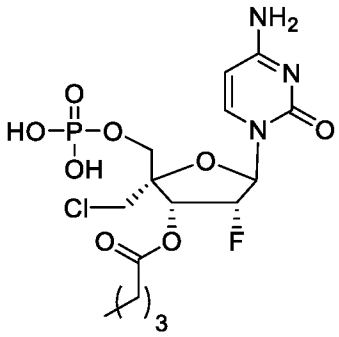
In certain embodiments, where a triphosphate, diphosphate, or monophosphate group is drawn or recited in a formula one or more of those phosphate groups can be replaced by a bioisostere or other phosphate described herein or otherwise known. Non-limiting examples of bioisosteres of phosphate () include , , , , , , , , and .

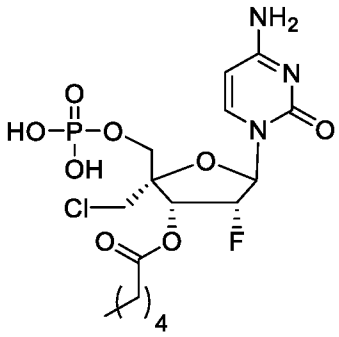
Also, when a 5'-triphosphate or 5'-diphosphate is illustrated herein, the corresponding 5'-monophosphate can also be used. Likewise, if a 5'-monophosphate is illustrated, the corresponding

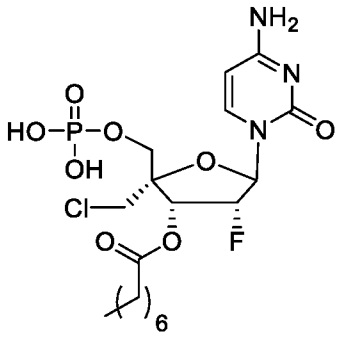
5'-diphosphate or 5'-triphosphate can be used or if a 5'-diphosphate is illustrated, the corresponding 5'-monophosphate or 5'-triphosphate can be used. In addition, a 5'-phosphate of the selected anti-viral nucleoside that is not a 5'-monophosphate, 5'-diphosphate or 5'-triphosphate can be used if it achieves the desired result.

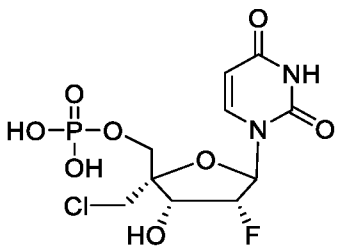
Non-limiting examples of compounds for use in the present invention include:

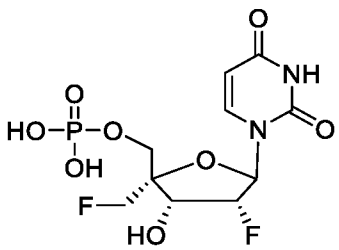


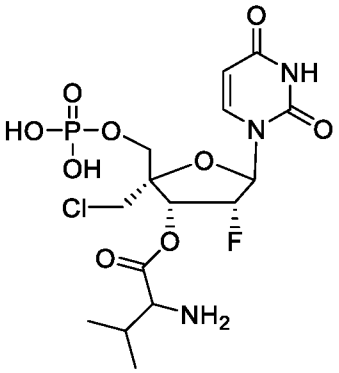


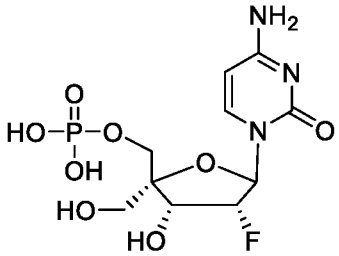


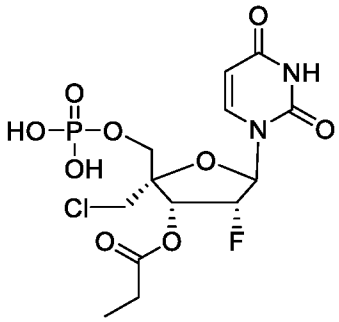


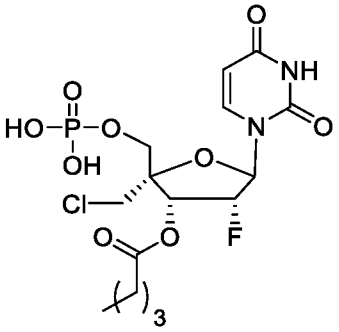


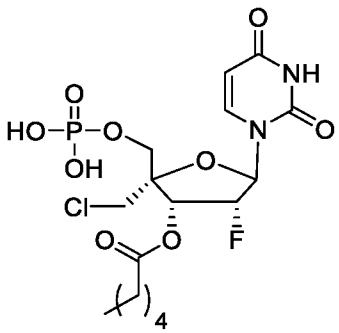


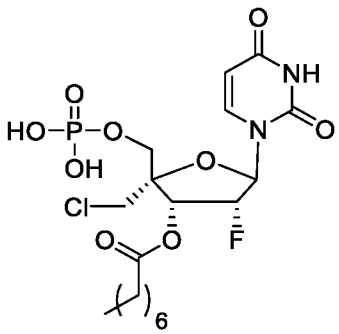


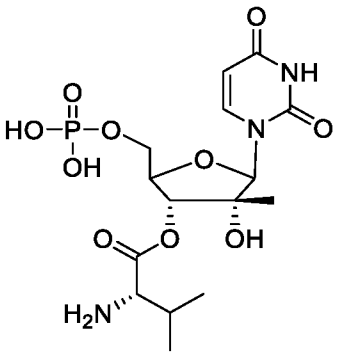


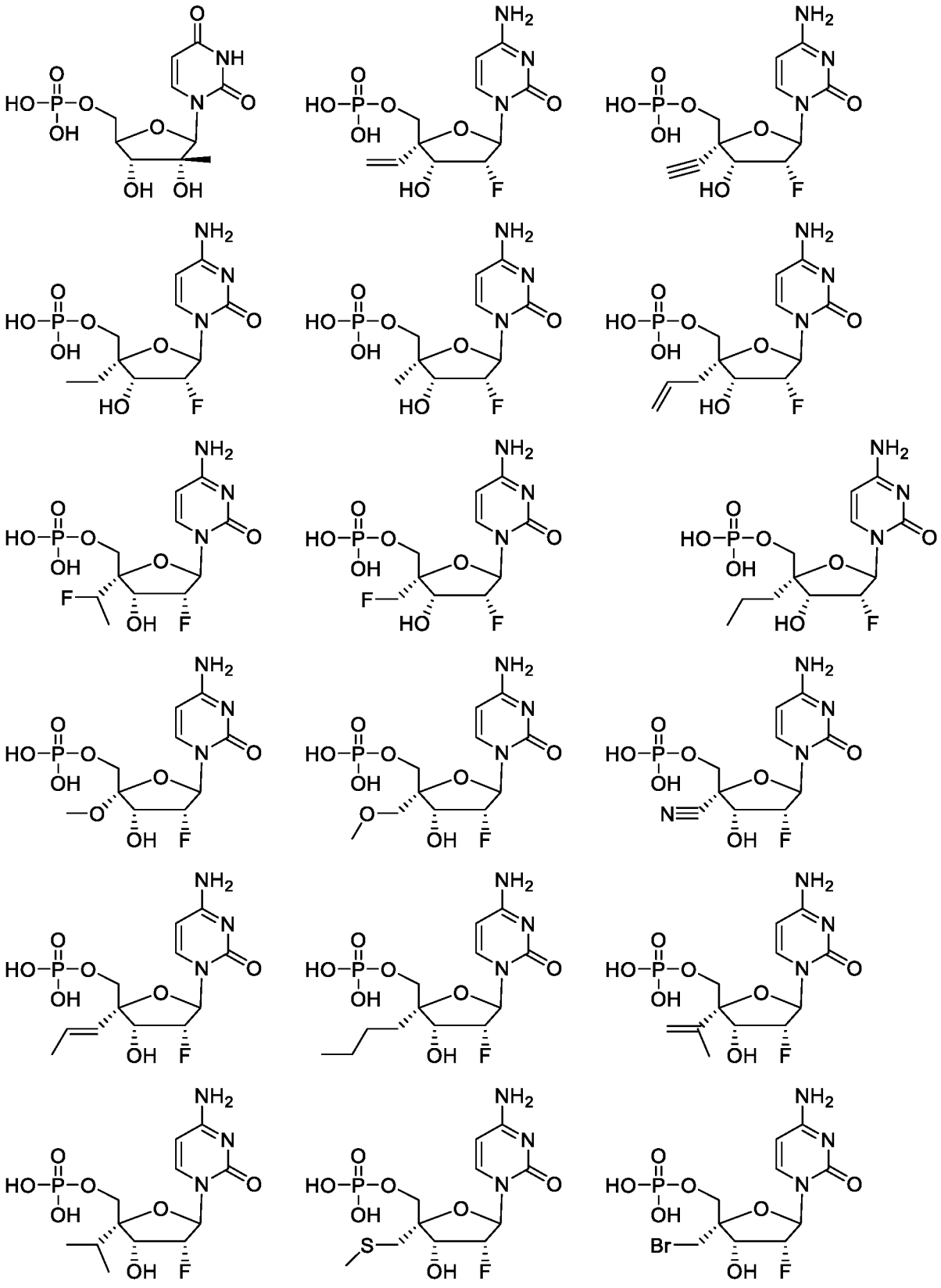


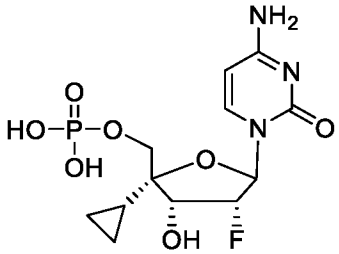




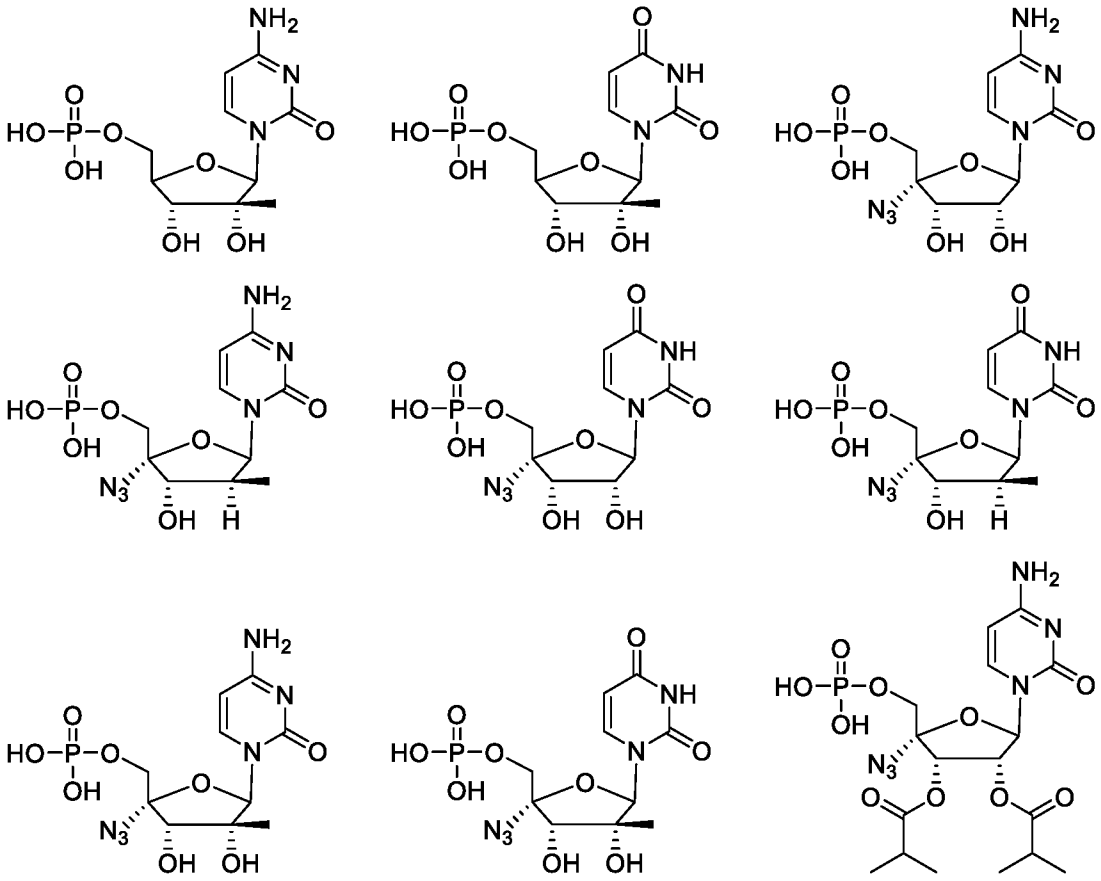


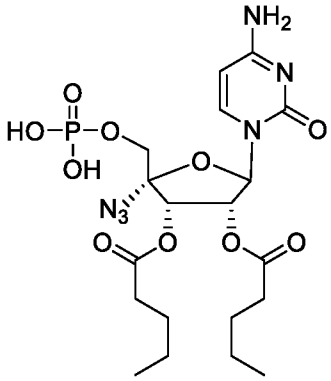
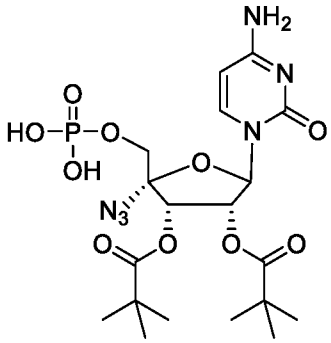
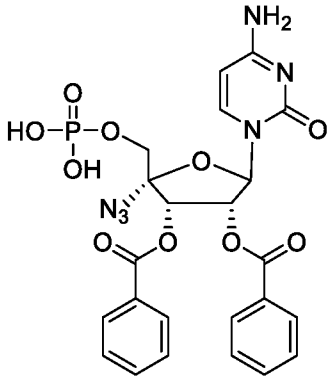




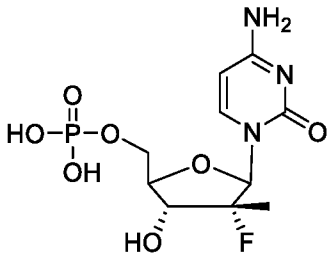
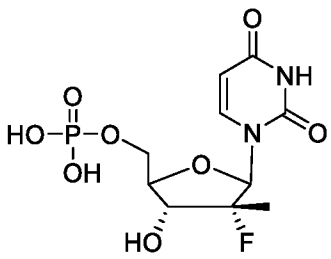
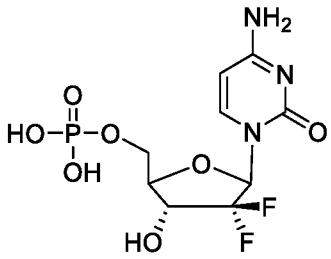
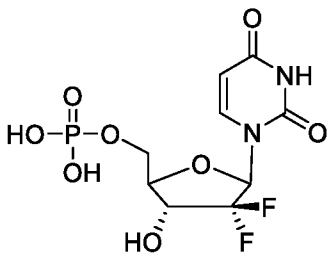
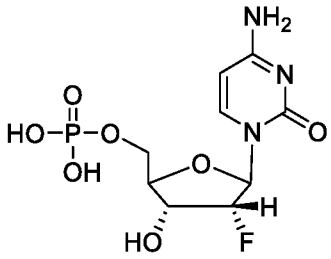
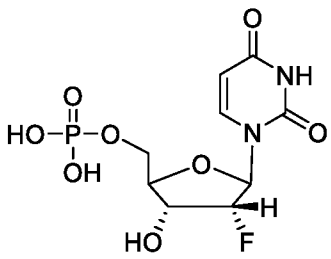
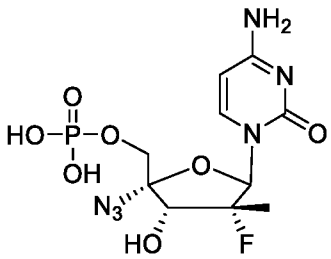
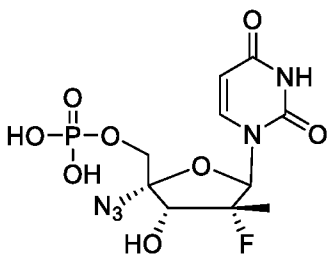
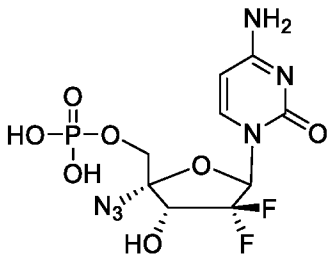
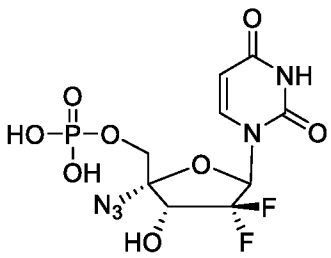
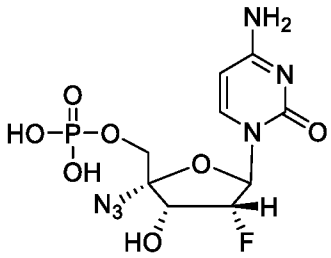
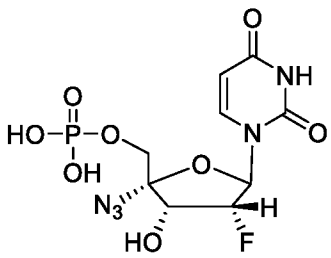


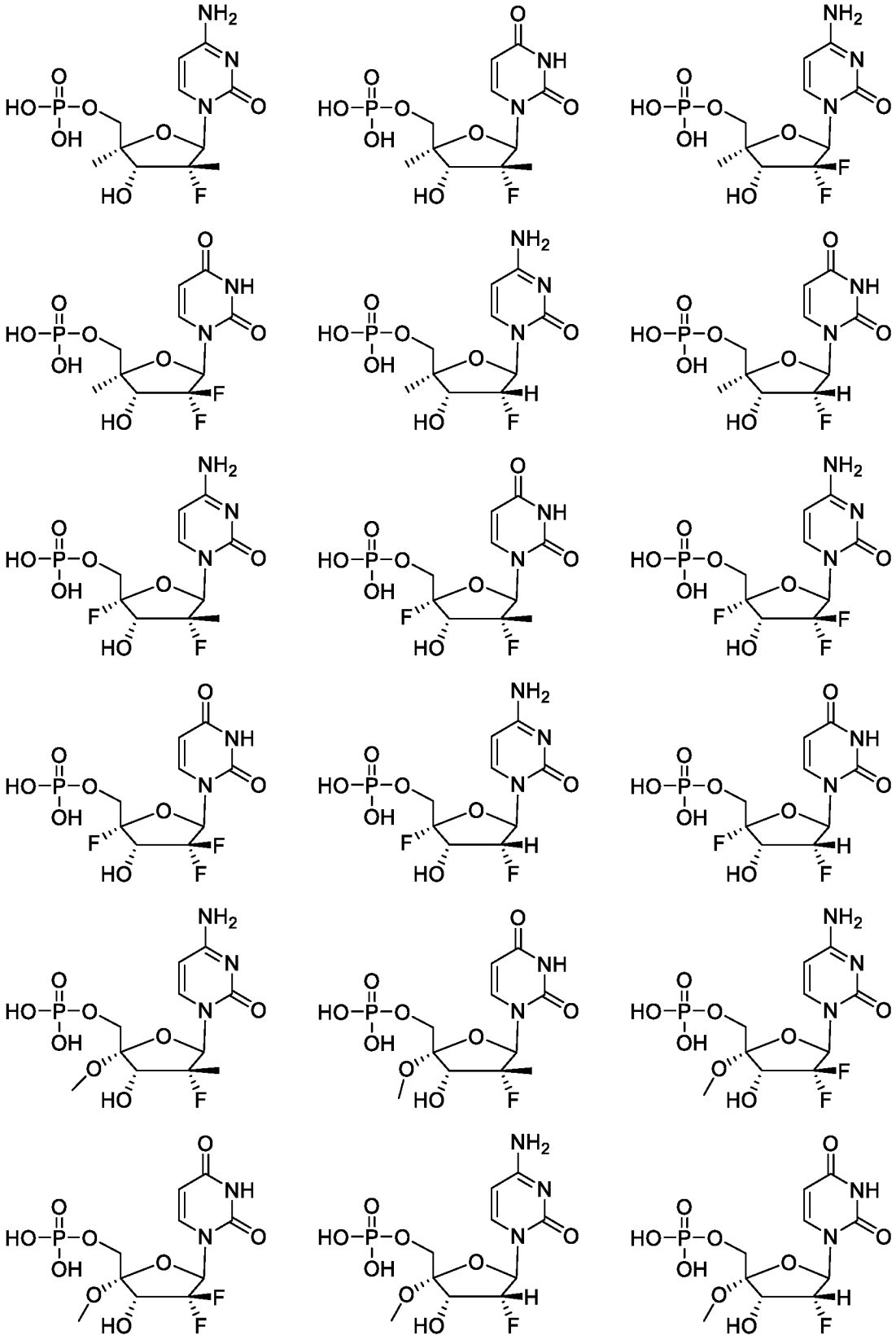
Additional non-limiting examples of compounds for use in the present invention include:

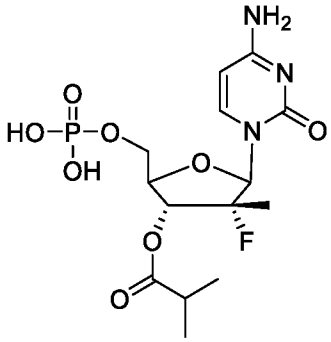


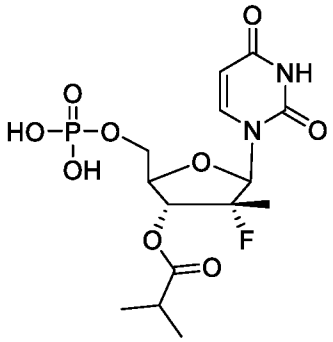




Additional non-limiting examples of compounds for use in the present invention include:

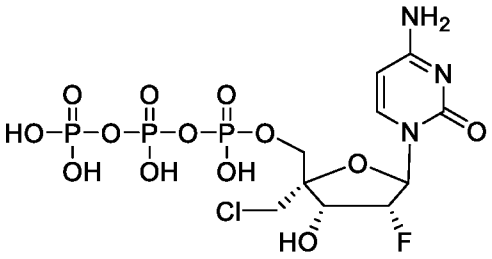













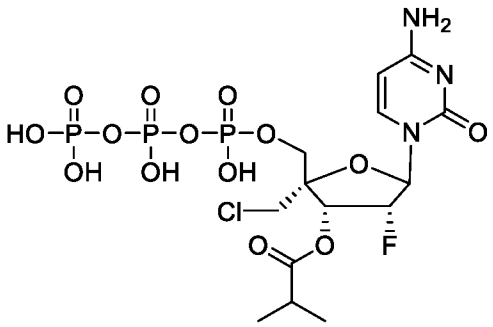


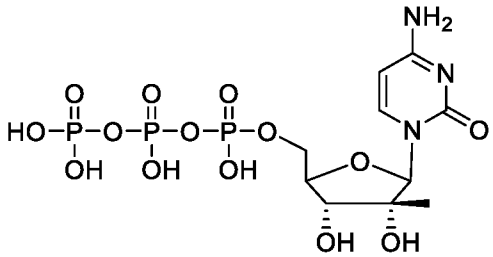


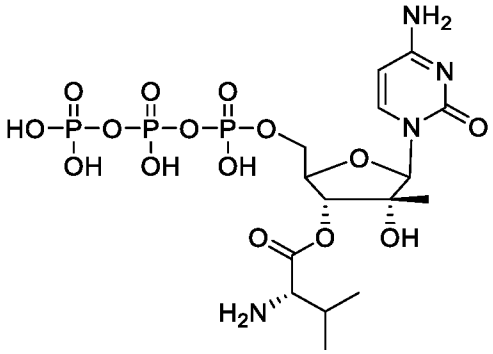


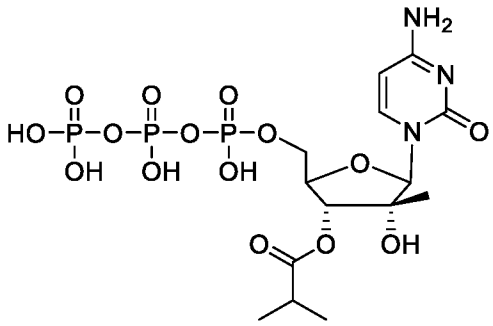
Non-limiting examples of compounds for use in the present invention include:

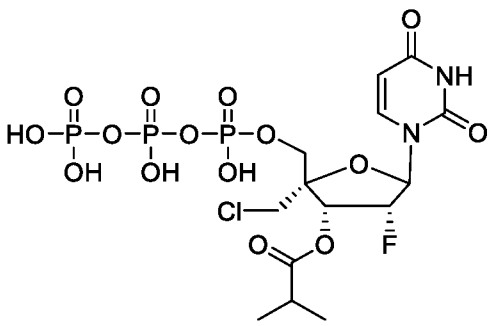


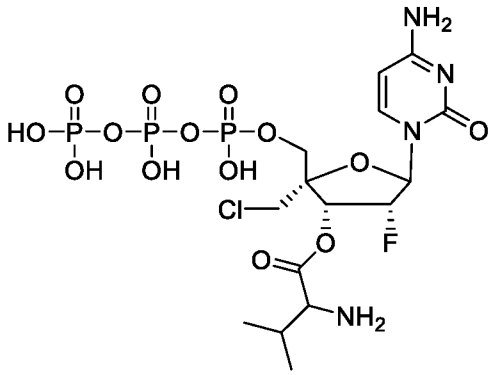


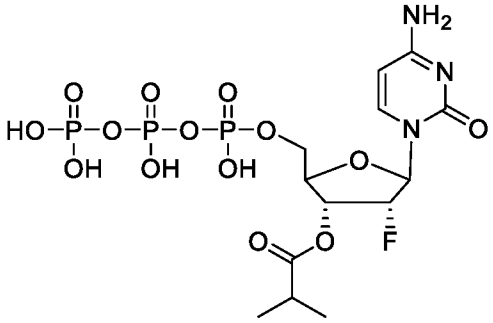


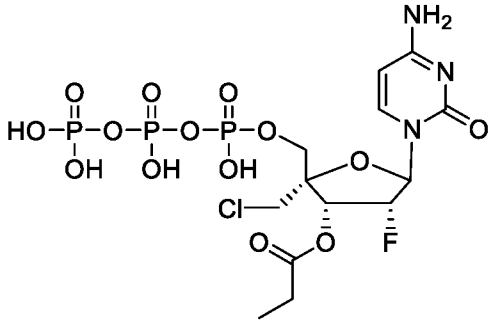


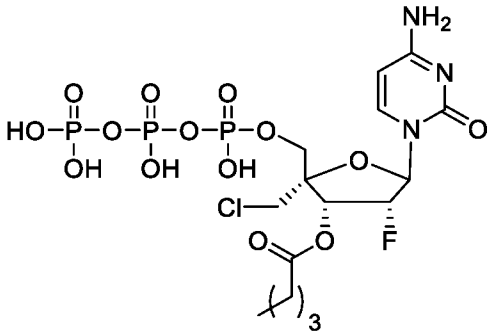


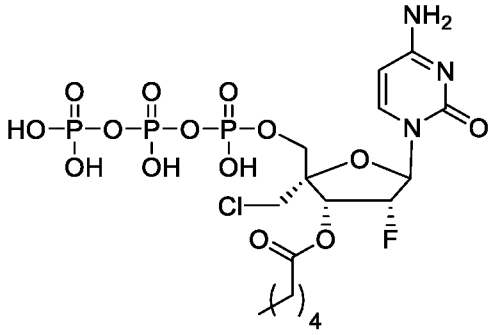


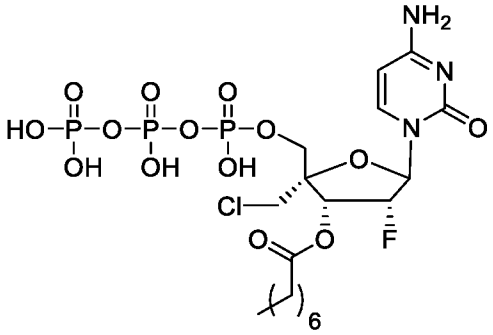


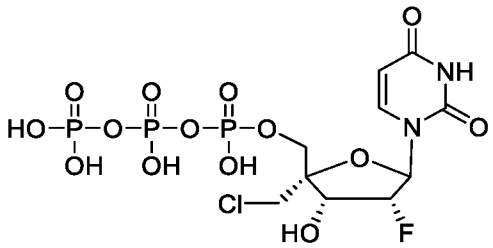


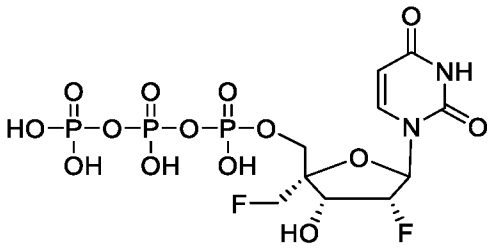


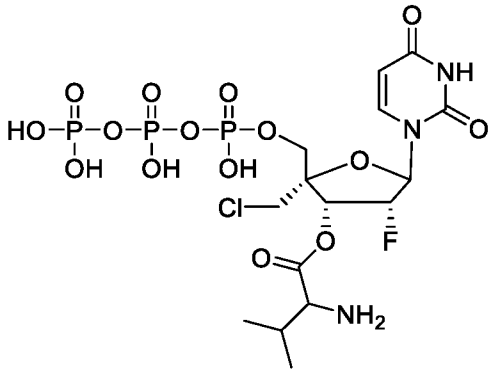


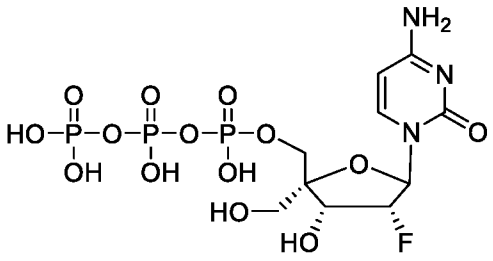


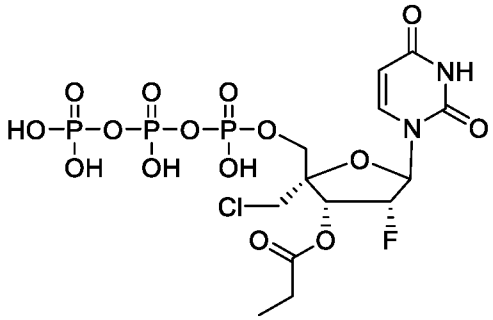


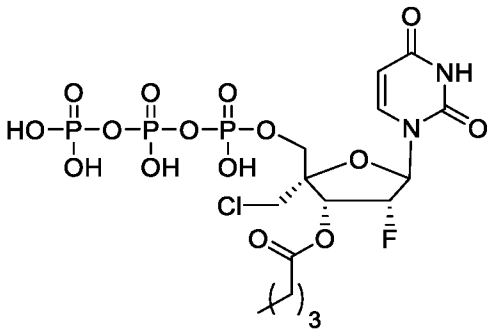


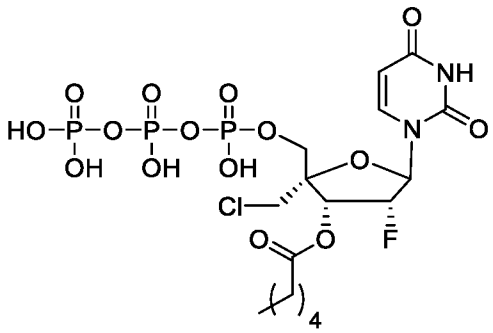


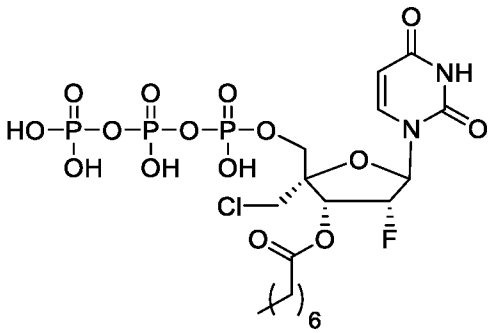


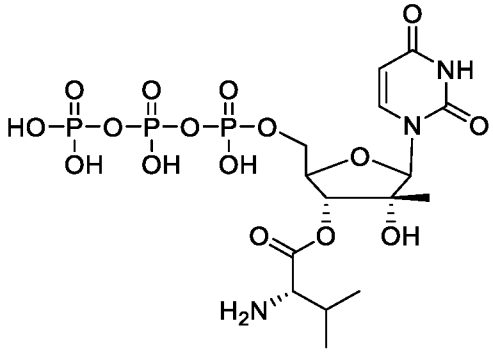


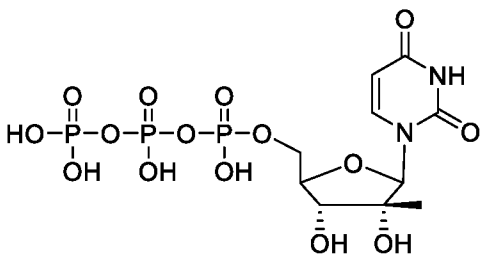


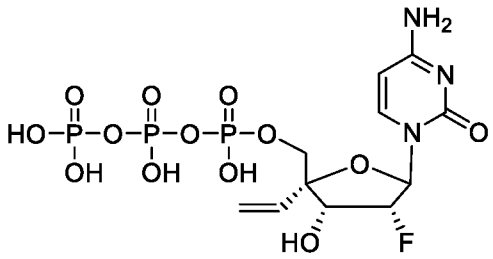


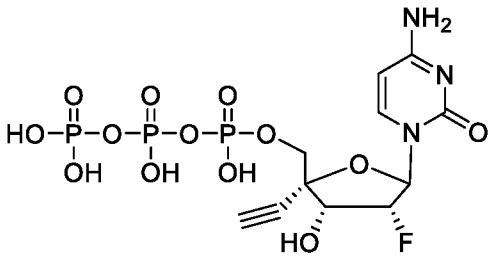


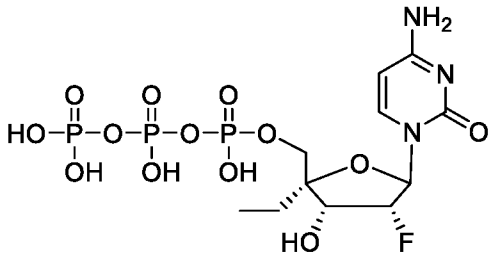


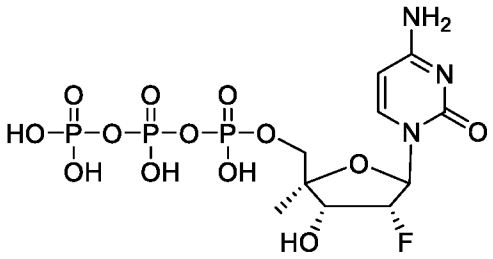


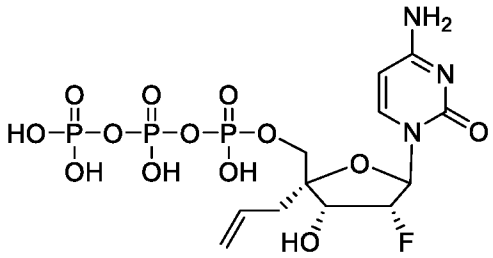


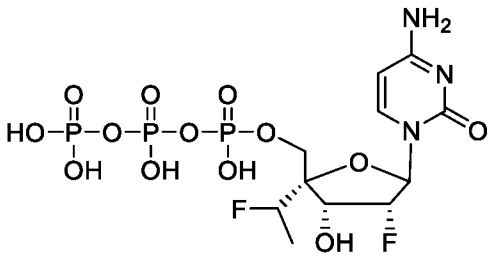


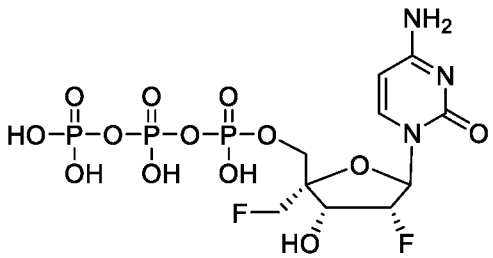


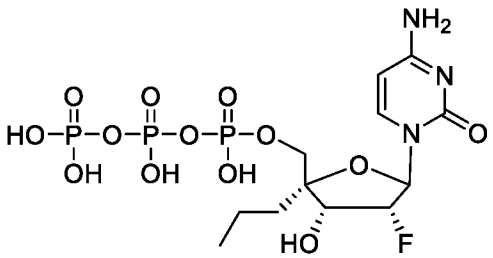


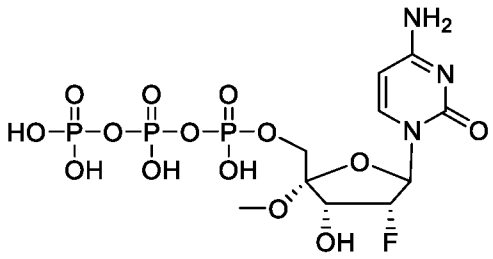


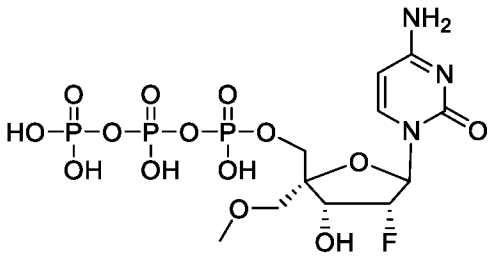


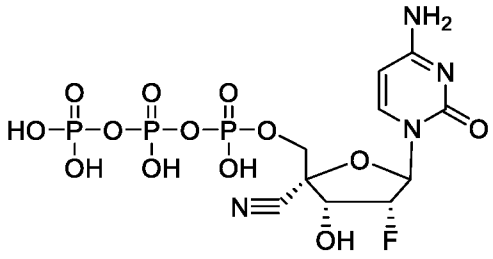


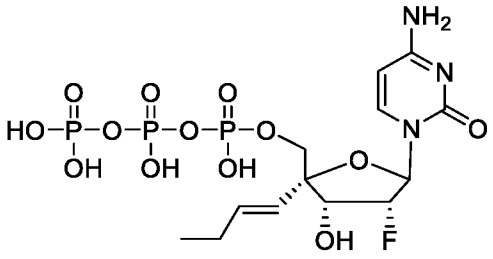


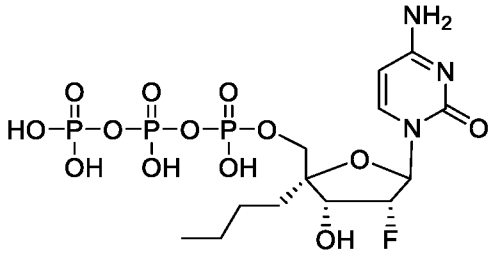
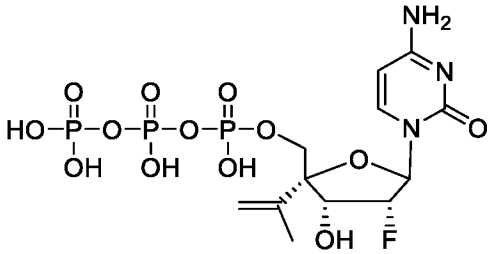
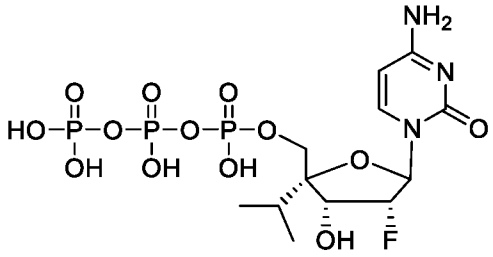
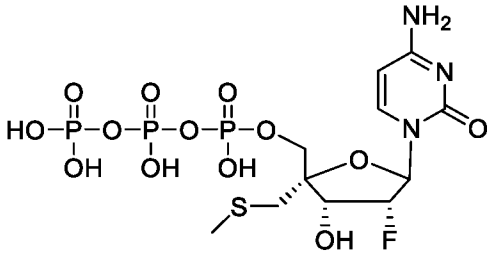
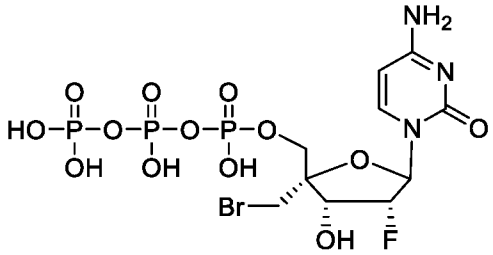
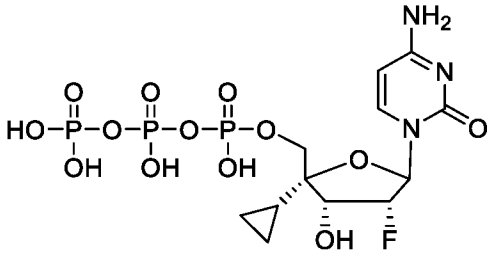




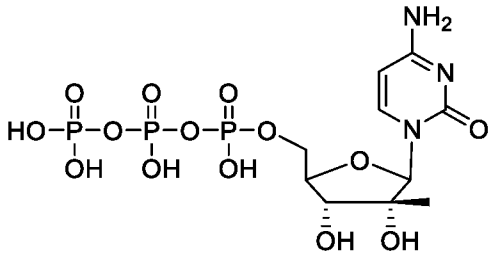
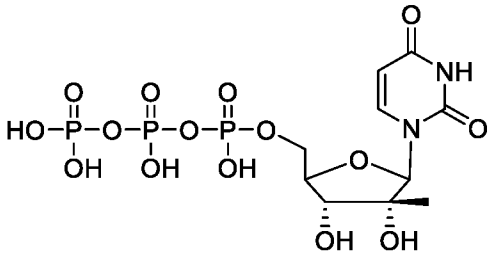
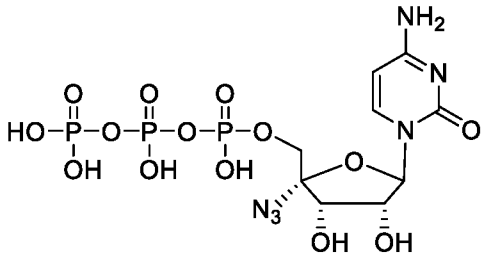
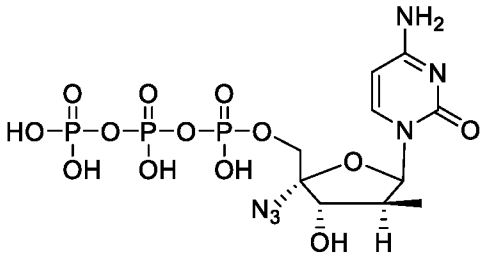


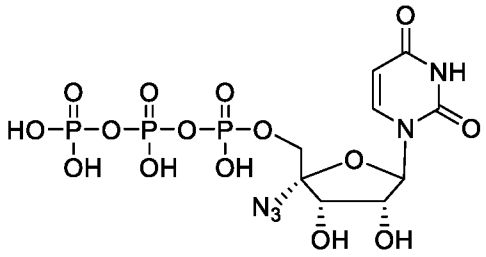


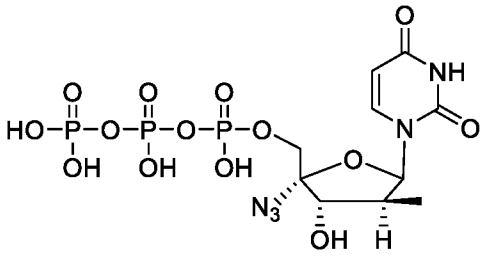


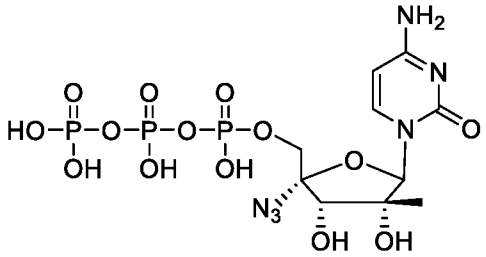







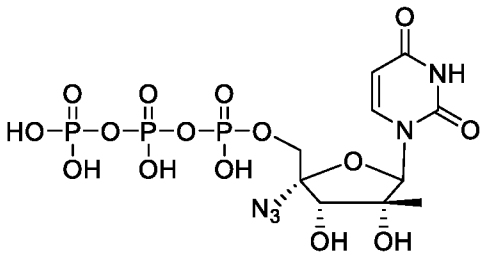
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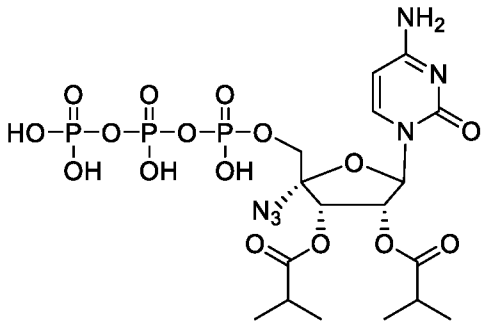





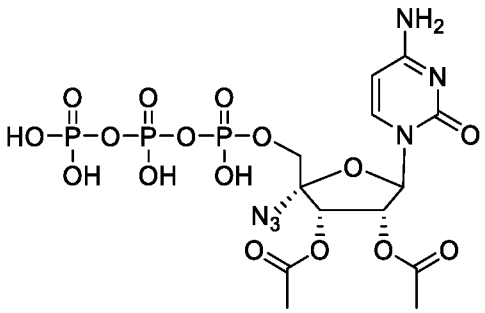


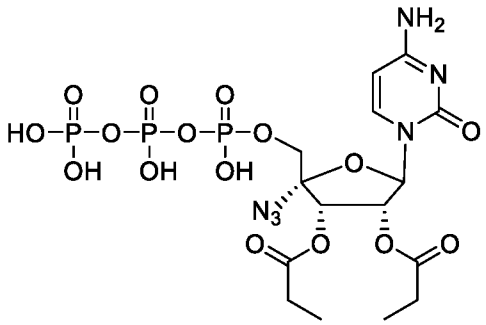


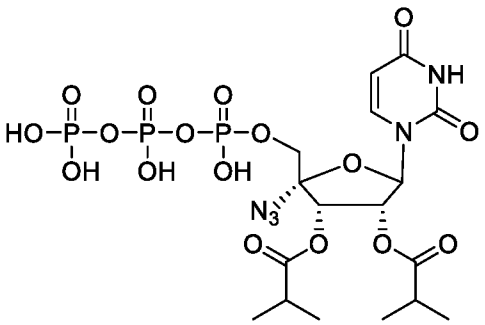


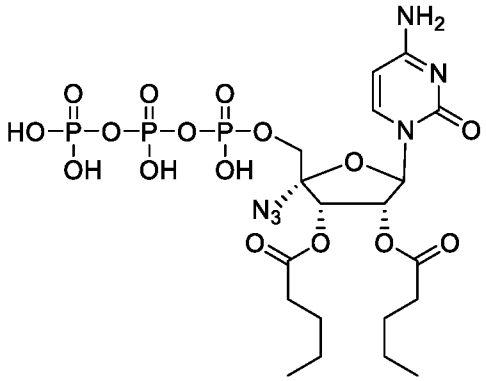


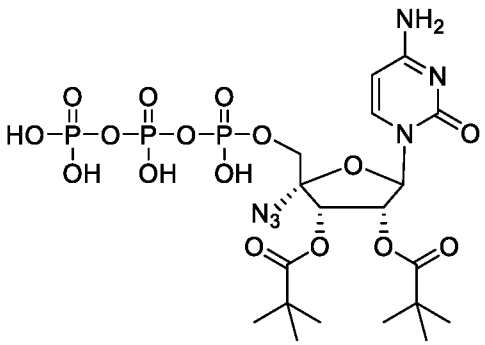


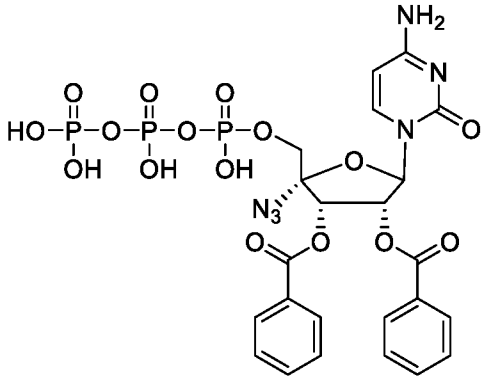




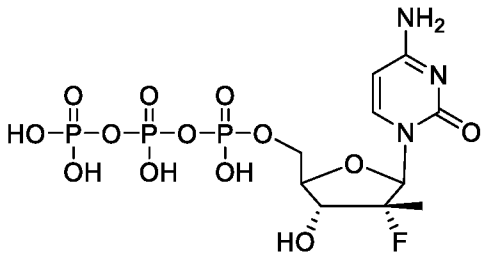
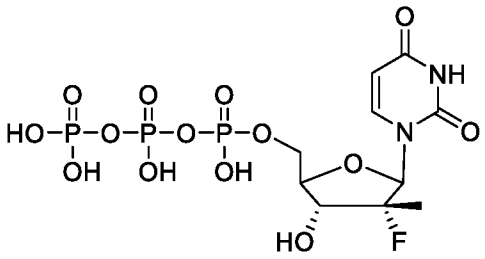
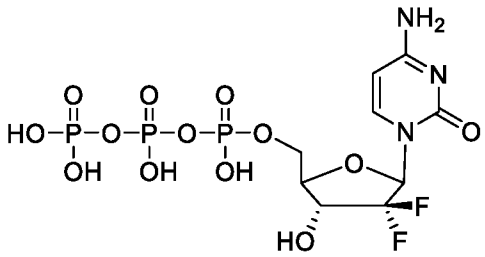
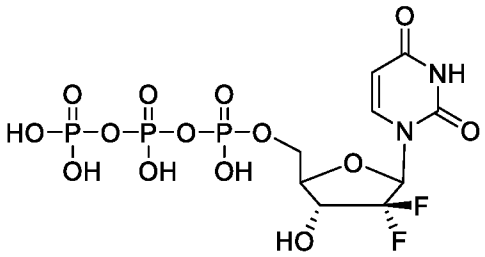
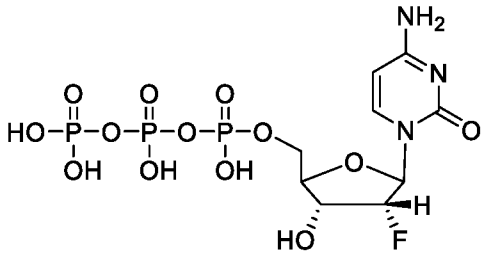
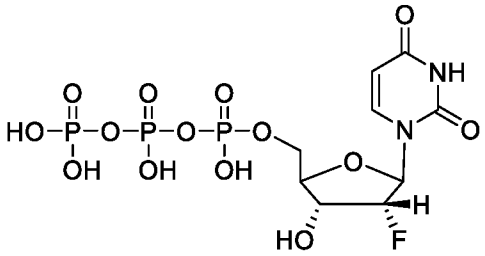
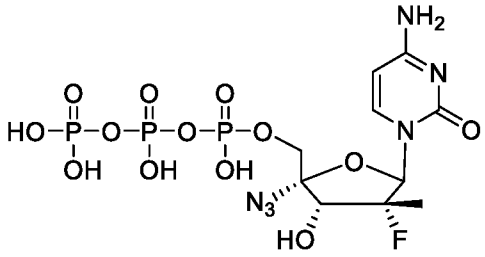
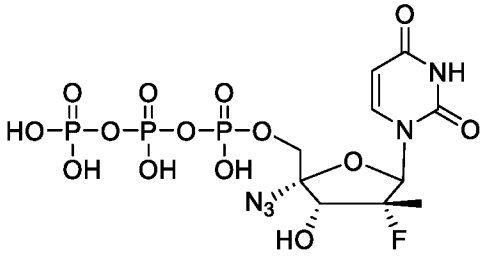


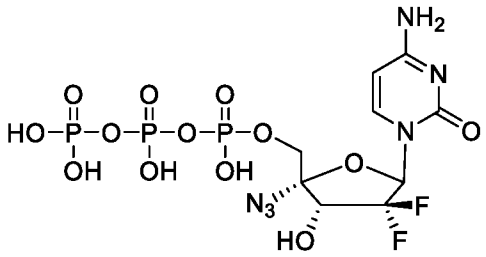
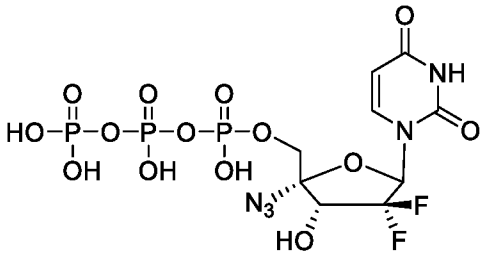
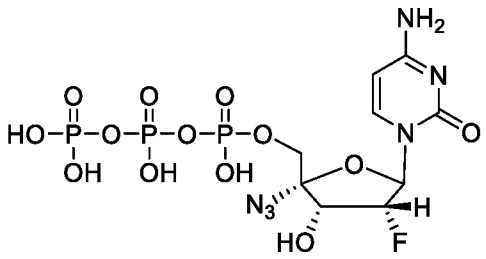
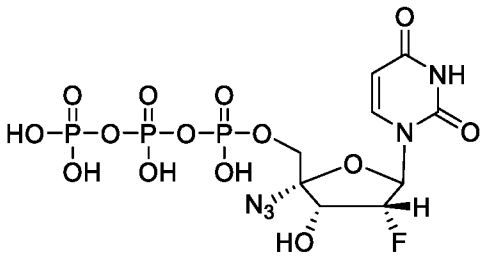
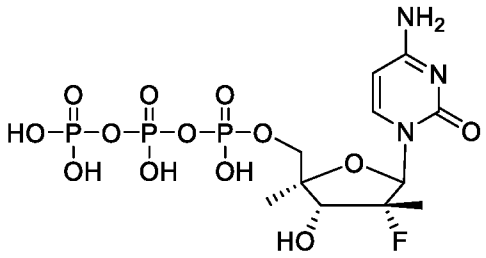
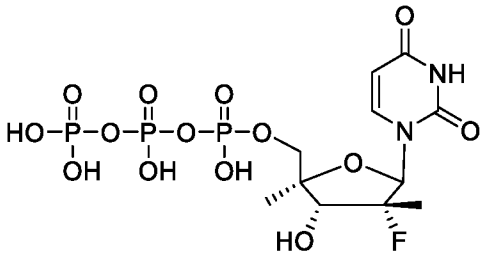
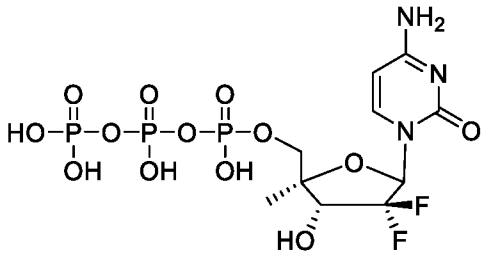
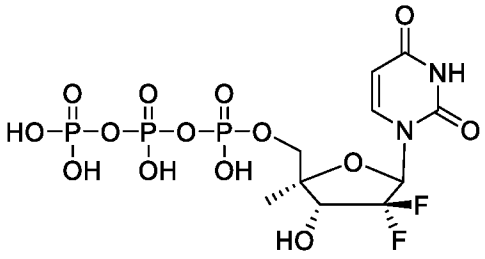
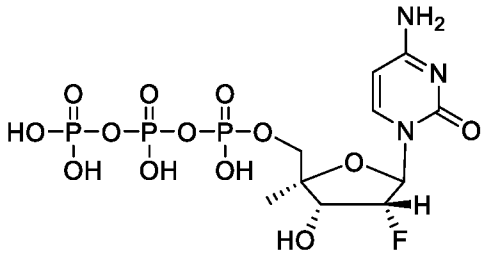
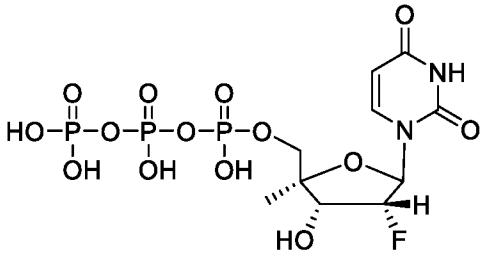
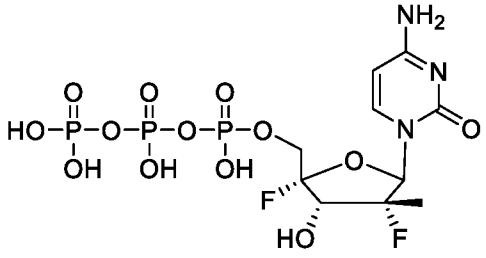
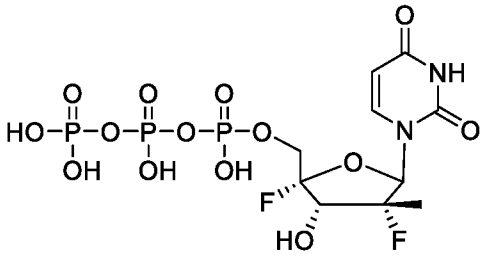




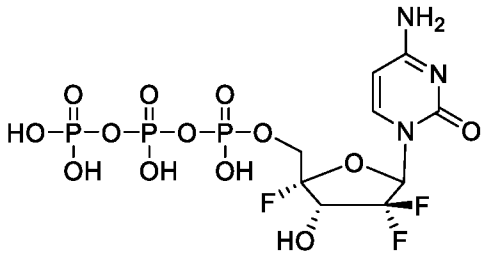


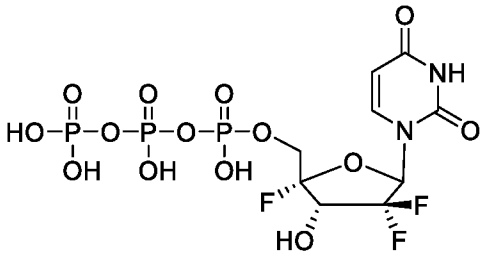
Additional non-limiting examples of a compound of Formula Ia or Formula Ib include:

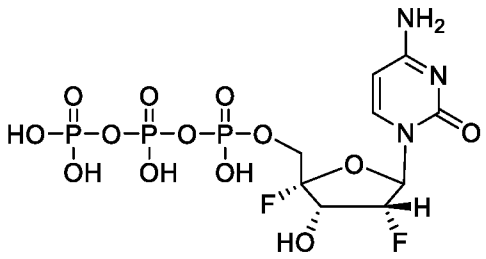









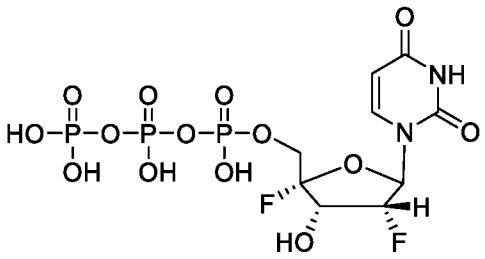













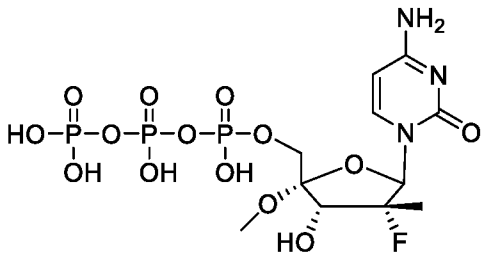
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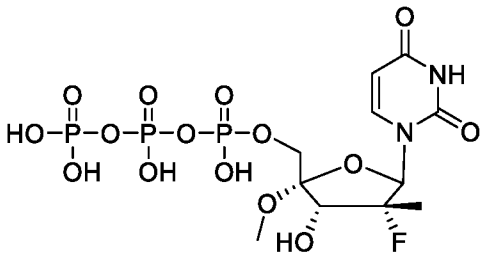


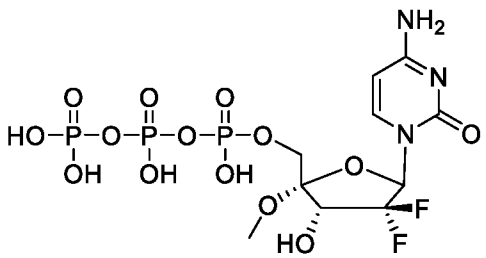


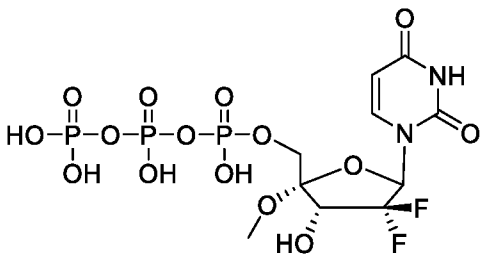


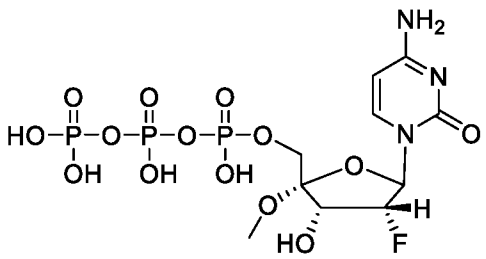


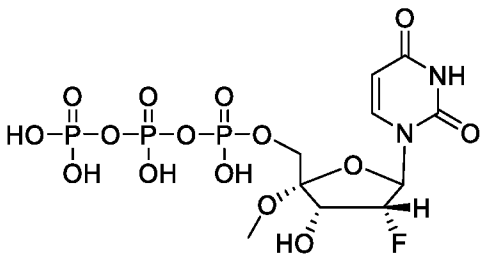


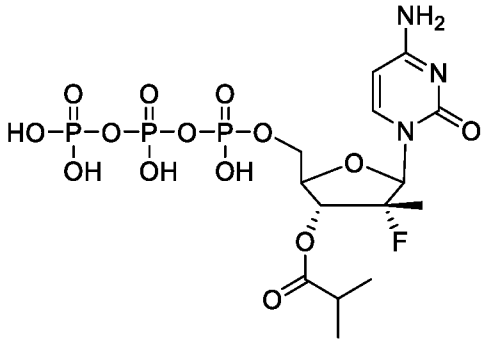


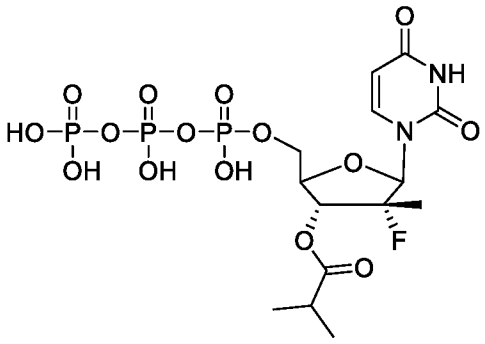




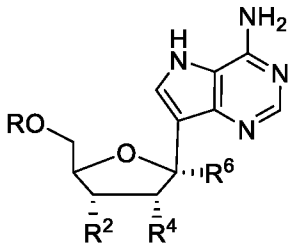




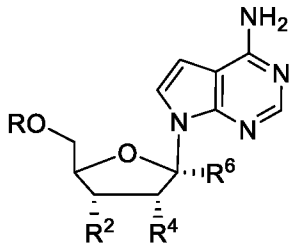




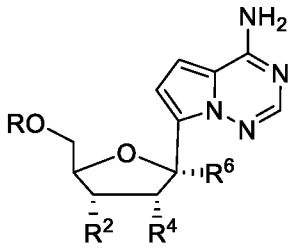
In one aspect, the active compound of the present invention is a compound of Formula Ic, Formula Id, or Formula Ie, which can be provided as a pharmaceutically acceptable salt:



Formula Ic

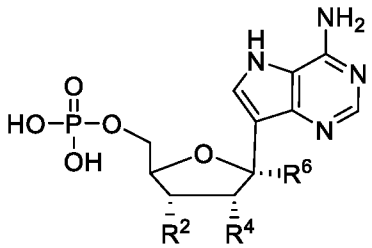


Formula Id

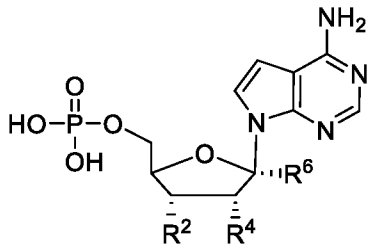


Formula Ie

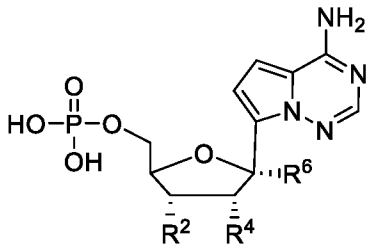
In one aspect, the active compound of the present invention is a compound of Formula Ic', Formula Id', or Formula Ie', which can be provided as a pharmaceutically acceptable salt:



Formula Ic'



Formula Id'



Formula Ie'

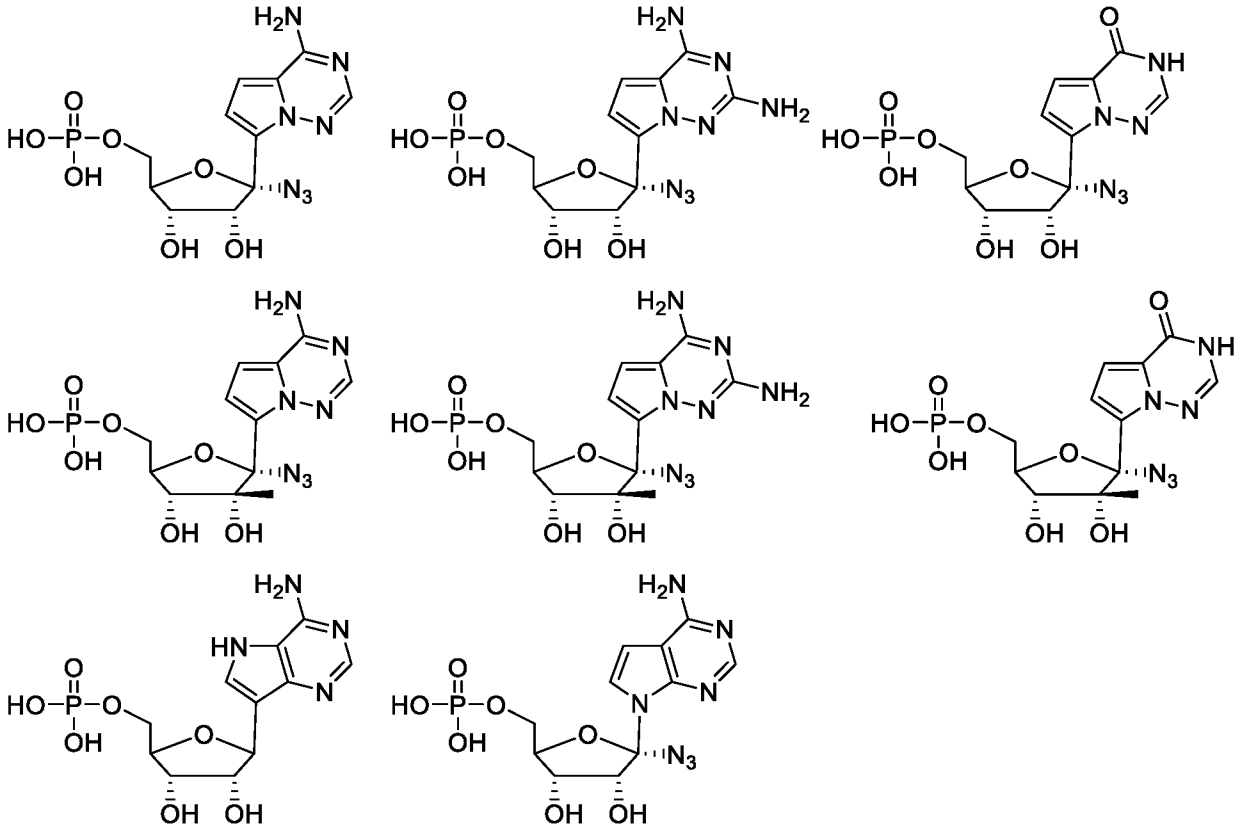
In certain embodiments of Formula Ic, Formula Id, or Formula Ie, R^6 is hydrogen.

In certain embodiments of Formula Ic, Formula Id, or Formula Ie, R^6 is cyano.

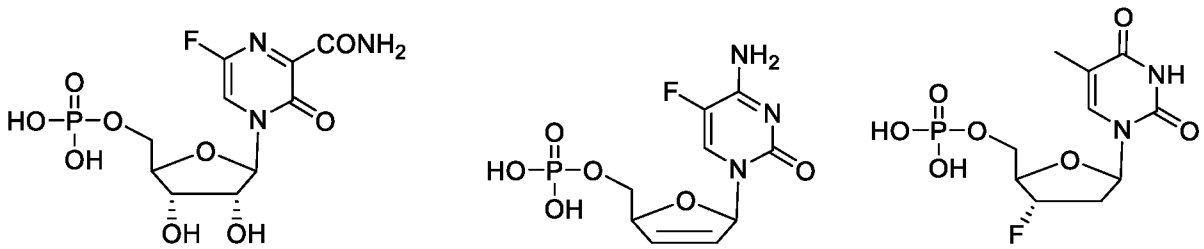
In certain embodiments of Formula Ic, Formula Id, or Formula Ie, R^2 is OH.

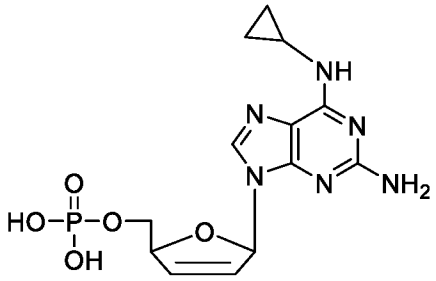
In certain embodiments of Formula Ic, Formula Id, or Formula Ie, R^4 is OH.

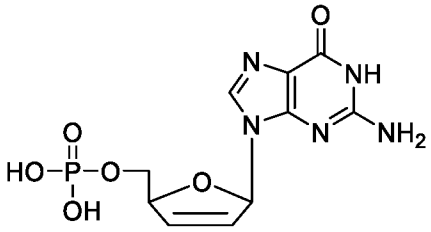
Non-limiting examples of compounds for use in the present invention include:

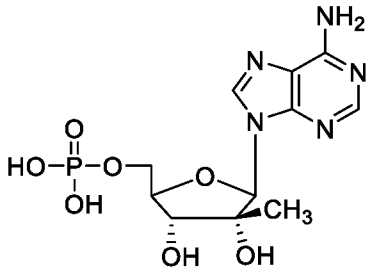


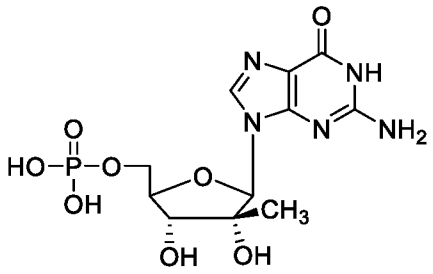
Additional non-limiting examples of compounds for use in the present invention include:

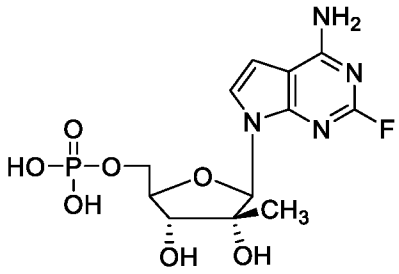


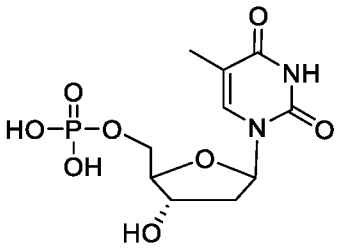


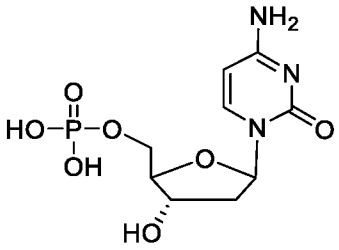


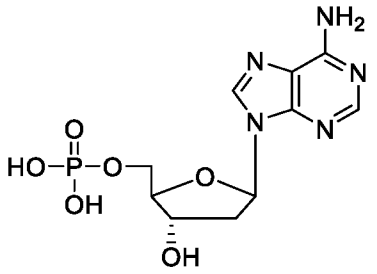


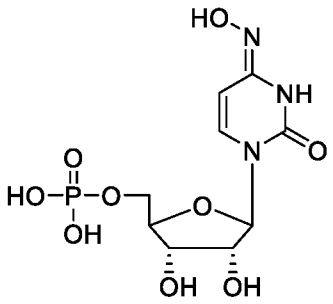




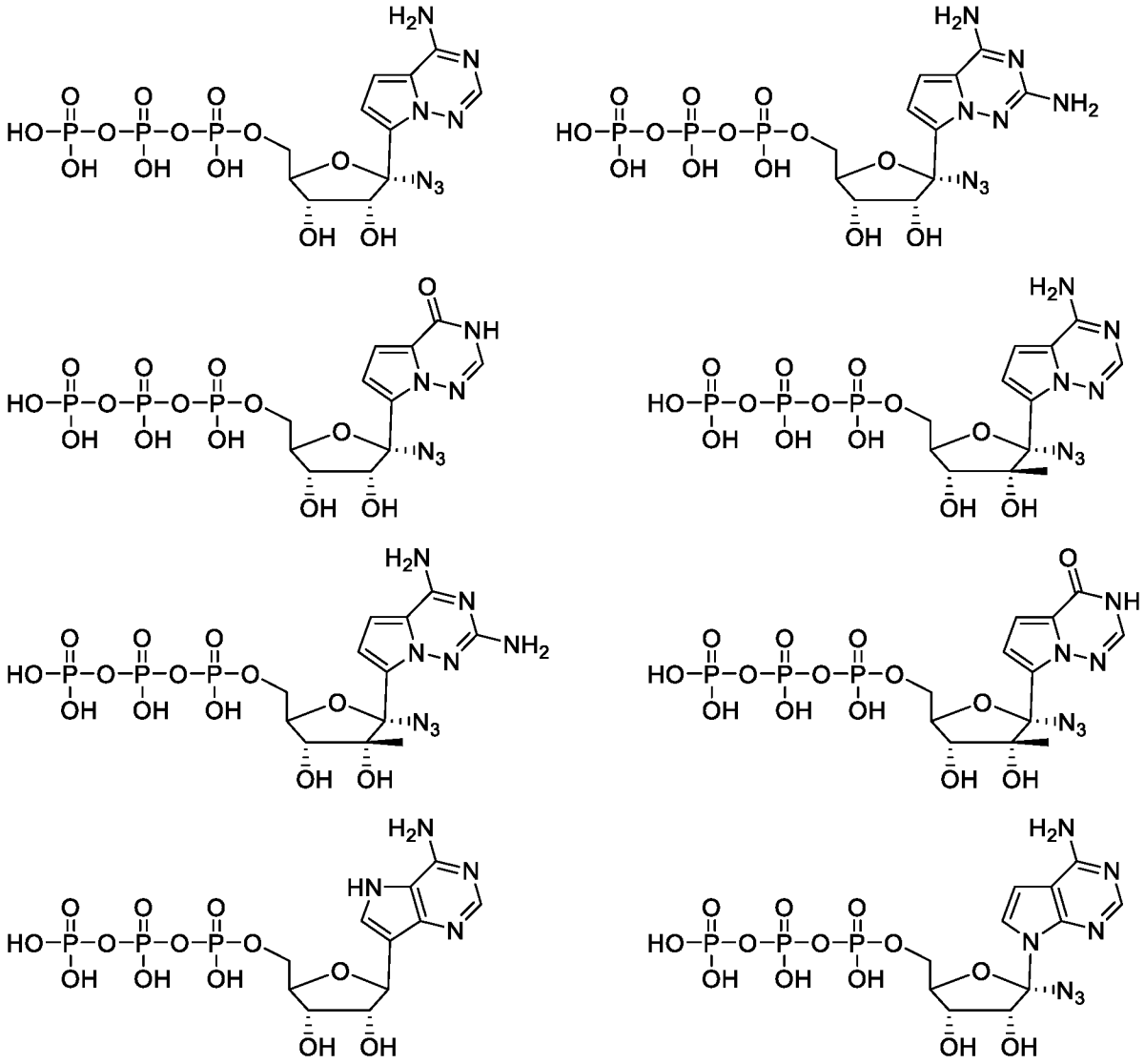




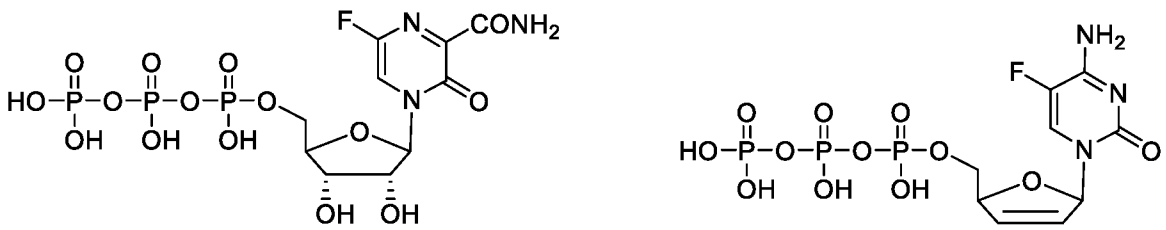


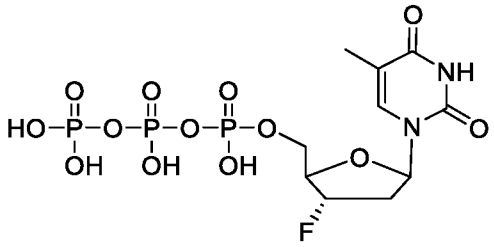


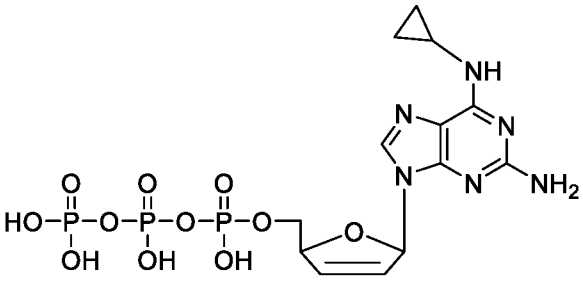
Additional non-limiting examples of a compounds for use in the present invention include:

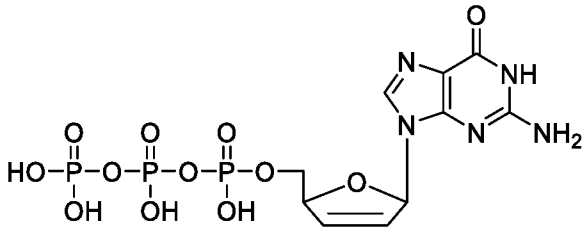


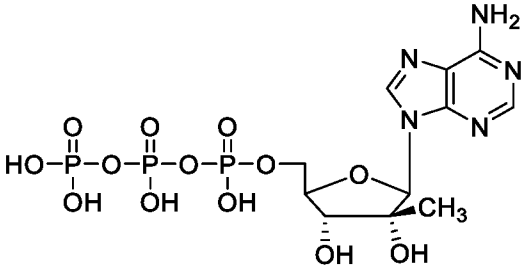
Additional non-limiting examples of compounds for use in the present invention include:

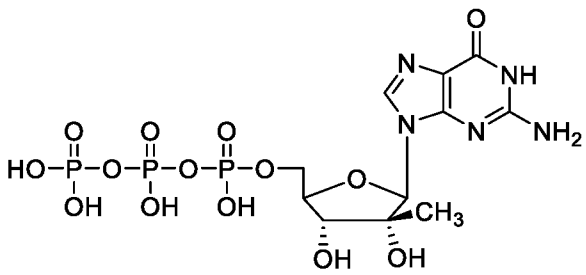


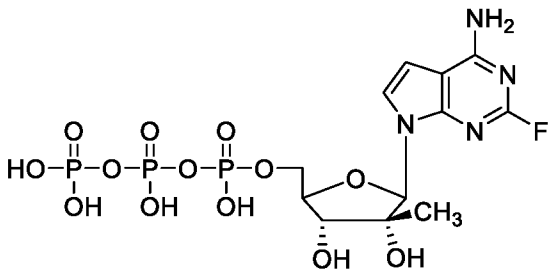




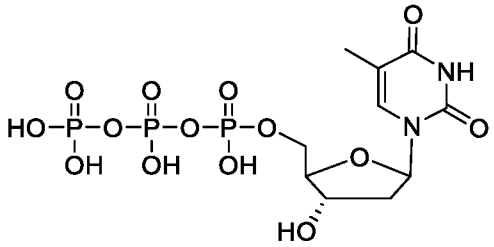


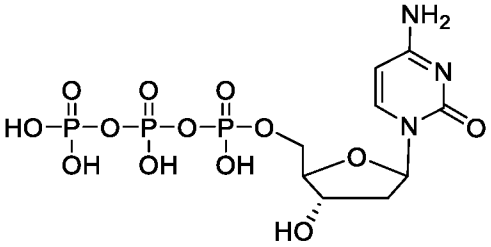


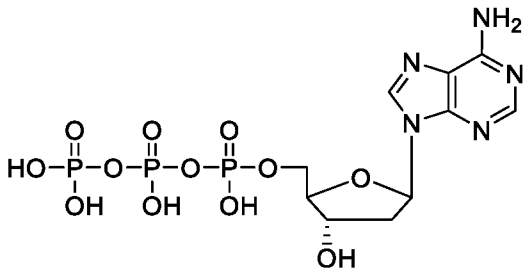


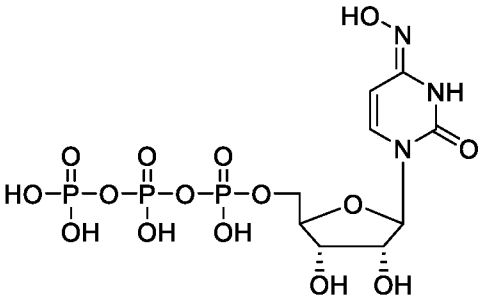


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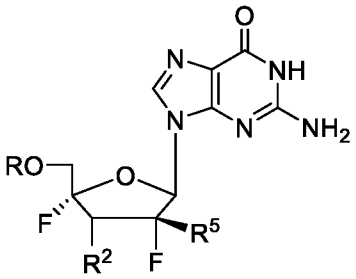




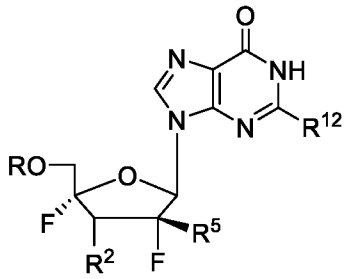




In certain aspects, the active compound of the present invention is a compound of Formula IIa or IIb, which can be provided as a pharmaceutically acceptable salt:



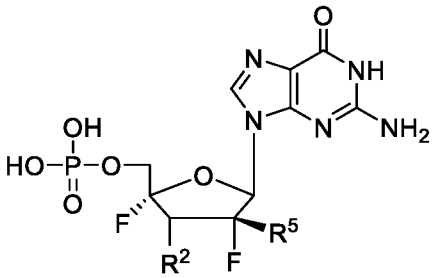
Formula IIa



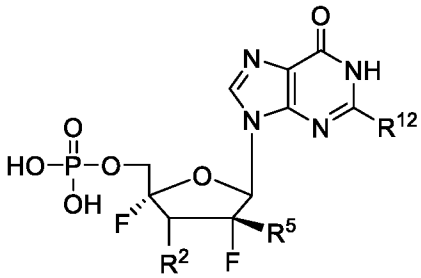
Formula IIb

wherein R, R^2, R^5, and R^{12} are as defined herein.

In other aspects, the active compound of the present invention is a compound of Formula IIa' or IIb', which can be provided as a pharmaceutically acceptable salt:



Formula IIa



Formula IIb

wherein R, R^2, R^5, and R^{12} are as defined herein.

In certain embodiments of Formula IIa or Formula IIb, R^5 is CH_3, CH_2F, CHF_2, CF_3, or ethynyl.

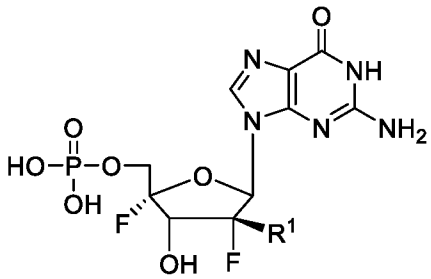
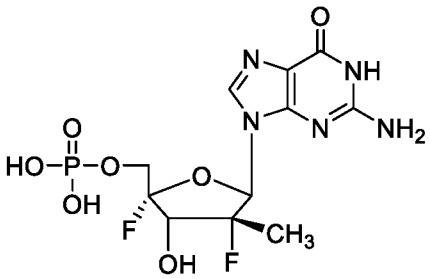
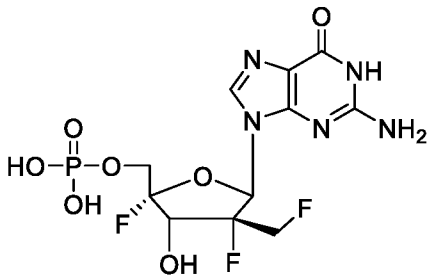
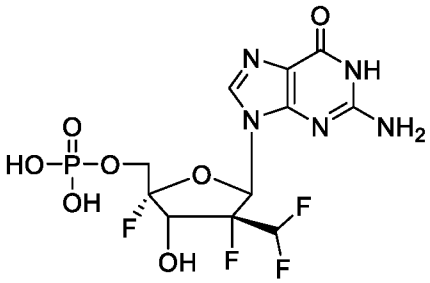
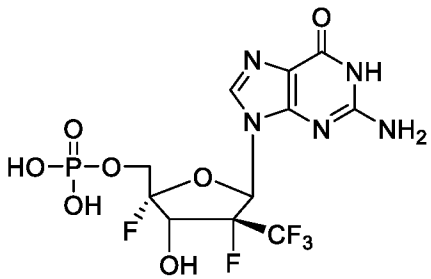
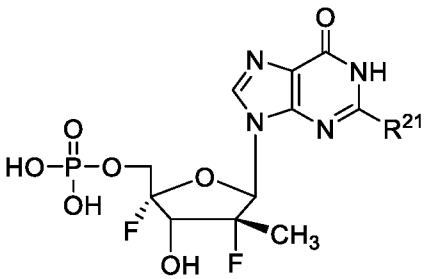
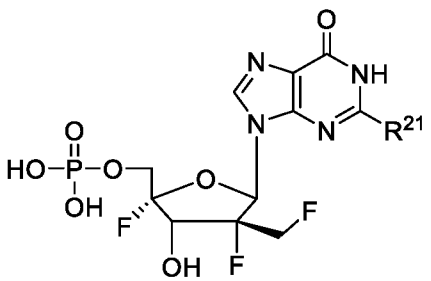
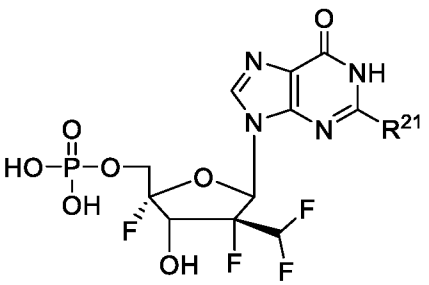
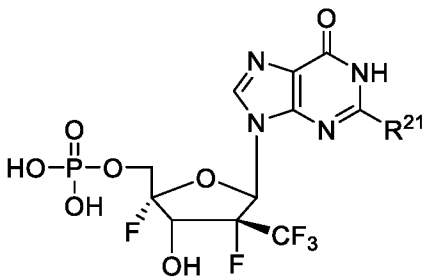
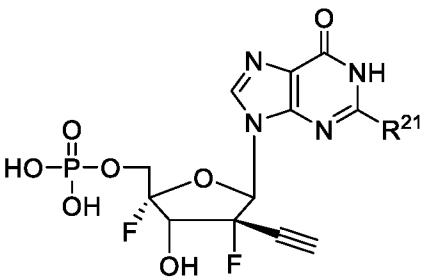
In certain embodiments of Formula IIa or Formula IIb, R^2 is hydrogen.

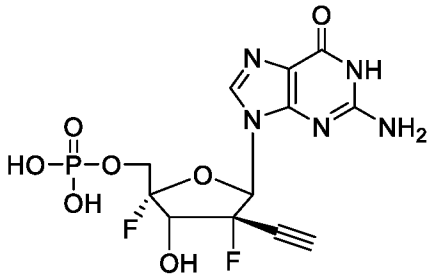
In certain embodiments of Formula IIa or Formula IIb, R^{12} is NR^7R^8, Cl, Br, F, N_3, -$NH(O)CH_3$, CN, $CONH_2$, SO_2NH_2, or CF_3.

In certain embodiments of Formula IIa or Formula IIb, R^7 is hydrogen, $C_{1\text{-}8}$alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, and pentyl), or -(C_0-C_2alkyl)(cycloalkyl).

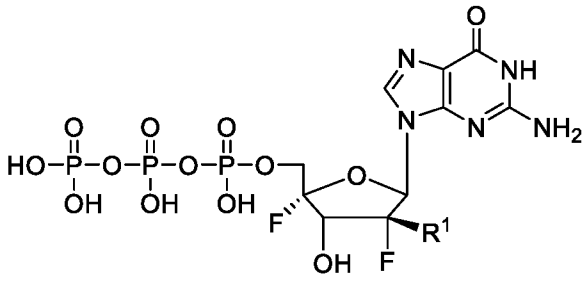
In certain embodiments of Formula IIa or Formula IIb, R^8 is $C_{1\text{-}8}$alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, and pentyl), -(C_0-C_2alkyl)(cycloalkyl), or -$C(O)R^{10C}$.

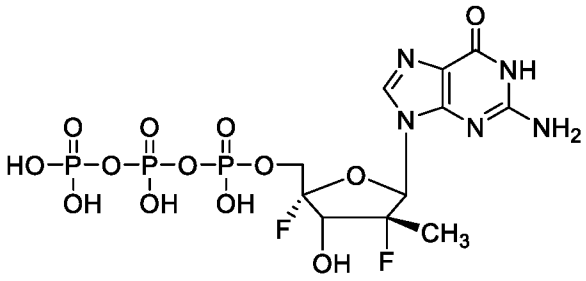
In certain embodiments the compound of the present invention is selected from:

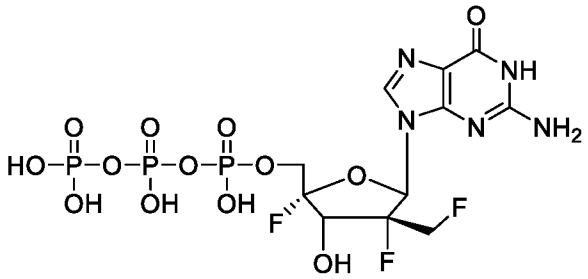











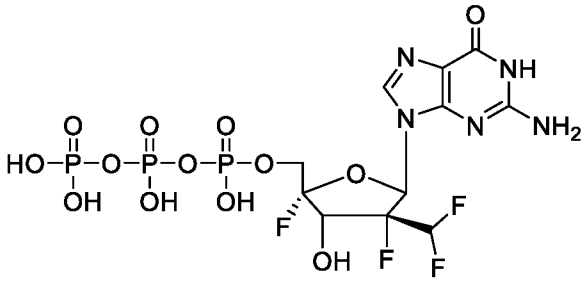


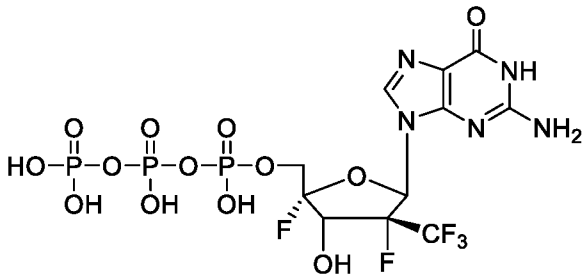
Non-limiting examples of a compound of Formula IIa or Formula IIb include:

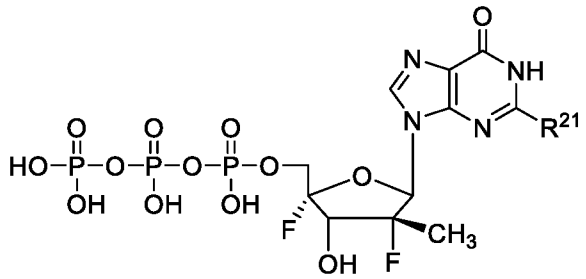


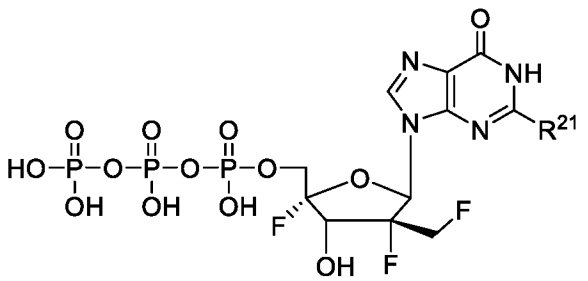


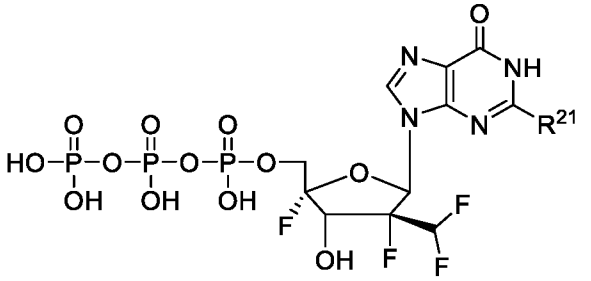


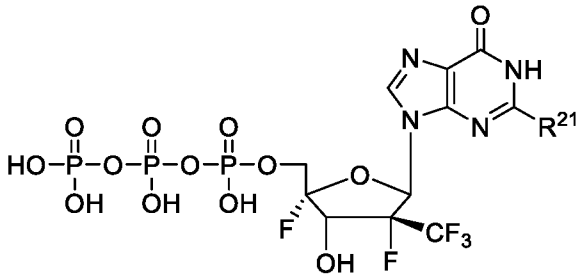


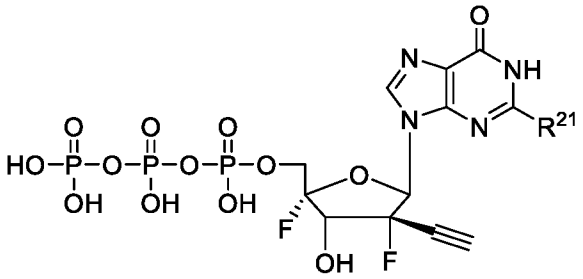


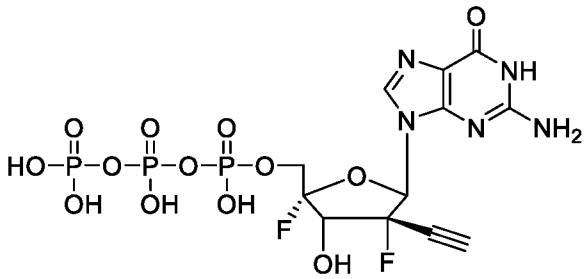




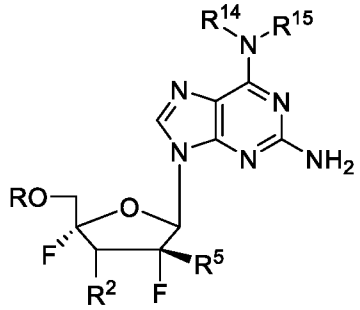




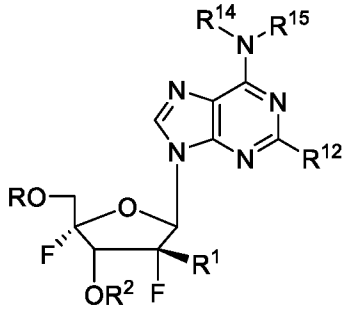




In certain aspects, the active compound of the present invention is a compound of Formula IIc or Formula IId, which can be provided as a pharmaceutically acceptable salt:



Formula IIc



Formula IId

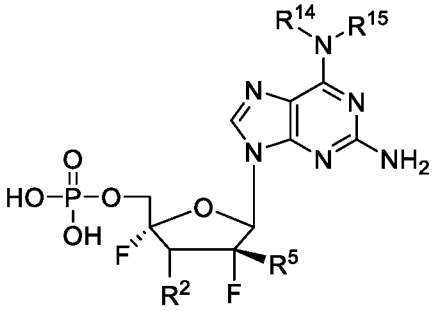
wherein R^2 and R^5 are as defined herein:

R^{14} is hydrogen, $C_{1\text{-}5}$alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, and pentyl) or -(C_0-C_2alkyl)(cycloalkyl); and

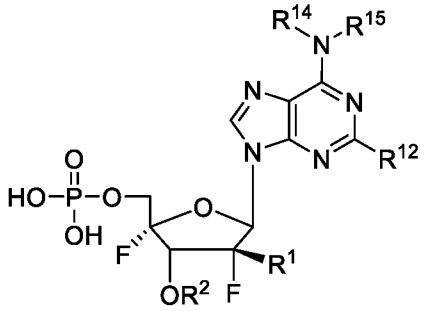
R^{15} is hydrogen, $C_{1\text{-}5}$alkyl (including methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, and pentyl), CHF_2, CH_2F, CF_3, -(C_0-C_2alkyl)(cycloalkyl), -$C(O)R^{10C}$; or

R^{14} and R^{15} together with the nitrogen to which they are bonded can form a heterocycle.

In other aspects, the active compound of the present invention is a compound of Formula IIc' or Formula IId', which can be provided as a pharmaceutically acceptable salt:

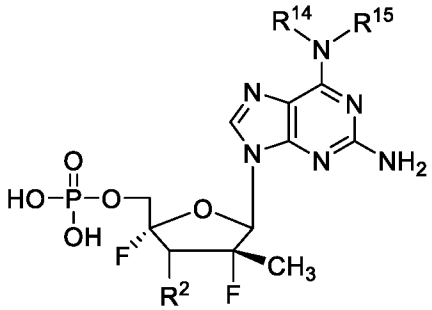


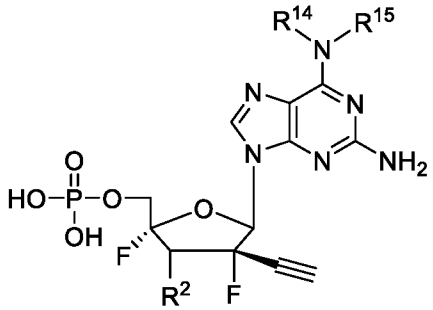
Formula IIc'

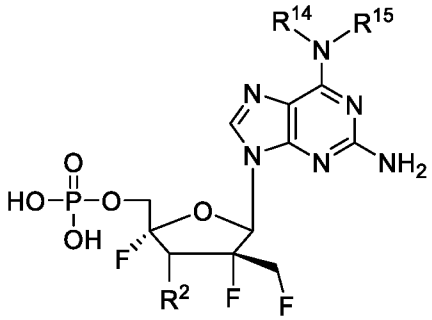


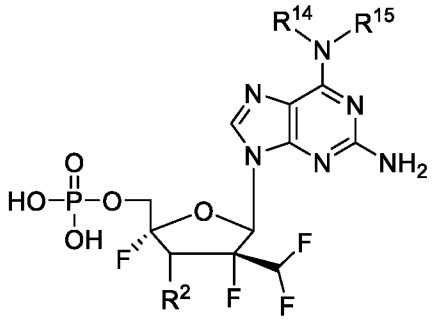
Formula IId'

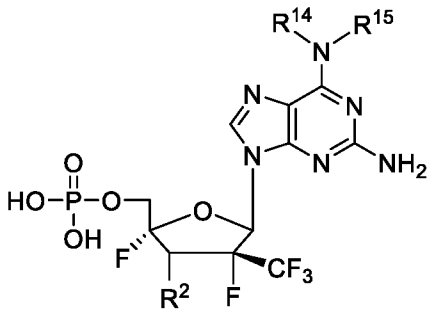
Non-limiting examples of a compound of Formula IIc' or Formula IId' include:

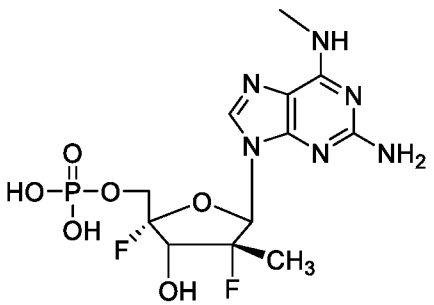


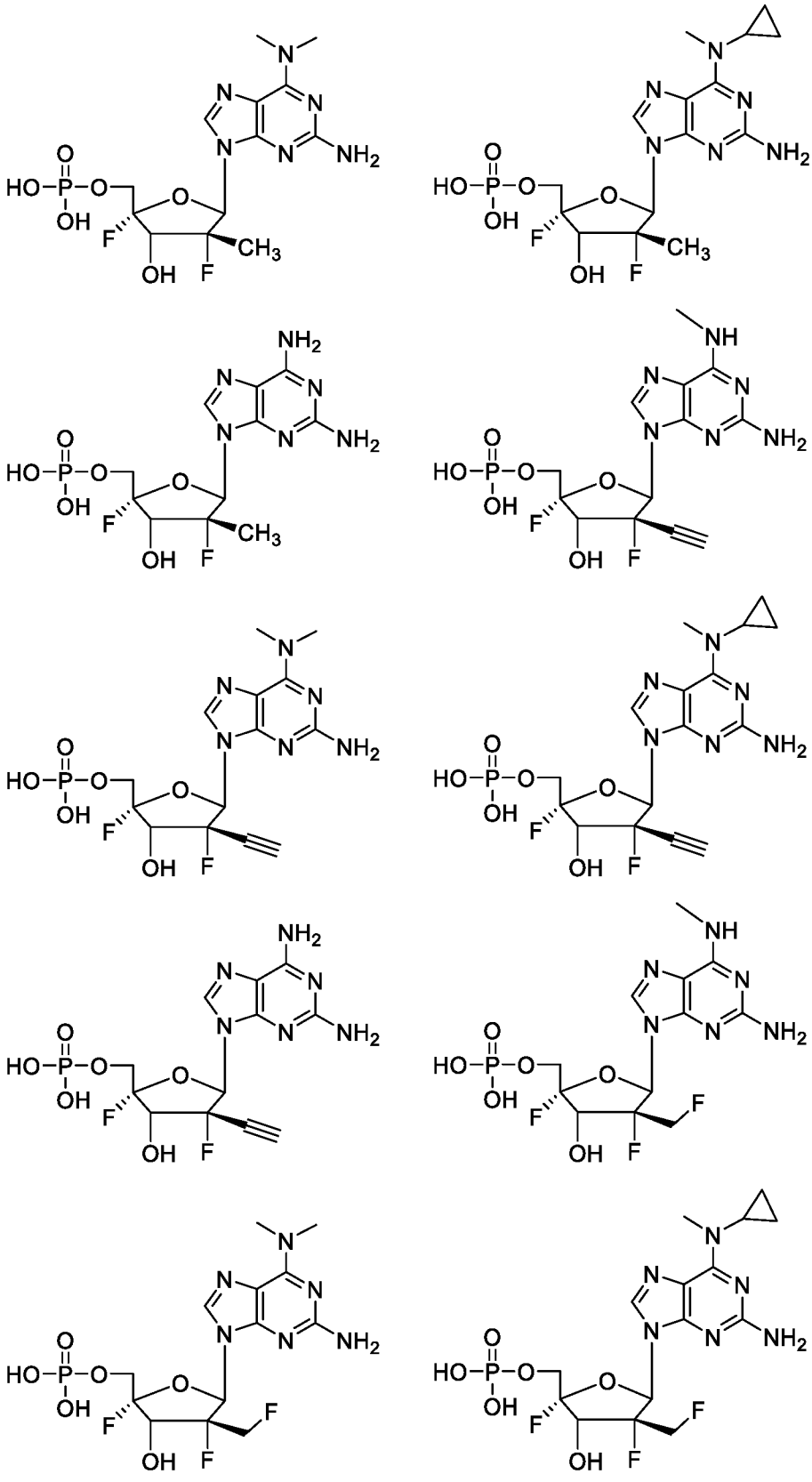


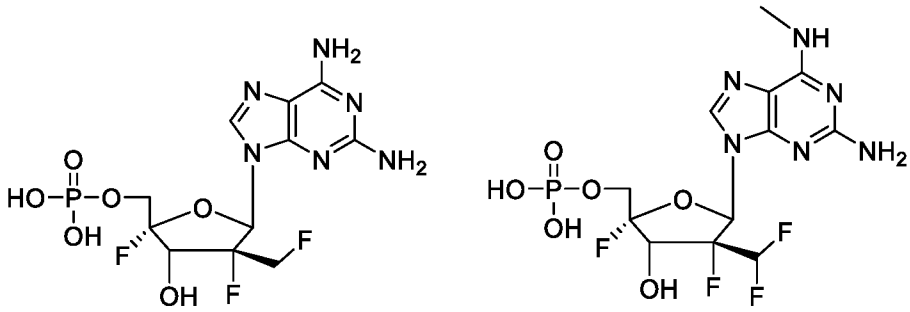


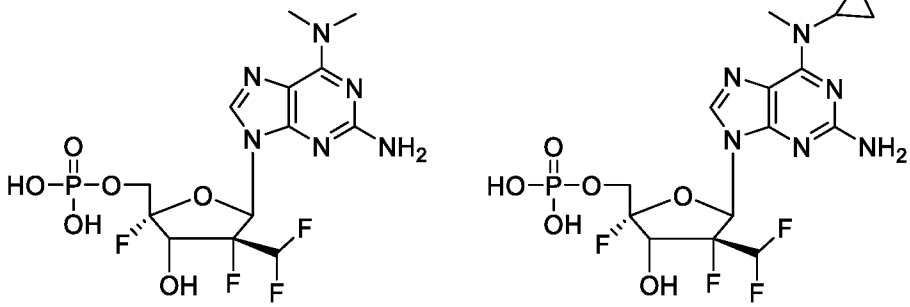


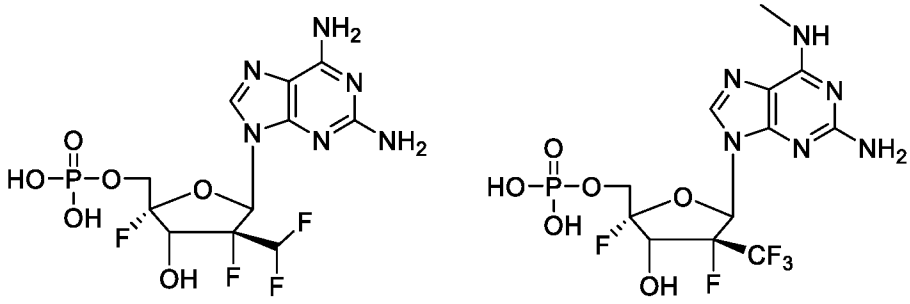


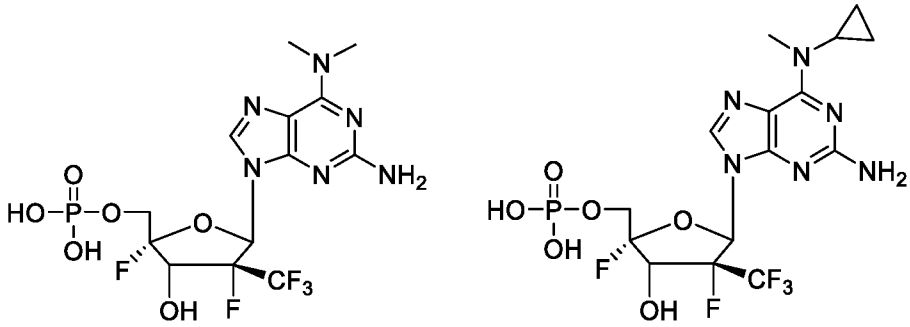


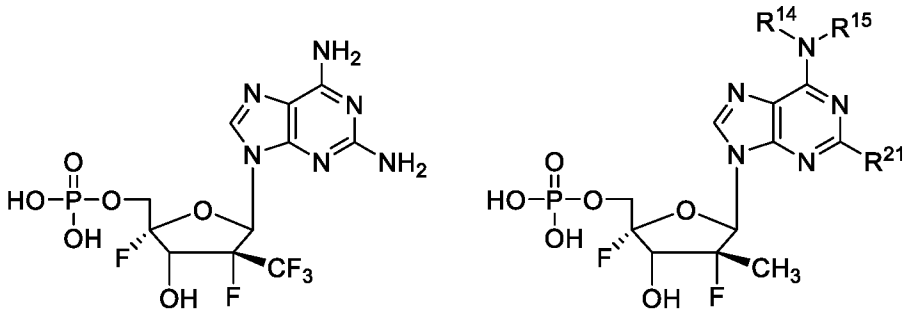


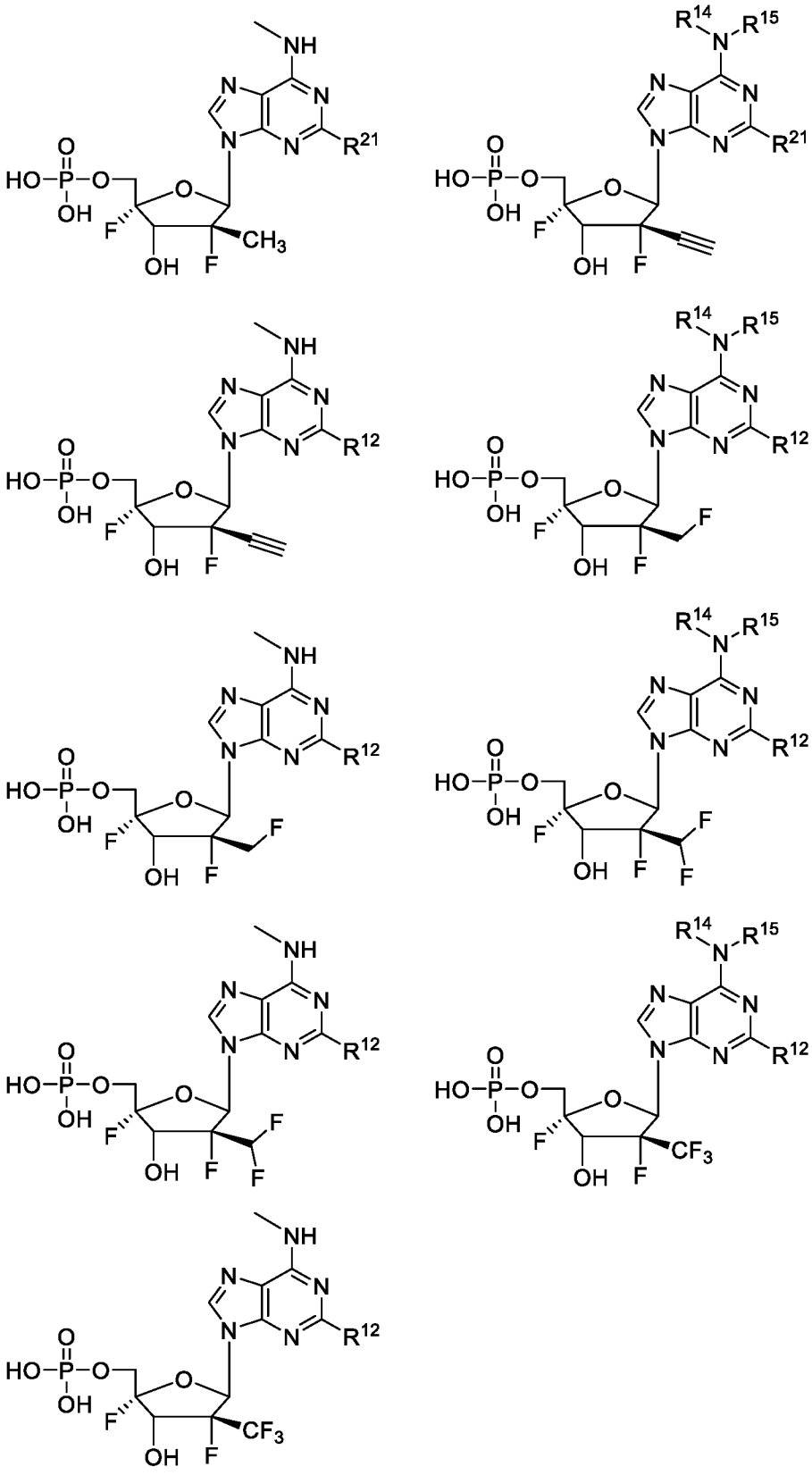




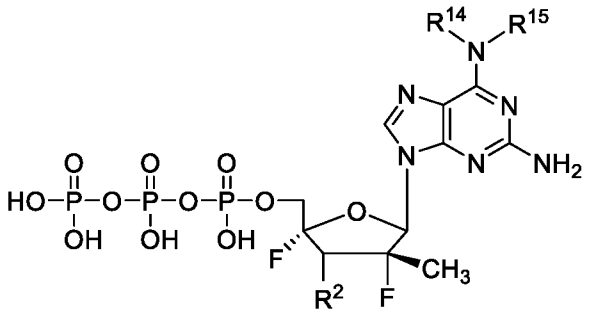


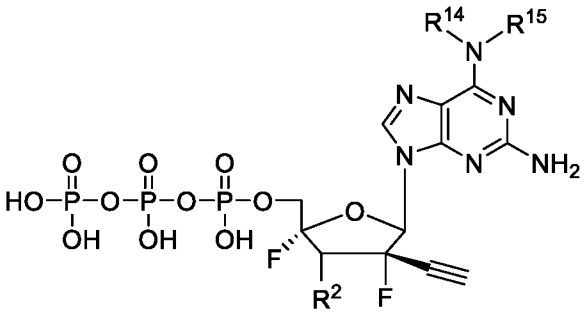


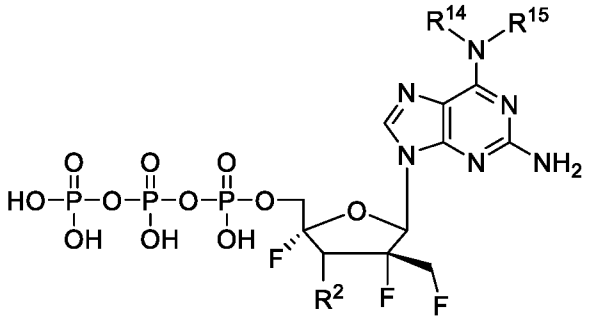


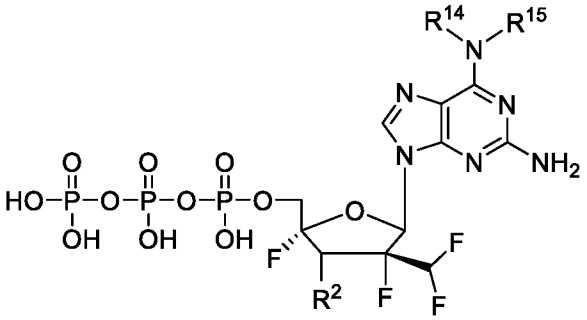


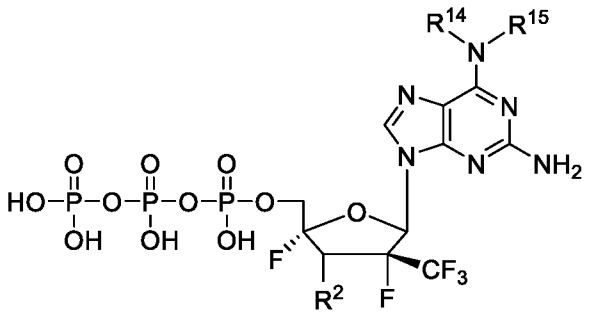
Non-limiting examples of a compound of Formula IIc or Formula IId include:

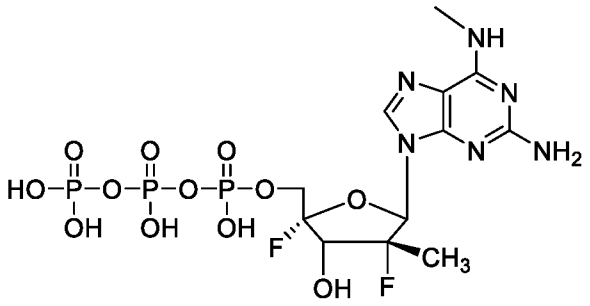


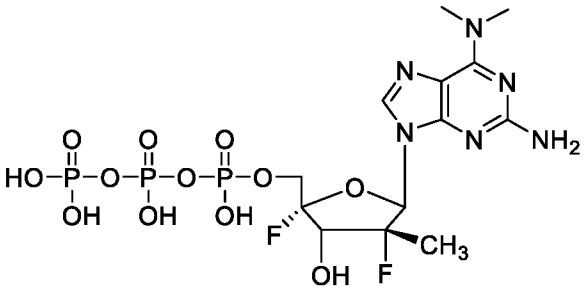


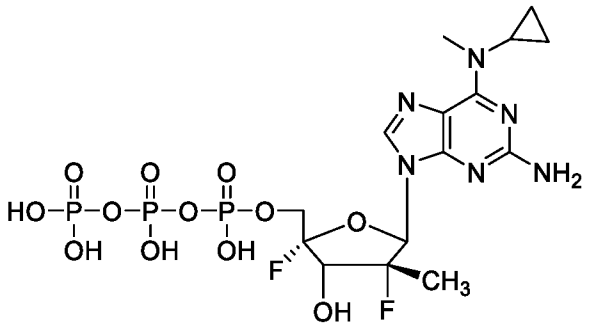


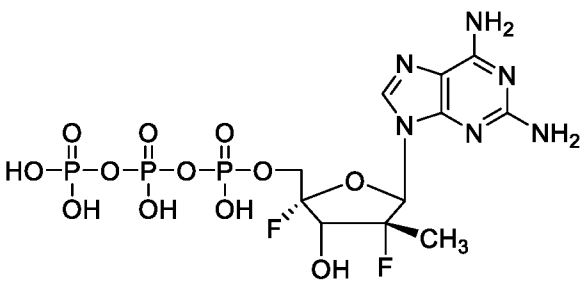




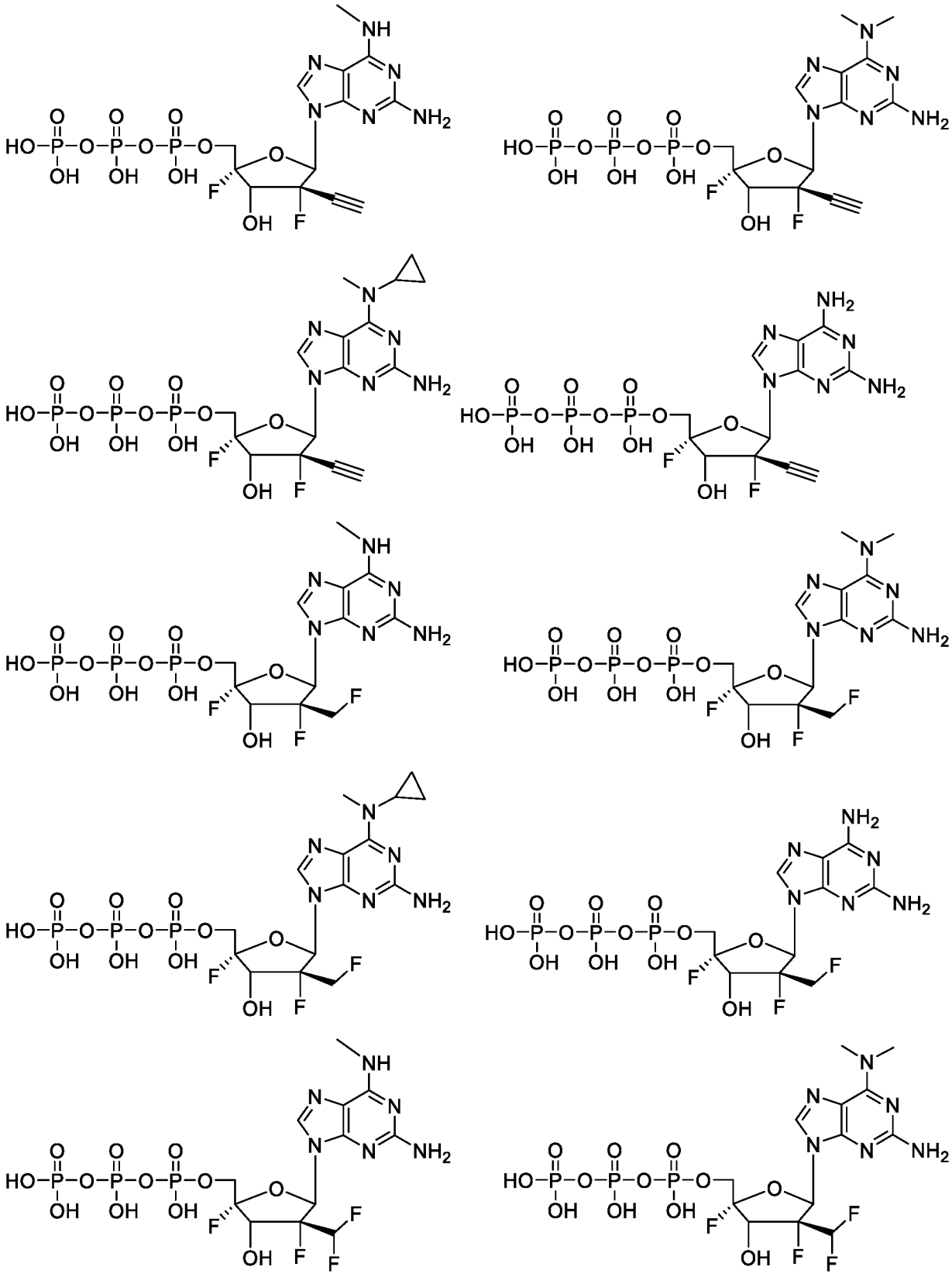


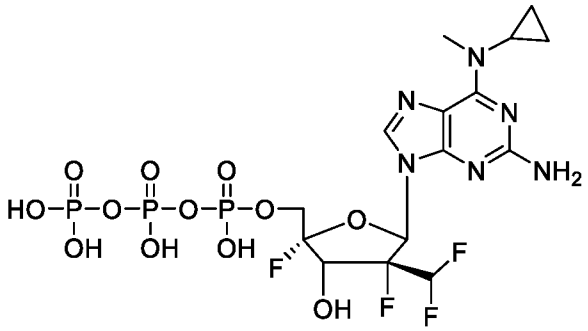


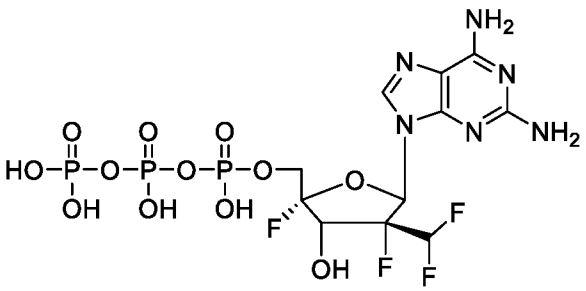


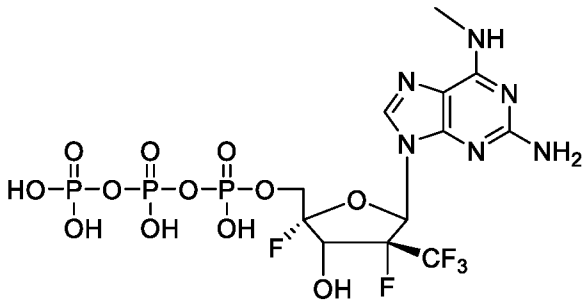


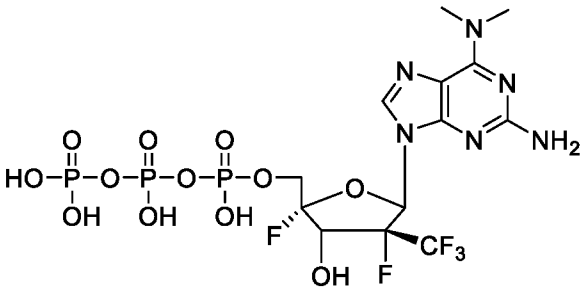
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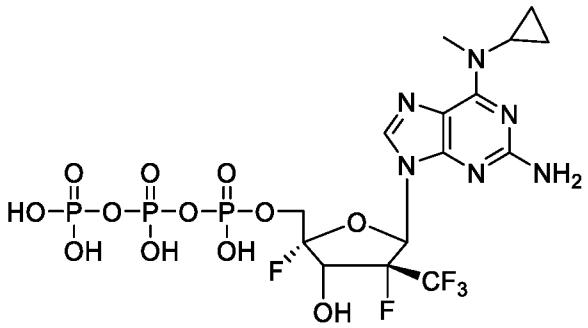


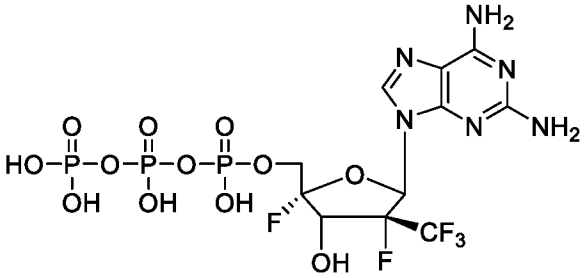


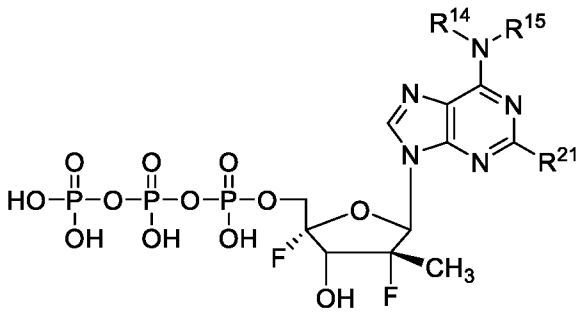


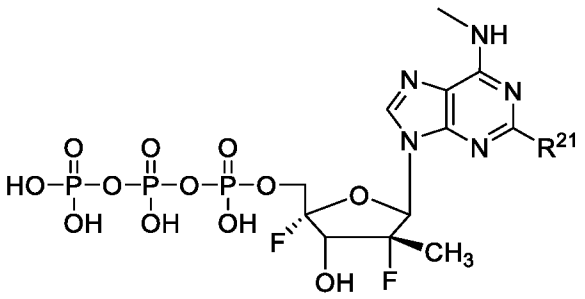


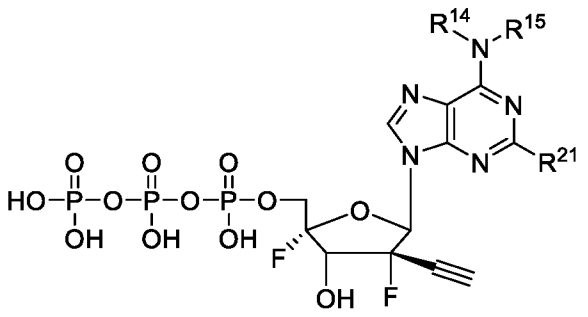


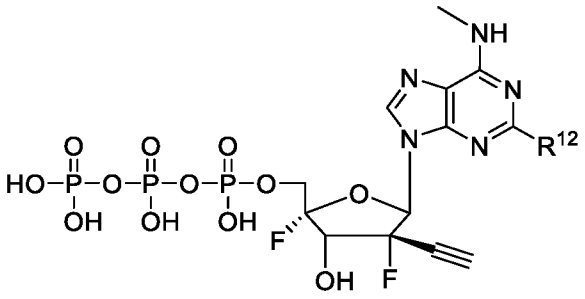




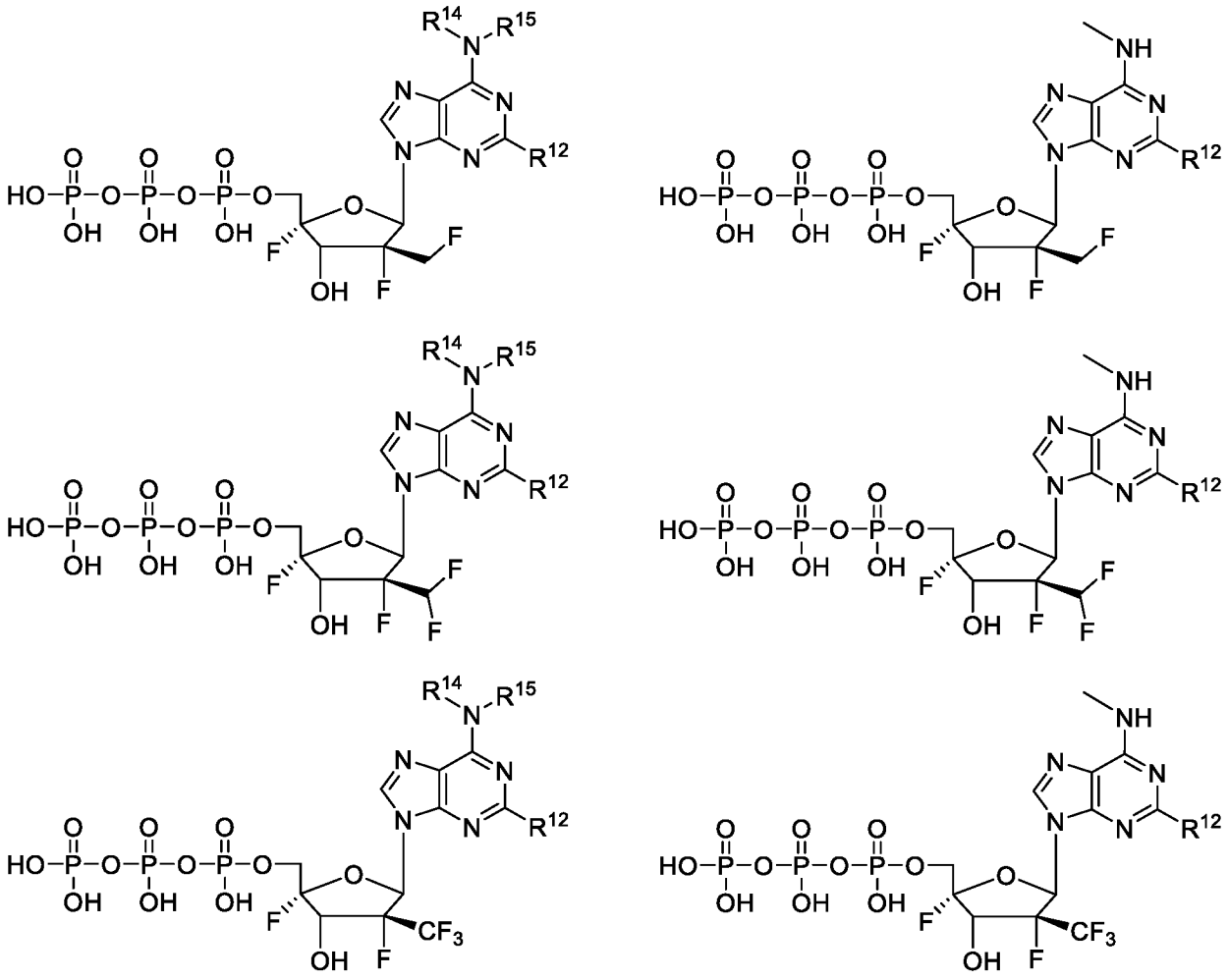




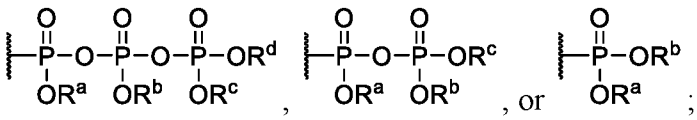




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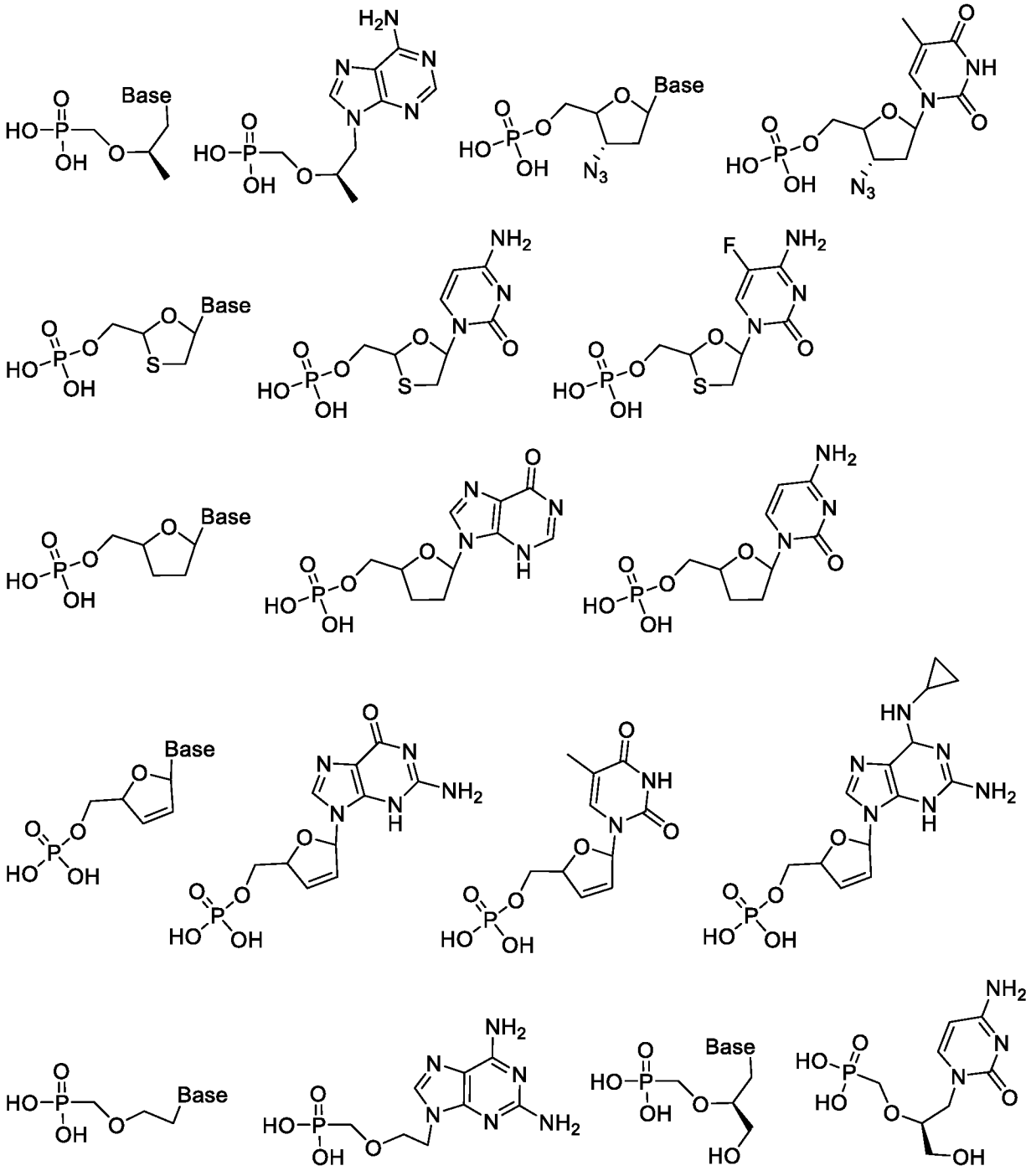


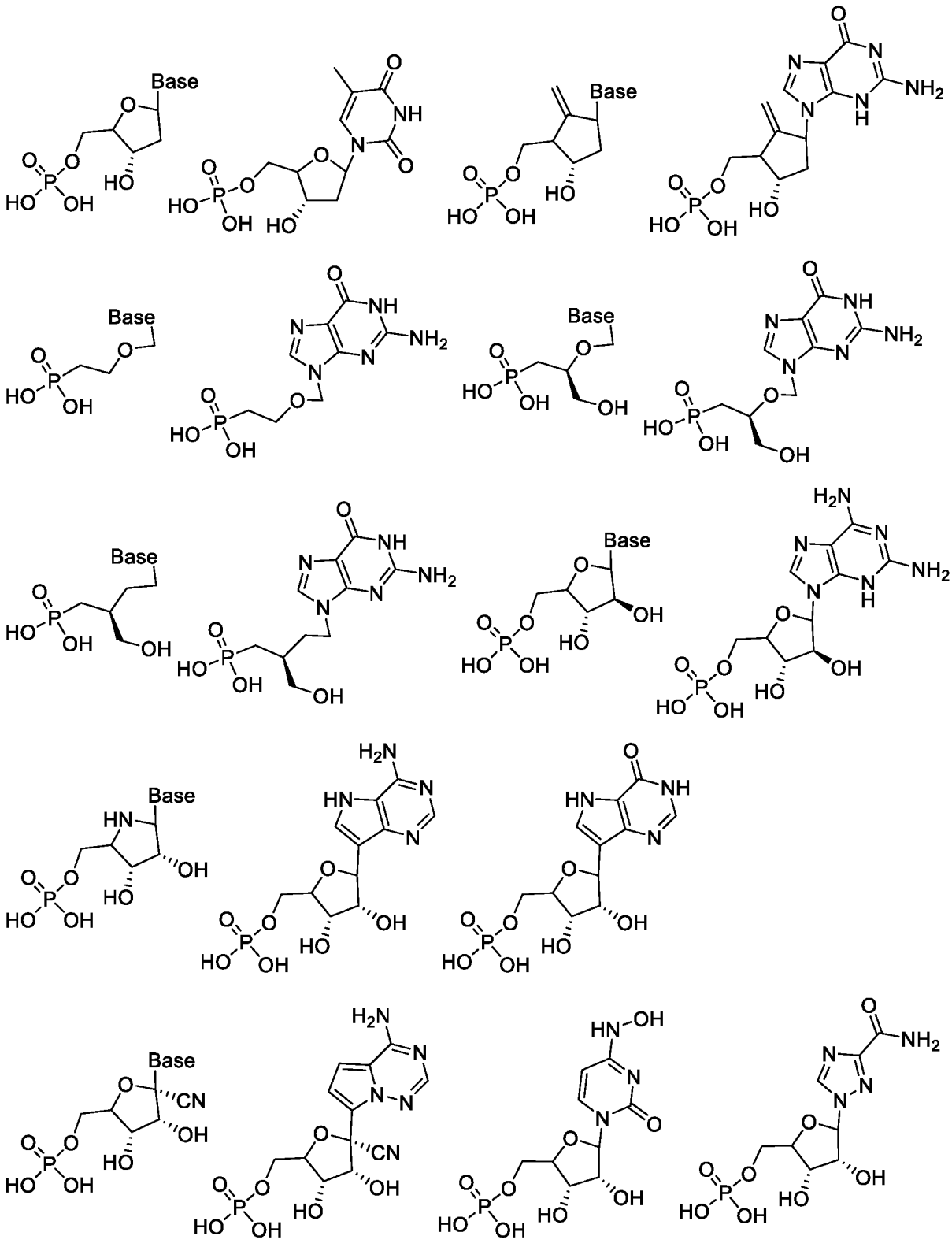
In an alternative embodiment in any of the Formulas described herein, R is selected from:

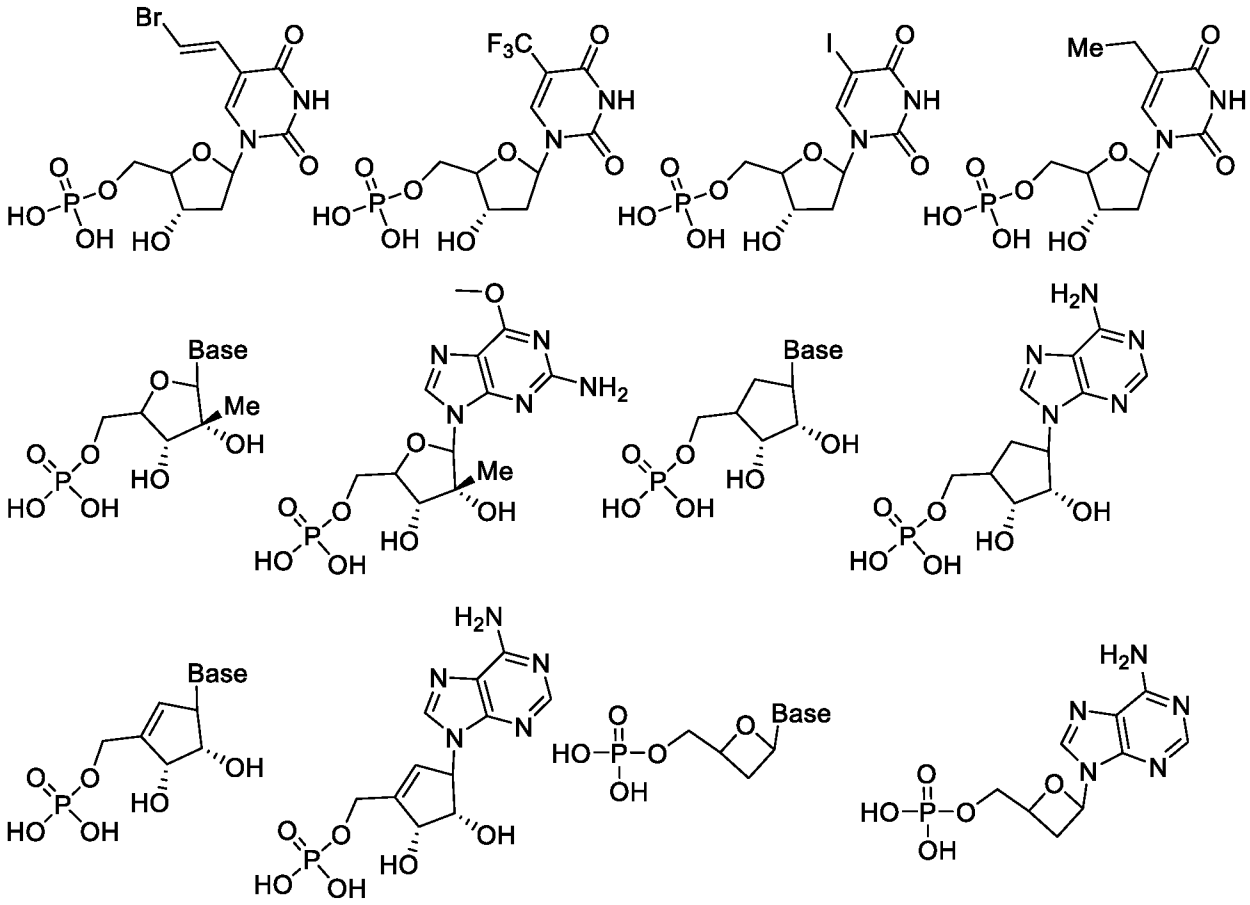


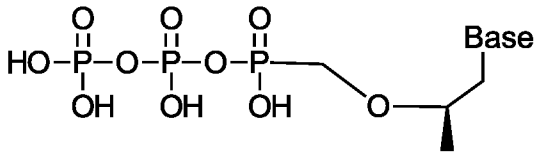
wherein R^a, R^b, R^c, and R^d are independently selected from hydrogen, a carbonyl linked amino acid, and -C(O)C_{1-6}alkyl.

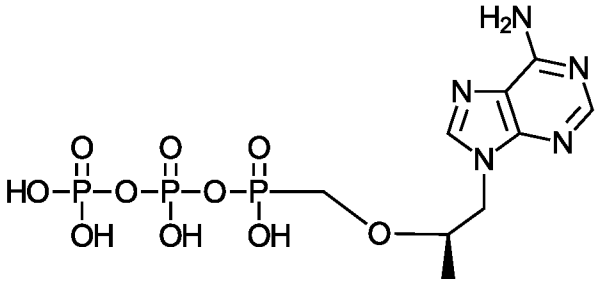
In one aspect, the active nucleos(t)ide analog is derived from the 5'-triphosphate metabolite of a known or FDA-approved nucleos(t)ide drug or 5'-diphosphate in the case of phosphonate analog. Non-limiting examples of these compounds include:

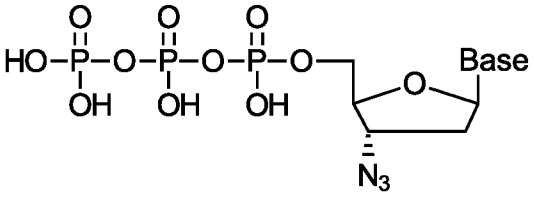


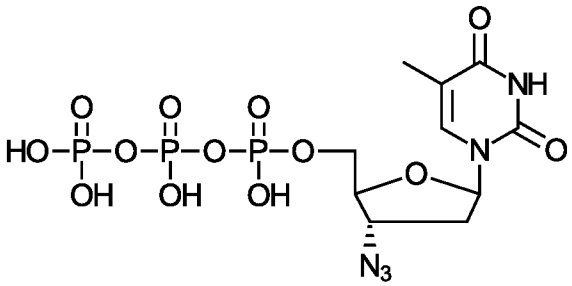


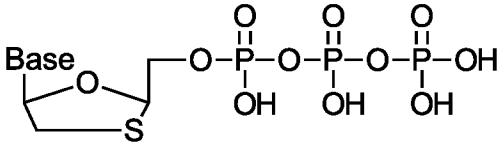


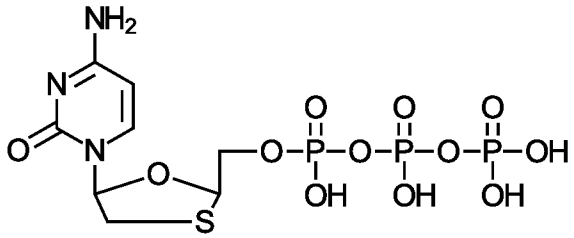

HO
P
O
OH
Base

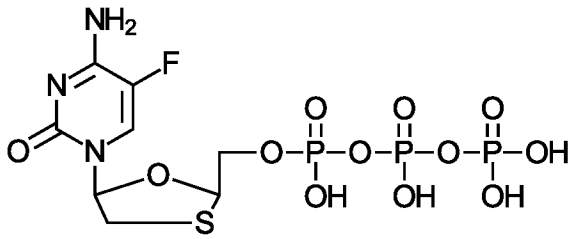

H2N
N
HO
P
O
OH

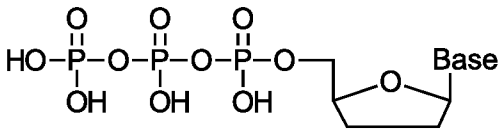

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P
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Base
N3

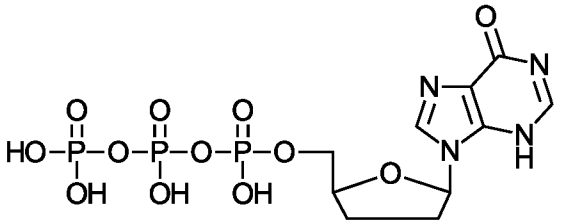

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NH
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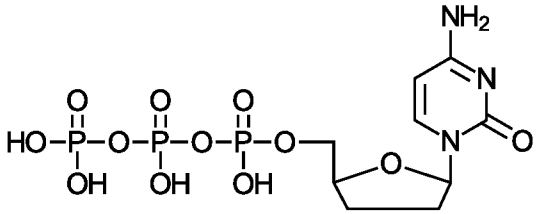

Base
O
P
OH
S

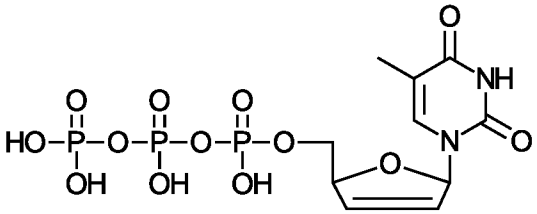

NH2
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OH
S

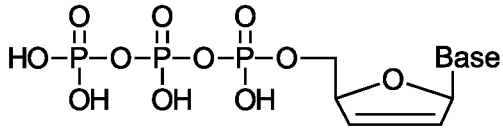

NH2
F
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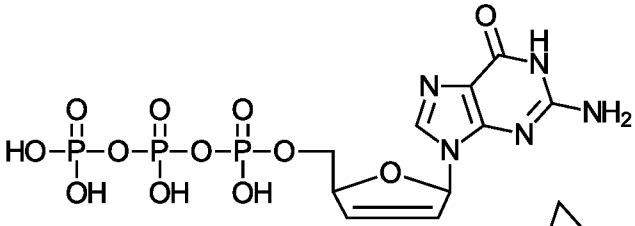

HO
P
O
OH
Base

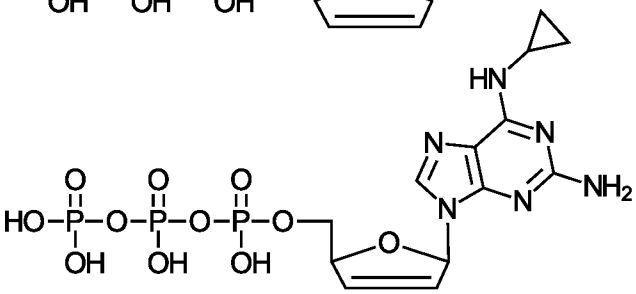

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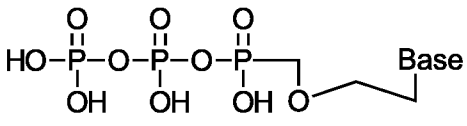

NH2
HO
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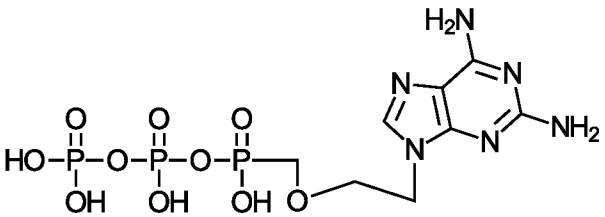

HO
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NH
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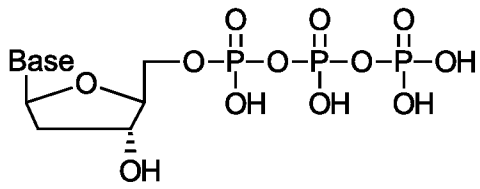

HO
P
O
OH
Base

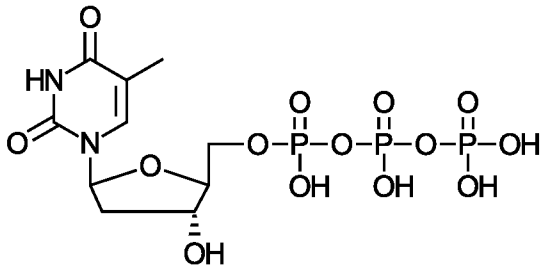

HO
P
O
OH
H
N
NH2

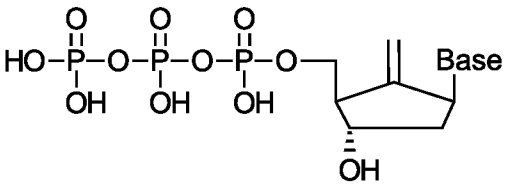

HO
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OH
HN
N
NH2

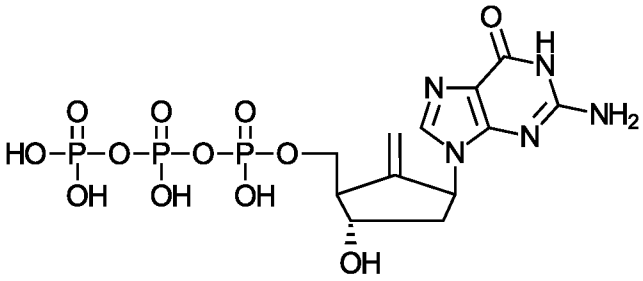

HO
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OH
Base

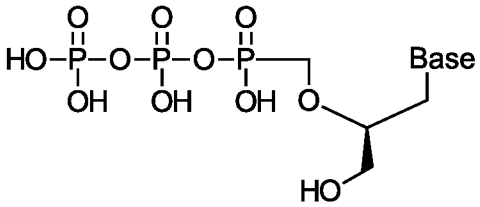

H2N
HO
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N
NH2

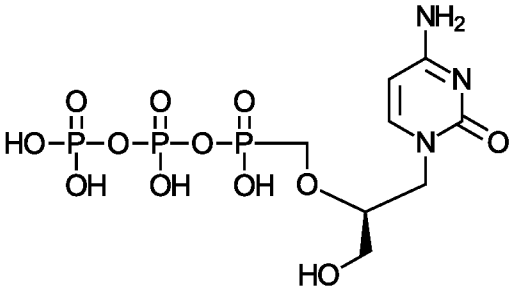

Base
O
O
P
OH
OH
OH
OH

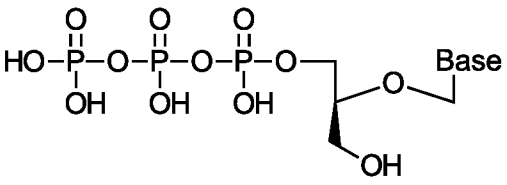

O
HN
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O
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OH
OH
OH

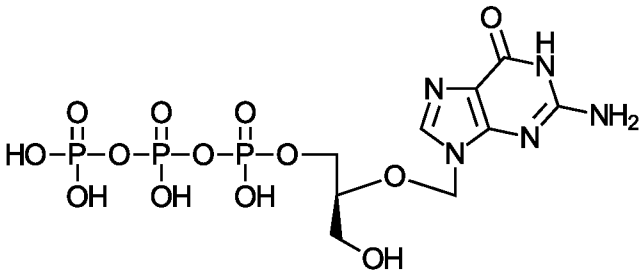

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OH
Base

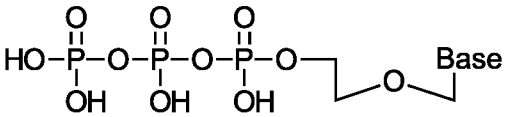

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OH
OH
OH
N
H
NH2

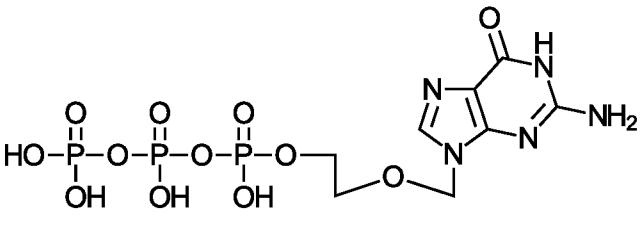

HO
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OH
OH
OH
Base
HO

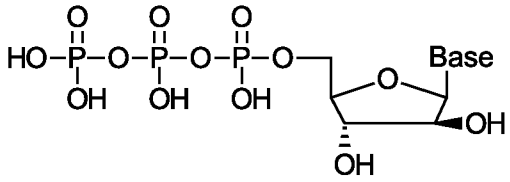

NH2
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O
HO
P
O
OH
OH
OH
HO

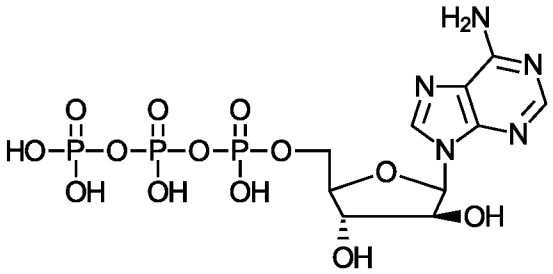

HO
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Base
OH

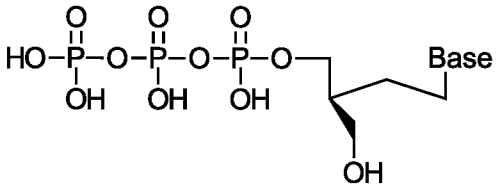

HO
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O
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OH
N
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NH2
OH

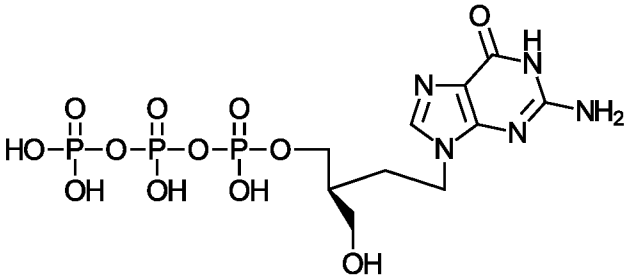

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OH
Base

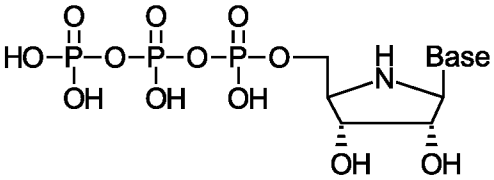

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O
OH
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OH
N
H
NH2

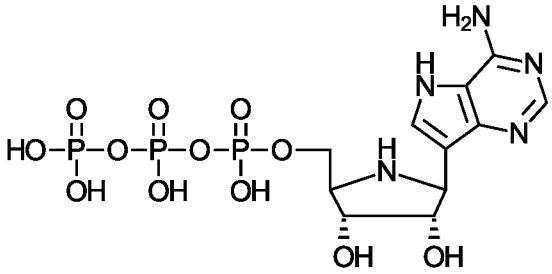

HO
P
O
OH
Base

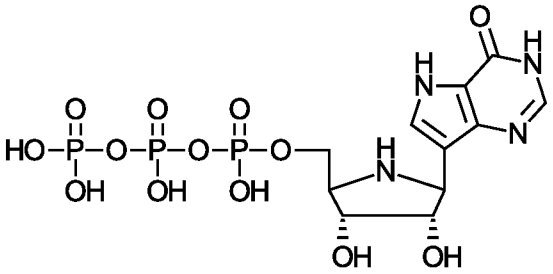

H2N
N
HO
P
O
OH

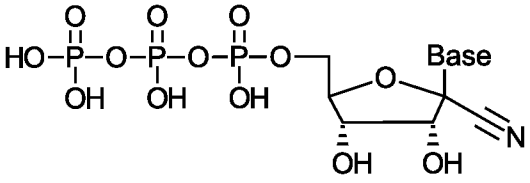

HO
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OH
Base

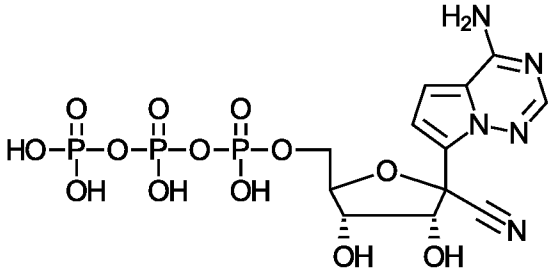

O
H
N
NH2
HO
P
O
OH

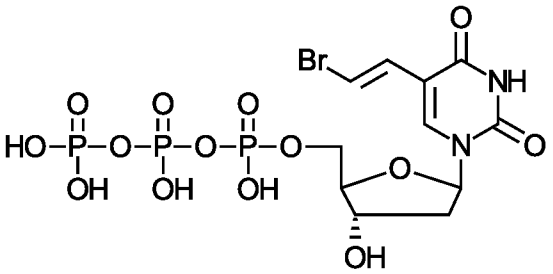

HO
P
O
OH
H
N
Base

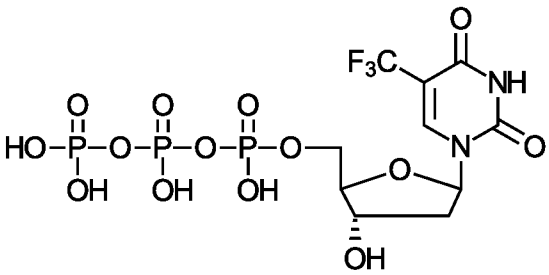

H2N
H
N
HO
P
O
OH

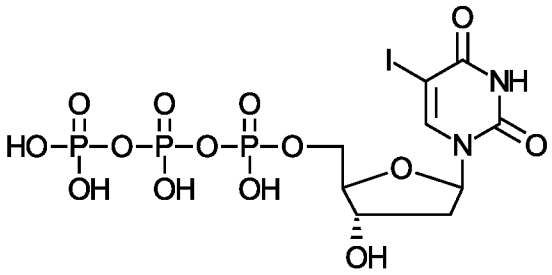

O
H
N
HO
P
O
OH

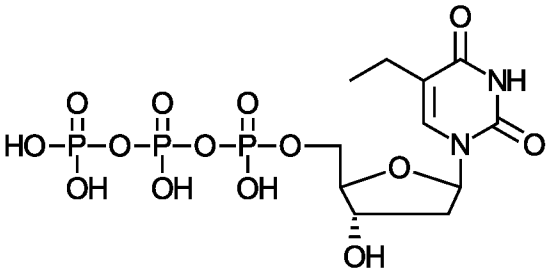

HO
P
O
OH
Base
N

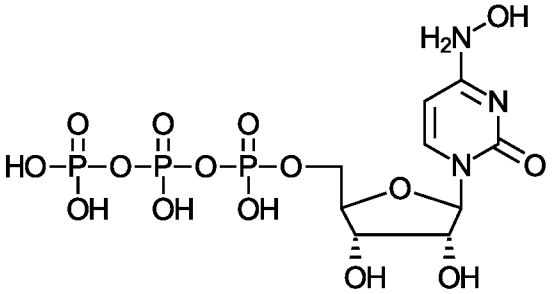

H2N
N
HO
P
O
OH

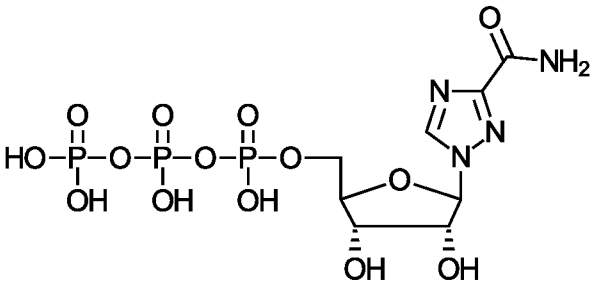


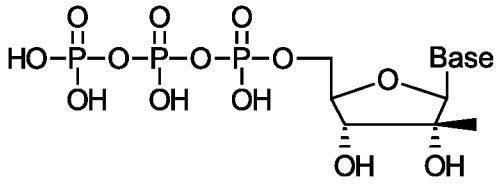


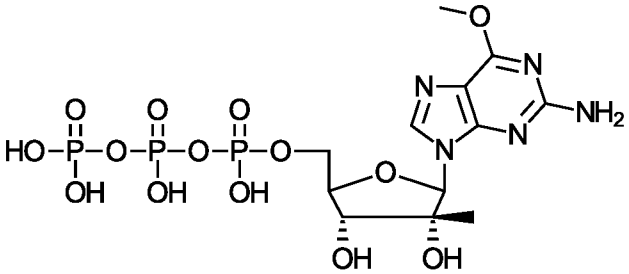


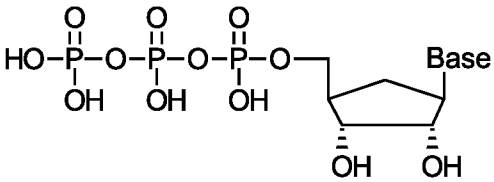


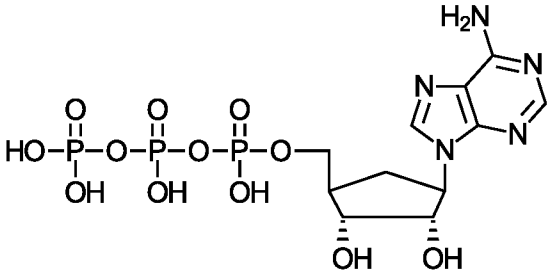


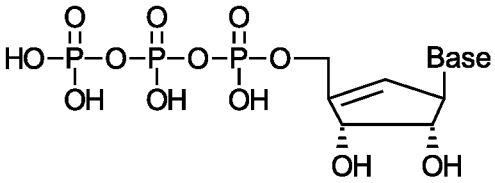


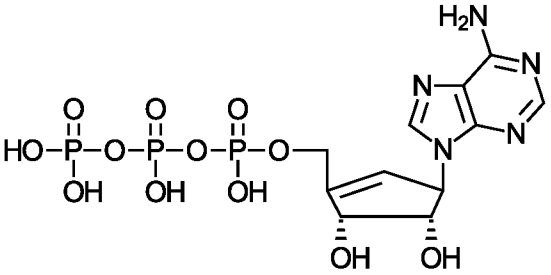

HO
P
O
OH
Base
OH
OH

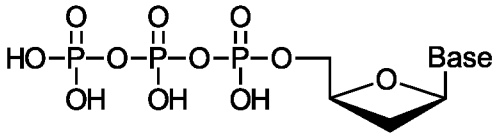

O
N
NH2
HO
P
O
OH
OH
OH

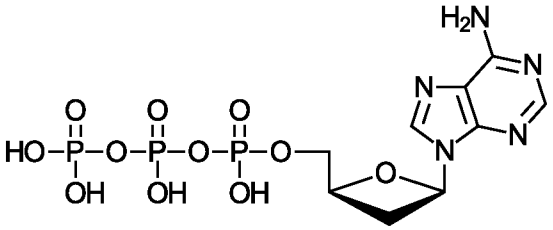

HO
P
O
OH
Base
OH
OH

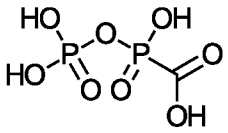

H2N
N
HO
P
O
OH
OH
OH

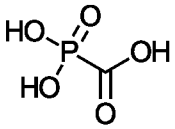

HO
P
O
OH
Base
OH
OH


H2N
N
HO
P
O
OH
OH
OH

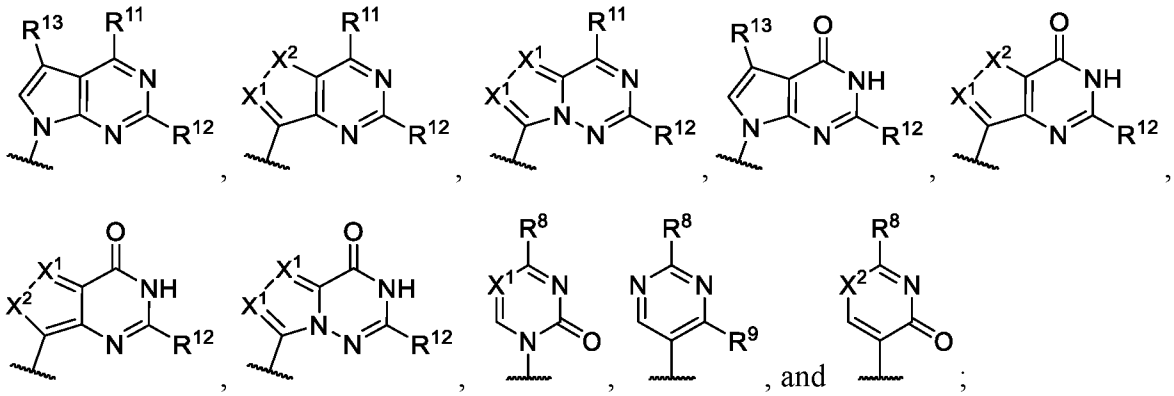

HO
P
O
OH
Base
O


H2N
N
HO
P
O
OH
O


HO
OH
P
O
HO
O
OH


HO
P
O
OH
HO
O

Where Base is selected from:



wherein

X^1 is independently N or CR^{13};

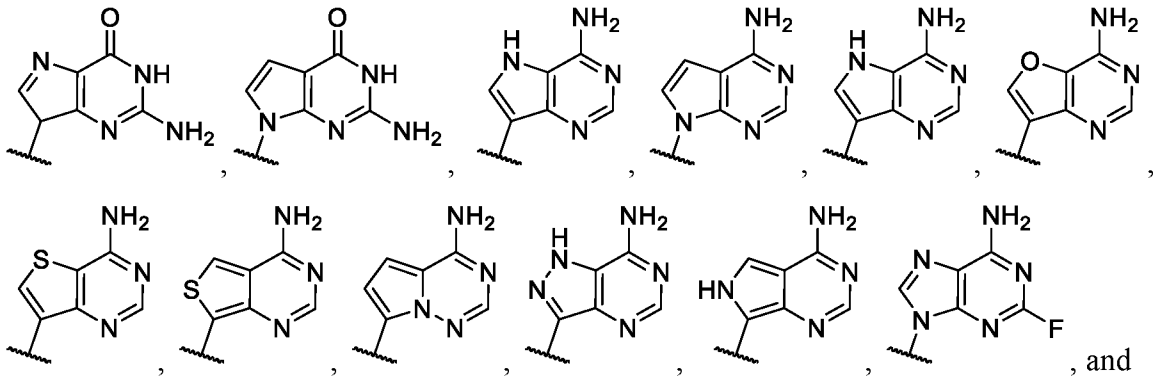
X^2 is independently NR^7, O, or $S(O)_n$

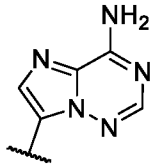
R^{11} is independently halogen, $-NR^7R^8$, $-N(R^7)OR^7$, $-NR^7NR^7R^8$, N_3, NO, NO_2, $C(O)R^{10C}$, CN, —CH(=NR^7), —CH=NNHR^7, —CH=N(OR^7), —$CH(OR^7)_2$, —C(=O)NR^7R^8, -C(=S)NR^7R^8, -C(=O)OR^7, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, -(C_0-C_2alkyl)(cycloalkyl), aryl, -(C_0-C_2alkyl)(heterocyclo), heteroaryl, —$C(O)R^{10C}$, —$S(O)_n$(C_1-C_8)alkyl, aryl(C_1-C_8alkyl)-, or $OR^{10;}$; and

R^{12} and R^{13} are independently H, halogen, $-NR^7R^8$, $-N(R^7)OR^7$, $-NR^7NR^7R^8$, N_3, NO, NO_2, $C(O)R^{10C}$, CN, —CH(=NR^7), —CH=NHNR^7, —CH=N(OR^7), —$CH(OR^7)_2$, —C(=O)NR^7R^8, -C(=S)NR^7R^8, —C(=O)OR^7, R^7, OR^{10}, or SR^8.

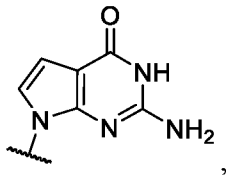
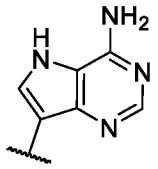
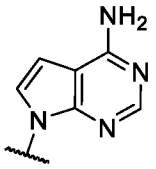
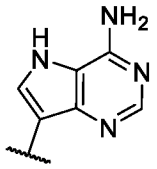
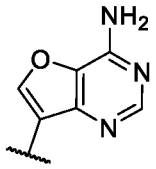
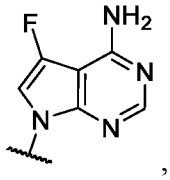
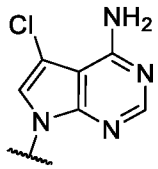
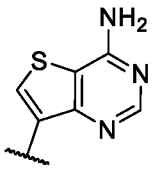
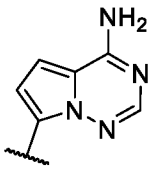
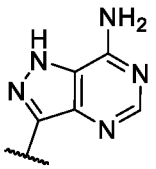
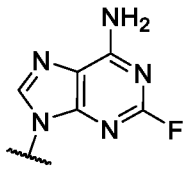
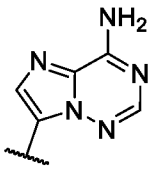
Embodiments of Base

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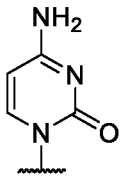
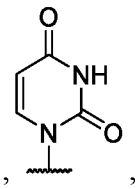
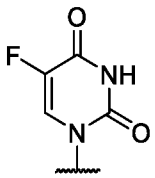
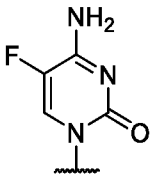
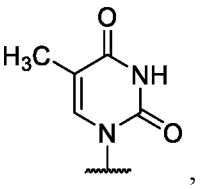
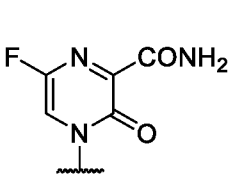
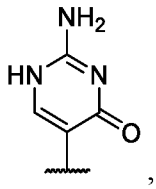
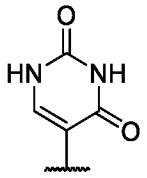
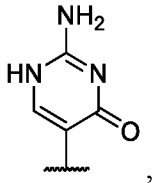
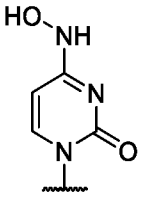



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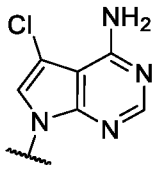
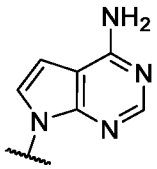
In certain embodiments, Base is selected from:


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In certain embodiments, Base is selected from:


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In certain embodiments Base is

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In certain embodiments R^{13} is halogen.

In certain embodiments R^{13} is F.

In certain embodiments R^{13} is Cl.

In certain embodiments R^{13} is Br.

In certain embodiments R^{13} is I.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate derivative of GS-44152.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 2'-C'methyladenosine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 7-deaza-2'-C-methyl-adenosine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 2'-C-methylguanosine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 2'-C-methylcytidine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 2'-C'methyluridine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate derivative of the non-phosphoramidated precursor of sofosbuvir.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 2'-C-ethynyladenosine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate derivative of NITD008.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate derivative of NITD449.

In certain non-limiting embodiments, the nucleoside is a 5'-monophosphate, diphosphate, or triphosphate derivative of NITD203.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 4'-C-azidocytidine.

In certain non-limiting embodiments, the nucleotide is a is a 5'-monophosphate, diphosphate, or triphosphate derivative of T-1106.

In certain non-limiting embodiments, the nucleotide is a is a 5'-monophosphate, diphosphate, or triphosphate derivative of BCX4430.

In certain non-limiting embodiments, the nucleotide is a is a 5'-monophosphate, diphosphate, or triphosphate derivative of RO-9187.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 6-methyl-7-deazaadenosine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of N6-(9-anthranylmethyl) adenosine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of N6-(1-pyrenylmethyl) adenosine.

In certain non-limiting embodiments, the nucleotide is a 3'-monophosphate, diphosphate, or triphosphate of Flex 1.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of ribavirin.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate derivative ETAR.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate derivative IM18.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 6-azauridine

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 5-(perylene-3-yl)ethynyl-arabino-uridine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 5-(perylene-3-yl)ethynyl-2'-deoxy-uridine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate, or triphosphate of 5-(pyren-1-yl)ethynyl-2'-deoxy-uridine.

In certain non-limiting embodiments, the nucleotide is a 5'-monophosphate, diphosphate or triphosphate of a nucleoside analog selected from the table below (from L. Eyer et al. "Nucleoside analogs as a rich source of antiviral agents active against arthropod-borne flaviviruses" Antivir Chem Chemother. 2018, **26**, 2040206618761299).

Heterobase identity/modification	Type of the glycosidic bond	Ribose substitution	Ribose position	Example
4-Aza-7,9-dideazaadenie	*C*-glycosidic	-CN (α)	*C*1′	GS-441524
4-Aza-7,9-dideazaadenie	*C*-glycosidic	-CN (α)	*C*1′	GS-5734

Adenine	*N*-glycosidic	-CH_3 (β)	*C*2′	2′-C-methyladenosine
7-Deazaadenine	*N*-glycosidic	-CH_3 (β)	*C*2′	7-Deaza-2′-*C*-methyladenosine
Guanine	*N*-glycosidic	-CH_3 (β)	*C*2′	2′-*C*-methylguanosine
Cytosine	*N*-glycosidic	-CH_3 (β)	*C*2′	2′-*C*-methylcytidine
Uracil	*N*-glycosidic	-CH_3 (β)	*C*2′	2′-*C*-methyluridine
6-O-methylguanine	*N*-glycosidic	-CH_3 (β)	*C*2′	INX-08189
Uracil	*N*-glycosidic	-F (α), CH_3 (β)	C2’	sofosbuvir
Adenine	*N*-glycosidic	-ethynyl (β)	*C*2′	2′-*C*-ethynyladenosine
7-Deazaadenine	*N*-glycosidic	-ethynyl (β)	*C*2′	NITD008
7-Deaza-7-carbamoyladenine	*N*-glycosidic	-ethynyl (β)	*C*2′	NITD449
Cytosine	*N*-glycosidic	-H (α), OH (β) + N_3 (α)	*C*2’ + *C4′*	4′-Azido
Cytosine	*N*-glycosidic	–N_3 (α)	C4’	4′-Azido-aracytidine
9-Deazaadenine	*C*-glycosidic	O exchanged for N	-	BCX4430
3-Oxopyrazine-2-carboxamide	*N*-glycosidic	No substitution	-	T-1106
6-Methyl-7-deazaadenine	*N*-glycosidic	No substitution	-	6-Methyl-7-deazadenosine
Uracil	*N*-glycosidic	Trityl	*C*2′ and *C*5′	2′,5′di-*O*-trityluridine
Uracil	*N*-glycosidic	Trityl	*C*3′ and *C*5′	3′,5′di-*O*-trityluridine

1'-Cyano nucleosides

GS-5734 (also known as Remdesivir) is a 1'-cyano nucleotide-5'-phosphoramidate that has been tested in the clinic against Ebola virus as well as SARS-CoV-2. In October of 2020, remdesivir became the first drug to be approved by the FDA for treating an infection of SARS-CoV-2 (COVID-19). The non-phosphoramidated precursor, GS-44152, has a free 5' hydroxyl group and thus is the appropriate moiety to use in the 5'-mono, di or triphosphate or other 5'-phosphate lipid nano or micro particles of the present invention. Prior to 2020 remdesivir has been developed by Gilead Sciences as a treatment against filovirus infections. It also displayed *in vitro* activity against flaviviruses. Other 1'-Cynano nucleosides include 1′-*C*-Cyano-2′-deoxyuridine, 1′-*C*-cyano-2′-deoxy-Cytidine, 1′-*C*-Cyanouridine, 1′-*C*-Cyano-2′-*C*-methyluridine, 1′-*C*-Cyano-4′-*C*-(fluoromethyl)uridine, 1′-*C*-Cyano-4′-*C*-(fluoromethyl)cytidine, 1′-*C*-cyano-4′-*C*-(fluoromethyl)-Uridine-2′,3′,5′-triacetate, 1′-*C*-Cyano-5-methyluridine, 1′-*C*-Cyano-2′,3′-*O*-(1-methylethylidene)uridine, 1′-*C*-Cyano-2′-*C*-methylcytidine, 1′-*C*-Cyano-4′-*C*-(fluoromethyl)-5′-uridylic acid, 1′-*C*-Cyano-2′-*O*-methyluridine, β-D-*arabino*-2-Hexulofuranosononitrile, 1′-*C*-cyano-adenosine, 2-*C*-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-2,5-anhydro-D-allononitrile, 2-*C*-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-2,5-anhydro-3-deoxy-3-fluoro-D-altrononitrile, 3-bromo-2,3-dideoxy-2-(3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl)- β-D-*arabino*-2-Hexulofuranosononitrile, 2,7-dideoxy-2-(3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl)- α-L-*talo*-2-Heptulofuranosononitrile, and 2,5-anhydro-2-*C*-(2,4-diaminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-3-*C*-methyl- D-Altrononitrile.

2'-C-Methyl nucleosides

2'-C-methyl nucleosides have a methyl group in the 2' position on the sugar core. This moiety has been shown to be important for activity against certain *Flaviviruses*. Examples of 2'-C-methyl nucleosides include INX-08189, 2′-*C*-Methylcytidine, 2′-*C*-Methyladenosine, 2′-*C*-Methyluridine, 7-Deaza-2′-*C*-methyladenosine, Valopicitabine, 2′-*C*-Methylguanosine, MK-3682, 2′-*C*-Methyl-6-*O*-methylguanosine, (2′*R*)- Cytidine, 6-Chloro-9-(2,3,5-tri-*O*-benzoyl-2-*C*-methyl-β-D-ribofuranosyl)-9*H*-purin-2-amine, (2′*R*)-2′-Chloro-2′-deoxy-2′-methyluridine, 1-(2-Deoxy-2-methyl-β-D-arabinofuranosyl)-2,4(1*H*,3*H*)-pyrimidinedione, 5-Iodo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine, 2′-*C*-Methyl-2′,3′-*O*-(1-methylethylidene)uridine, 6-Chloro-9-(2-*C*-methyl-β-D-ribofuranosyl)-9*H*-purin-2-amine, 4-

Chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine, 4-Amino-1-(2-deoxy-2-methyl-β-D-arabinofuranosyl)-2(1*H*)-pyrimidinone, and 6-*O*-Ethyl-2′-*C*-methylguanosine.

2'-Fluoro-2'-C-Methyl Nucleosides

2'-Fluoro-2'-C-methyl nucleosides have been successfully developed as therapeutics against HCV. One 2'-fluoro-2'-C-methyl nucleotide phosphoramidate is sofosbuvir, developed by Pharmasset and Gilead Sciences. The 5'-OH precursor compound is known as PSI-331007. The non-phosphoramidated version of sofosbuvir has a free 5' hydroxyl group and thus is the appropriate moiety to use in the 5'-mono, di or triphosphate or other 5'-phosphate lipid nano or micro particles of the present invention.

Non-limiting examples of 2'fluoro-2'C-methyl nucleosides include 2′-Deoxy-2′-fluoro-2′-*C*-methylcytidine, *N*-benzoyl-2′-deoxy-2′-fluoro-2′-methyl-, 3′,5′-dibenzoate, (2′*R*)-2′-Deoxy-6-*O*-ethyl-2′-fluoro-2′-methylguanosine, (2′*R*)-2′-Deoxy-2′-fluoro-2′-methyluridine, Mericitabine, 2′-Deoxy-2′-fluoro-2′-*C*-methylcytidine, (2′*R*)-2′-Deoxy-6-*O*-ethyl-2′-fluoro-2′-methylguanosine, (2′*R*)-2′-Deoxy-2′-fluoro-2′-methylguanosine, (2′*R*)-2′-Deoxy-2′-fluoro-2′-methyl-6-*O*-methylguanosine, (2′*R*)-2′-Deoxy-2′-fluoro-4′-*C*-fluoro-2′-methyluridine, (2′*R*)-*N*-Benzoyl-2′-deoxy-2′-fluoro-2′-methylcytidine, PSI 7411, 2′-deoxy-2′-fluoro-2′-methyl-, 3′,5′-diacetate, (2′*R*)-Uridine, (2′*R*)-2′-Deoxy-2′-fluoro-2′-methyladenosine, (2′*R*)-2-Amino-2′-deoxy-2′-fluoro-*N*,2′-dimethyladenosine, 2′-deoxy-2′-fluoro-2′-methyl-, 3′-(4-oxopentanoate), (2′*R*)- Uridine, (2′*R*)-2′-Deoxy-2′-fluoro-4′-*C*-fluoro-2′-methylcytidine, 7-[(2*R*)-2-deoxy-2-fluoro-2-methyl-β-D-*erythro*-pentofuranosyl]-5-fluoro-7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-amine, 2′-deoxy-6-*O*-ethyl-2′-fluoro-2′-methyl-, cyclic 3′,5′-(1-methylethyl phosphate), (2′*R*)- Guanosine, (2′*R*)-2′-Deoxy-2′-fluoro-4′-*C*-fluoro-2′-methylguanosine, and (2′*R*)-2′-Deoxy-2′-fluoro-2′-methyl-6-*O*-(phenylmethyl)guanosine.

2'-C'-ethynyl nucleosides

Nucleosides with a 2'-ethynyl substitution, such as NITD008, have been identified as inhibitors of Dengue fever, West Nile virus, and Zika virus. Non-limiting examples of 2'-C-ethynyl nucleosides include 2′-*C*-Ethynyluridine, 7-(2-*C*-Ethynyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine, 2′-*C*-Ethynyladenosine, (2′*R*)-2′-Deoxy-2′-ethynyl-2′-fluorouridine, (2′*R*)-2′-Deoxy-2′-ethynyl-2′-methyluridine, 2′-*C*-Ethynyl-4′-*C*-fluorouridine, 2′-*C*-

Ethynylcytidine, (2′*R*)-2′-Deoxy-2′-ethynyl-2′-fluoro-4′-*C*-fluorouridine, 2′-*C*-Ethynyl-4′-*C*-fluoroguanosine, (2′*R*)-2′-Deoxy-2′-ethynyl-2′-fluoro-4′-*C*-fluoroguanosine, 4-Chloro-7-(2-*C*-ethynyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine, 2′,3′-*O*-Cyclopentylidene-2′-*C*-ethynyluridine, 2′-*C*-Ethynylguanosine, 1-(2-Deoxy-2-ethynyl-β-D-arabinofuranosyl)-2,4(1*H*,3*H*)-pyrimidinedione, 1-(2-Deoxy-2-ethynyl-β-D-arabinofuranosyl)-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione, 1-(2-*C*-Ethynyl-β-D-arabinofuranosyl)-2,4(1*H*,3*H*)-pyrimidinedione, 2′-*C*-Ethynyl-4′-*C*-fluoro-2′,3′-*O*-(methoxymethylene)uridine, 4′-*C*-(Chloromethyl)-2′-*C*-ethynyluridine, 2′-*C*-Ethynyl-4′-*C*-(fluoromethyl)uridine, 2′-*C*-Ethynyl-4′-*C*-methyladenosine, 2′-*C*-Ethynyl-2′,3′-*O*-(1-methylethylidene)adenosine, 2′,3′-*O*-Cyclopentylidene-2′-*C*-ethynyl-4′-*C*-(hydroxymethyl)uridine, 4′-*C*-(Chloromethyl)-2′-*C*-ethynyladenosine, 2′-*C*-Ethynyl-4′-*C*-(fluoromethyl)adenosine, 7-(2-*C*-Ethynyl-β-D-ribofuranosyl)-5-fluoro-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine, 4′-*C*-(Chloromethyl)-2′,3′-*O*-cyclopentylidene-2′-*C*-ethynyluridine, 4′-*C*-(Chloromethyl)-2′-*C*-ethynyl-2′,3′-*O*-(methoxymethylene)adenosine, 2′-*C*-Ethynyl-4′-*C*-(fluoromethyl)-2′,3′-*O*-(methoxymethylene)adenosine, (2′*R*)-2-Amino-*N*-cyclopropyl-2′-deoxy-2′-ethynyl-2′-fluoroadenosine, and 3′-Deoxy-2′-*C*-ethynyl-3′-fluoro-4′-*C*-fluoroguanosine.

2'-O-substituted nucleosides

Non-limiting examples of 2'-O-substituted nucleosides include 2′-*O*-Methyladenosine, 2′-*O*-Methyluridine, 2′-*O*-Methylcytidine, 2′-*O*-Methylguanosine, 2′-*O*-[2-[2-(Dimethylamino)ethoxy]ethyl]-5-methyluridine, 2′,3′-*O*-Isopropylideneuridine, 2′-*O*-Methoxyethyl-5-methyluridine, 2′-*O*-[2-[(Dimethylamino)oxy]ethyl]-5-methyluridine, 5-Methyl-2′-*O*-methyluridine, N^6,2′-*O*-Dimethyladenosine, 2′-*O*-Methylinosine, WAG 994, 2′-*O*-Methylpseudouridine, 2-Amino-2′-*O*-methyladenosine, 5-(2-Amino-2-oxoethyl)-2′-*O*-methyluridine, *N*,*N*-Dimethyl-2′-*O*-methyladenosine, 5-Formyl-2′-*O*-methylcytidine, 2′-*O*-2-Propyn-1-yladenosine, 2′-*O*-2-Propen-1-yluridine, 2′-*O*-2-Propen-1-ylguanosine, -Methyl-2′,3′,5′-tri-*O*-methyluridine, 5-methyl-2′-*O*-propyl-Uridine, 2′-*O*-ethyl- Adenosine, and 2′-*O*-butyl- Adenosine.

4'-Azido substituted nucleosides

4'-azido nucleotides have been identified as inhibitors of HCV, henipaviruses, and paramyxoviruses (RO-1479 and RO-9187). Other non-limiting examples of 4'-azido nucleosides include Balapiravir, 4′-Azidocytidine, 2′-Deoxy-2′-β-fluoro-4′-azidocytidine, 4′-*C*-Azidouridine, 4′-Azidothymidine, 4-Amino-1-(4-*C*-azido-β-D-arabinofuranosyl)-2(1*H*)-pyrimidinone, 4′-*C*-Azido-2′-deoxy-2′-fluorocytidine, 4-Amino-1-(4-*C*-azido-2-deoxy-2-methyl-β-D-arabinofuranosyl)-2(1*H*)-pyrimidinone, 1-[4-*C*-Azido-2-deoxy-2-fluoro-β-D-arabinofuranosyl]-2,4(1*H*,3*H*)-pyrimidinedione, 4′-Azido-2′-deoxyadenosine, 4′-Azido-3′-deoxythymidine, 4′-Azido-2′-deoxycytidine, 4′-*C*-Azido-2′-deoxy-2′,2′-difluorocytidine, 4′-Azido-2′-deoxyuridine, 4′-Azido-2′-deoxyinosine, 4′-*C*-Azido-2′-deoxy-2′-fluorouridine, 4′-Azido-5-chloro-2′-deoxyuridine, 4′-Azido-2′-deoxyguanosine, 1-(4-*C*-Azido-2-deoxy-2-methyl-β-D-arabinofuranosyl)-2,4(1*H*,3*H*)-pyrimidinedione, 1-(4-*C*-Azido-β-D-arabinofuranosyl)-2,4(1*H*,3*H*)-pyrimidinedione, 4′-*C*-Azidoadenosine, 4′-*C*-Azido-2′,3′-*O*-(1-methylethylidene)uridine, 4′-*C*-Azido-3′-deoxy-3′-fluorocytidine, 4′-*C*-Azido-2′-deoxy-2′,2′-difluorouridine, 4′-*C*-Azido-2′,3′-*O*-(1-methylethylidene)cytidine, 1-(4-*C*-azido-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-(cyclopropylamino)- 2(1*H*)-Pyrimidinone, 1-(4-*C*-azido-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-(methylamino)- 2(1*H*)-Pyrimidinone, 7-(4-*C*-azido-2-deoxy-2-fluoro-β-D-arabinofuranosyl)- 7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-amine, 7-(4-*C*-azido-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-fluoro-7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-amine, 4′-*C*-Azido-3′-deoxy-3′-fluorouridine, 4′-*C*-Azidoinosine, 1-(4-*C*-azido-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-(dimethylamino)- 2(1*H*)-Pyrimidinone, 1-(4-*C*-azido-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-(hydroxyamino)- 2(1*H*)-Pyrimidinone, and 1-(4-*C*-azido-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-(ethylamino)- 2(1*H*)-Pyrimidinone.

Heterocyclic base-modified nucleosides

Non-limiting examples of heterocyclic base-modified nucleosides include T-1106, T-705, 6-methyl-7-deazaadenosine, 6-methyl-1-deazaadenosine, 6-methyl-4-deazaadenosine, N6-(9-anthracenylmethyl)adenosine, N6-(-pyrenylmethyl)adenosine, 5'3'-O-*tert*-butyldiphenylsilyl modified adenosine, and 5',2'-O-*tert*-butyldiphenylsilyl modified adenosine.

Tritylated nucleosides

Non-limiting examples of tritylated nucleosides include 2',5'-di-O-trityluridine, 3',5'-di-O-trityluridine, and 2'-deoxy-3',5'-di-O-trityluridine. The corresponding compounds with a free 5' hydroxyl group can be converted to a 5'-mono, di or triphosphate or other 5'-phosphate for the lipid nano or micro particles of the present invention.

III. Cationic Lipid Carriers

As described, the 5'-monophosphate, 5'-diphosphate or 5'-triphosphate or another 5'-phosphate of an antiviral nucleoside or nucleotide is delivered into a cell for a therapeutic or prophylactic use in an effective amount to a host, including a human, in need thereof encased in an ionizable or permanently ionized cationic lipid nano- or micro- particle.

As further described herein, the cationic lipid carrier can have one component or 2, 3, 4 or or more components to collectively achieve the desired results. Nonlimiting components included an ionizable lipid, ionized lipid, non-ionized lipid (for example, a sterol), PEG lipid, structural lipid, or phospholipid.

In an alternative embodiment, the cationic lipid nanoparticle can be a cationic liposome. The cationic lipid nanoparticle carrier, for example, can be composed of one or more amphipatic lipids that for a sphere surrounding a hydrophobic core. The lipid nanoparticle generally, regardless of structure, is typically of a size, for example, including but not limited to at least about 40 to up to about 1,000 nm or more in diameter (including but not limited to up to about 50, 75, 100, 150, 200, 400, 500, 750 or 1,000 nm or more).

The cationic lipid nano or micro particle can be ionizable as a function of pH, or can be permanently ionized. As nonlimiting examples, lipid amines are ionizable as a function of pH, however, lipid quaternary amines are permanently ionized. The counterion to the quaternary amine of the lipid can be any counterion that achieves the desired purpose, as further described herein.

Numerous examples of cationic lipids that can be used in the cationic lipid nano or micro particles are generally known and are described herein. General categories of cationic lipids include but are not limited to long chain amino ethers, long chain carbamates, long chain amino or carbamate esters or diesters, pegylated amines or carbamates that may also have an ester, long chain alkyl, alkenyl or alkynyl esters or ethers generally of an amine or carbamate or other

ionizable nitrogen moiety. Any lipid carbonyl(s) can be protected, for example, as a ketal, acetal, acylal, or dithiane.

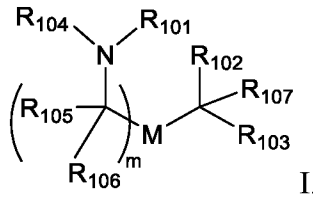
The lipid amine can be in the form of a primary, secondary, tertiary or quaternary amine. The amine can be protected, for example, with/as a carbobenzyloxy group, a methoxybenzyl carbonyl, a t-butyloxycarbonyl, acyl, acetyl, benzoyl, benzyl, carbamate, methoxybenzyl, phenyl, tosyl, as a sulfonamide or amide.

Specific examples of cationic lipids are described in more detail below and include, as nonlimiting illustrative examples, PEG-DMG, PEG-DSG, DODMA, DODAP, DOTMA, DOTAP, KC2 and MC3.

Ionizable Lipids

As used herein "ionizable lipid" refers to a lipid that can be ionized under certain conditions. Typically, the ionizing condition is the pH of the solution. Ionizable lipids are typically lipids containing a basic nitrogen, such that the nitrogen bears a positive or partial positive charge at physiological pH.

In certain embodiments, the ionizable lipid component is a compound of Formula I:



I.

or a salt, isotope, or isomer thereof, wherein, for example:

R_{101} is selected from the group consisting of C_{5-30}alkyl, C_{5-20}alkenyl, —R*YR″, —YR″, and —R″M′R′;

R_{102} and R_{103} are independently selected from the group consisting of H, C_{1-14}alkyl, C_{2-14}alkenyl, —R*YR″, —YR″, and —R*OR″, or R_{102} and R_{103}, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_{104} is selected from the group consisting of a C_{3-6}carbocycle, —$(CH_2)_nQ$, —$(CH_2)_nCHQR_{100}$, —$CHQR_{100}$, —$CQ(R_{100})_2$, and unsubstituted C_{1-6}alkyl, where Q is selected from a carbocycle, heterocycle, —OR_{100}, —$O(CH_2)_nN(R_{100})_2$, —$C(O)OR_{100}$, —$OC(O)R_{100}$, —CX_3, —CX_2H, —CXH_2, —CN, —$N(R_{100})_2$, —$C(O)N(R_{100})_2$, —$N(R_{100})C(O)R_{100}$, —$N(R_{100})S(O)_2R_{100}$, —

$N(R_{100})C(O)N(R_{100})_2$, —$N(R_{100})C(S)N(R_{100})_2$, —$N(R_{100})R_{108}$, —$O(CH_2)_nOR_{100}$, —$N(R_{100})C(=NR_{109})N(R_{100})_2$, —$N(R_{100})C(=CHR_{109})N(R_{100})_2$, —$OC(O)N(R_{100})_2$, —$N(R_{100})C(O)OR_{100}$, —$N(OR_{100})C(O)R_{100}$, —$N(OR_{100})S(O)_2R_{100}$, —$N(OR_{100})C(O)OR_{100}$, —$N(OR_{100})C(O)N(R_{100})_2$, —$N(OR_{100})C(S)N(R_{100})_2$, —$N(OR_{100})C(=NR_{109})N(R_{100})_2$, —$N(OR_{100})C(=CHR_{109})N(R_{100})_2$, —$C(=NR_{109})N(R_{100})_2$, —$C(=NR_{109})R_{100}$, —$C(O)N(R_{100})OR_{100}$, and —$C(R_{100})N(R_{100})_2C(O)OR_{100}$, and each n is independently selected from 1, 2, 3, 4, and 5;

each R_{105} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R_{106} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

M and M′ are independently selected from —C(O)O—, —OC(O)—, —C(O)N(R′)—, —N(R′)C(O)—, —C(O)—, —C(S)—, —C(S)S—, —SC(S)—, —CH(OH)—, —P(O)(OR′)O—, —$S(O)_2$—, —S—S—, an aryl group, and a heteroaryl group;

R_{107} is selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

R_{108} is selected from the group consisting of C_{3-6}carbocycle and heterocycle;

R_{109} is selected from the group consisting of H, CN, NO_2, C_{1-6}alkyl, —OR_{100}, —$S(O)_2R_{100}$, —$S(O)_2N(R)_2$, C_{2-6}alkenyl, C_{3-6}carbocycle and heterocycle;

each R_{100} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R′ is independently selected from the group consisting of C_{1-18}alkyl, C_{2-18}alkenyl, —R*YR″, —YR″, and H;

each R″ is independently selected from the group consisting of C_{3-14}alkyl and C_{3-14}alkenyl;

each R* is independently selected from the group consisting of C_{1-12}alkyl and C_{2-12}alkenyl;

each Y is independently a C_{3-6}carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

In some embodiments, a subset of compounds of Formula (I) includes those in which when R_{104} is —$(CH_2)_nQ$, —$(CH_2)_nCHQR_{100}$, —$CHQR_{100}$, or —$CQ(R_{100})_2$, then (i) Q is not —$N(R_{100})_2$ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

In another embodiment, another subset of compounds of Formula (I) includes those in which R_{101} is selected from the group consisting of C_{5-30}alkyl, C_{5-20}alkenyl, —R*YR″, —YR″, and —R″M′R′;

R_{102} and R_{103} are independently selected from the group consisting of H, C_{1-14}alkyl, C_{2-14}alkenyl, —R*YR″, —YR″, and —R*OR″, or R_{102} and R_{103}, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_{104} is selected from the group consisting of a C_{3-6}carbocycle, —$(CH_2)_nQ$, —$(CH_2)_nCHQR_{100}$, —$CHQR_{100}$, —$CQ(R_{100})_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, —OR_{100}, —$O(CH_2)_nN(R_{100})_2$, —$C(O)OR_{100}$, —$OC(O)R_{100}$, —CX_3, —CX_2H, —CXH_2, —CN, —$C(O)N(R_{100})_2$, —$N(R_{100})C(O)R_{100}$, —$N(R_{100})S(O)_2R_{100}$, —$N(R_{100})C(O)N(R_{100})_2$, —$N(R_{100})C(S)N(R_{100})_2$, —$CR_{100}N(R_{100})_2C(O)OR_{100}$, —$N(R_{100})R_{108}$, —$O(CH_2)_nOR_{100}$, —$N(R_{100})C(=NR_{109})N(R_{100})_2$, —$N(R_{100})C(=CHR_{109})N(R_{100})_2$, —$OC(O)N(R_{100})_2$, —$N(R_{100})C(O)OR_{100}$, —$N(OR_{100})C(O)R_{100}$, —$N(OR_{100})S(O)_2R_{100}$, —$N(OR_{100})C(O)OR_{100}$, —$N(OR_{100})C(O)N(R_{100})_2$, —$N(OR_{100})C(S)N(R_{100})_2$, —$N(OR_{100})C(=NR_{109})N(R_{100})_2$, —$N(OR_{100})C(=CHR_{109})N(R_{100})_2$, —$C(=NR_{109})N(R_{100})_2$, —$C(=NR_{109})R_{100}$, —$C(O)N(R_{100})OR_{100}$, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C_{1-3}alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;

each R_{105} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R_{106} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

M and M′ are independently selected from —C(O)O—, —OC(O)—, —C(O)N(R′)—, —N(R′)C(O)—, —C(O)—, —C(S)—, —C(S)S—, —SC(S)—, —CH(OH)—, —P(O)(OR′)O—, —$S(O)_2$—, —S—S—, an aryl group, and a heteroaryl group;

R_{107} is selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

R_{108} is selected from the group consisting of C_{3-6}carbocycle and heterocycle;

R_{109} is selected from the group consisting of H, CN, NO_2, C_{1-6}alkyl, —OR_{100}, —$S(O)_2R_{100}$, —$S(O)_2N(R_{100})_2$, C_{2-6}alkenyl, C_{3-6}carbocycle and heterocycle;

each R_{100} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R′ is independently selected from the group consisting of C_{1-18}alkyl, C_{2-18}alkenyl, —R*YR″, —YR″, and H;

each R″ is independently selected from the group consisting of C_{3-14}alkyl and C_{3-14}alkenyl;

each R* is independently selected from the group consisting of C_{1-12}alkyl and C_{2-12}alkenyl;

each Y is independently a C_{3-6}carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts, isotopes, or isomers thereof.

In yet other embodiments, another subset of compounds of Formula (I) includes those in which:

R_{101} is selected from the group consisting of C_{5-30}alkyl, C_{5-20}alkenyl, —R*YR″, —YR″, and —R″M′R′;

R_{102} and R_{103} are independently selected from the group consisting of H, C_{1-14}alkyl, C_{2-14}alkenyl,

—R*YR″, —YR″, and —R*OR″, or R_{102} and R_{103}, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_{104} is selected from the group consisting of a C_{3-6}carbocycle, —$(CH_2)_nQ$, —$(CH_2)_nCHQR_{100}$, —$CHQR_{100}$, —$CQ(R_{100})_2$, and unsubstituted C_{1-6}alkyl, where Q is selected from a C_{3-6}carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, —OR_{100}, —$O(CH_2)_nN(R_{100})_2$, —$C(O)OR_{100}$, —$OC(O)R_{100}$, —CX_3, —CX_2H, —CXH_2, —CN, —$C(O)N(R_{100})_2$, —$N(R_{100})C(O)R_{100}$, —$N(R_{100})S(O)_2R_{100}$, —$N(R_{100})C(O)N(R_{100})_2$, —$N(R_{100})C(S)N(R_{100})_2$, —$CR_{100}N(R_{100})_2C(O)OR_{100}$, —$N(R_{100})R_{108}$, —$O(CH_2)_nOR_{100}$, —$N(R_{100})C(=NR_{109})N(R_{100})_2$, —$N(R_{100})C(=CHR_{109})N(R_{100})_2$, —$OC(O)N(R_{100})_2$, —$N(R_{100})C(O)OR_{100}$, —$N(OR_{100})C(O)R_{100}$, —$N(OR_{100})S(O)_2R_{100}$, —$N(OR_{100})C(O)OR_{100}$, —$N(OR_{100})C(O)N(R_{100})_2$, —$N(OR_{100})C(S)N(R_{100})_2$, —$N(OR_{100})C(=NR_{109})N(R_{100})_2$, —$N(OR_{100})C(=CHR_{109})N(R_{100})_2$, —$C(=NR_{109})R_{100}$, —$C(O)N(R_{100})OR_{100}$, and —$C(=NR_{109})N(R_{100})_2$, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R_{104} is —$(CH_2)_nQ$ in which n is 1 or 2, or (ii) R_{104} is —

$(CH_2)_nCHQR_{100}$ in which n is 1, or (iii) R_{104} is —$CHQR_{100}$, and —$CQ(R_{100})_2$, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;

each R_{105} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R_{106} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

M and M′ are independently selected from —C(O)O—, —OC(O)—, —C(O)N(R′)—, —N(R′)C(O)—, —C(O)—, —C(S)—, —C(S)S—, —SC(S)—, —CH(OH)—, —P(O)(OR′)O—, —$S(O)_2$—, —S—S—, an aryl group, and a heteroaryl group;

R_{107} is selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

R_{108} is selected from the group consisting of C_{3-6}carbocycle and heterocycle;

R_{109} is selected from the group consisting of H, CN, NO_2, C_{1-6}alkyl, —OR_{100}, —$S(O)_2R_{100}$, —$S(O)_2N(R_{100})_2$, C_{2-6}alkenyl, C_{3-6}carbocycle and heterocycle;

each R_{100} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R′ is independently selected from the group consisting of C_{1-18}alkyl, C_{2-18}alkenyl, —R*YR″, —YR″, and H;

each R″ is independently selected from the group consisting of C_{3-14}alkyl and C_{3-14}alkenyl;

each R* is independently selected from the group consisting of C_{1-12}alkyl and C_{2-12}alkenyl;

each Y is independently a C_{3-6}carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts, isotopes, or isomers thereof.

In still other embodiments, another subset of compounds of Formula (I) includes those in which

R_{101} is selected from the group consisting of C_{5-30}alkyl, C_{5-20}alkenyl, —R*YR″, —YR″, and —R″M′R′;

R_{102} and R_{103} are independently selected from the group consisting of H, C_{1-14}alkyl, C_{2-14}alkenyl,

—R*YR″, —YR″, and —R*OR″, or R_{102} and R_{103}, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_{104} is selected from the group consisting of a C_{3-6}carbocycle, —$(CH_2)_nQ$, —$(CH_2)_nCHQR_{100}$, —$CHQR_{100}$, —$CQ(R_{100})_2$, and unsubstituted C_{1-6}alkyl, where Q is selected from a C_{3-6}carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, —OR_{100}, —$O(CH_2)_nN(R_{100})_2$, —$C(O)OR_{100}$, —$OC(O)R_{100}$, —CX_3, —CX_2H, —CXH_2, —CN, —$C(O)N(R_{100})_2$, —$N(R_{100})C(O)R_{100}$, —$N(R_{100})S(O)_2R_{100}$, —$N(R_{100})C(O)N(R_{100})_2$, —$N(R_{100})C(S)N(R_{100})_2$, —$CR_{100}N(R_{100})_2C(O)OR_{100}$, —$N(R_{100})R_{108}$, —$O(CH_2)_nOR_{100}$, —$N(R_{100})C(=NR_{109})N(R_{100})_2$, —$N(R_{100})C(=CHR_{109})N(R_{100})_2$, —$OC(O)N(R_{100})_2$, —$N(R_{100})C(O)OR_{100}$, —$N(OR_{100})C(O)R_{100}$, —$N(OR_{100})S(O)_2R_{100}$, —$N(OR_{100})C(O)OR_{100}$, —$N(OR_{100})C(O)N(R_{100})_2$, —$N(OR_{100})C(S)N(R_{100})_2$, —$N(OR_{100})C(=NR_{109})N(R_{100})_2$, —$N(OR_{100})C(=CHR_{109})N(R_{100})_2$, —$C(=NR_{109})R_{100}$, —$C(O)N(R_{100})OR_{100}$, and —$C(=NR_{109})N(R_{100})_2$, and each n is independently selected from 1, 2, 3, 4, and 5;

each R_{105} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R_{106} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

M and M′ are independently selected from —C(O)O—, —OC(O)—, —C(O)N(R′)—, —N(R′)C(O)—, —C(O)—, —C(S)—, —C(S)S—, —SC(S)—, —CH(OH)—, —P(O)(OR′)O—, $—S(O)_2—$, —S—S—, an aryl group, and a heteroaryl group;

R_{107} is selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

R_{108} is selected from the group consisting of C_{3-6}carbocycle and heterocycle;

R_{109} is selected from the group consisting of H, CN, NO_2, C_{1-6}alkyl, —OR_{100}, —$S(O)_2R_{100}$, —$S(O)_2N(R_{100})_2$, C_{2-6}alkenyl, C_{3-6}carbocycle and heterocycle;

each R_{100} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R′ is independently selected from the group consisting of C_{1-18}alkyl, C_{2-18}alkenyl, —R*YR″, —YR″, and H;

each R″ is independently selected from the group consisting of C_{3-14}alkyl and C_{3-14}alkenyl;

each R* is independently selected from the group consisting of C_{1-12}alkyl and C_{2-12}alkenyl;

each Y is independently a C_{3-6}carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts, isotopes, or isomers thereof.

In yet other embodiments, another subset of compounds of Formula (I) includes those in which

R_{101} is selected from the group consisting of C_{5-30}alkyl, C_{5-20}alkenyl, —R*YR″, —YR″, and —R″M′R′;

R_{102} and R_{103} are independently selected from the group consisting of H, C_{2-14}alkyl, C_{2-14}alkenyl,

—R*YR″, —YR″, and —R*OR″, or R_{102} and R_{103}, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_{104} is $—(CH_2)_nQ$ or $—(CH_2)_nCHQR_{100}$, where Q is $—N(R_{100})_2$, and n is selected from 3, 4, and 5;

each R_{105} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R_{106} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

M and M′ are independently selected from —C(O)O—, —OC(O)—, —C(O)N(R′)—, —N(R′)C(O)—, —C(O)—, —C(S)—, —C(S)S—, —SC(S)—, —CH(OH)—, —P(O)(OR′)O—, $—S(O)_2—$, —S—S—, an aryl group, and a heteroaryl group;

R_{107} is selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R_{100} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R′ is independently selected from the group consisting of C_{1-18}alkyl, C_{2-18}alkenyl, —R*YR″, —YR″, and H;

each R″ is independently selected from the group consisting of C_{3-14}alkyl and C_{3-14}alkenyl;

each R* is independently selected from the group consisting of C_{1-12}alkyl and C_{1-12}alkenyl;

each Y is independently a C_{3-6}carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts, isotopes, or isomers thereof.

In still other embodiments, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30}alkyl, C_{5-20}alkenyl, —R*YR″, —YR″, and —R″M′R′;

R_{102} and R_{103} are independently selected from the group consisting of C_{1-14} alkyl, C_{2-14} alkenyl, —R*YR″, —YR″, and —R*OR″, or R_{102} and R_{103}, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_{104} is selected from the group consisting of —$(CH_2)_nQ$, —$(CH_2)_nCHQR_{100}$, —$CHQR_{100}$, and —$CQ(R_{100})_2$, where Q is —$N(R_{100})_2$, and n is selected from 1, 2, 3, 4, and 5;

each R_{105} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R_{106} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

M and M′ are independently selected from —C(O)O—, —OC(O)—, —C(O)N(R′)—, —N(R′)C(O)—, —C(O)—, —C(S)—, —C(S)S—, —SC(S)—, —CH(OH)—, —P(O)(OR′)O—, —$S(O)_2$—, —S—S—, an aryl group, and a heteroaryl group;

R_{107} is selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R_{100} is independently selected from the group consisting of C_{1-3}alkyl, C_{2-3}alkenyl, and H;

each R′ is independently selected from the group consisting of C_{1-18}alkyl, C_{2-18}alkenyl, —R*YR″, —YR″, and H;

each R″ is independently selected from the group consisting of C_{3-14}alkyl and C_{3-14}alkenyl;

each R* is independently selected from the group consisting of C_{1-12}alkyl and C_{1-12}alkenyl;

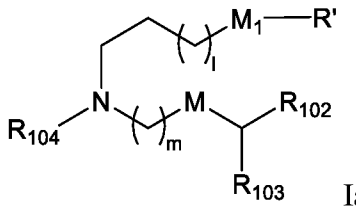
each Y is independently a C_{3-6}carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts, isotopes, or isomers thereof.

In certain embodiments, a subset of compounds of Formula (I) includes those of Formula (Ia):



Ia.

or a salt, isotope, or isomer thereof,

wherein l is selected from 1, 2, 3, 4, and 5;

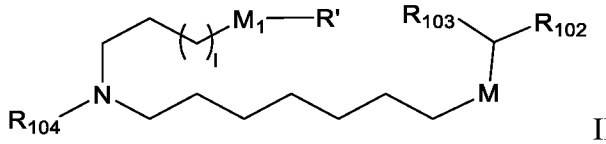
m is selected from 5, 6, 7, 8, and 9;

M_1 is a bond or M′;

R_{104} is unsubstituted C_{1-3}alkyl, or $—(CH_2)_nQ$, in which Q is OH, $—NHC(S)N(R_{100})_2$, $—NHC(O)N(R_{100})_2$, $—N(R_{100})C(O)R_{100}$, $—N(R_{100})S(O)_2R_{100}$, $—N(R_{100})R_{108}$, $—NHC(=NR_{109})N(R_{100})_2$, $—NHC(=CHR_{109})N(R_{100})_2$, $—OC(O)N(R_{100})_2$, $—N(R_{100})C(O)OR_{100}$, heteroaryl or heterocycloalkyl;

M and M′ are independently selected from —C(O)O—, —OC(O)—, —C(O)N(R′)—, —P(O)(OR′)O—, —S—S—, an aryl group, and a heteroaryl group; and R_{102} and R_{103} are independently selected from the group consisting of H, C_{1-14}alkyl, and C_{2-14}alkenyl.

In certain embodiments, a subset of compounds of Formula (I) includes those of Formula (II):



II.

or a salt, isotope, or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5;

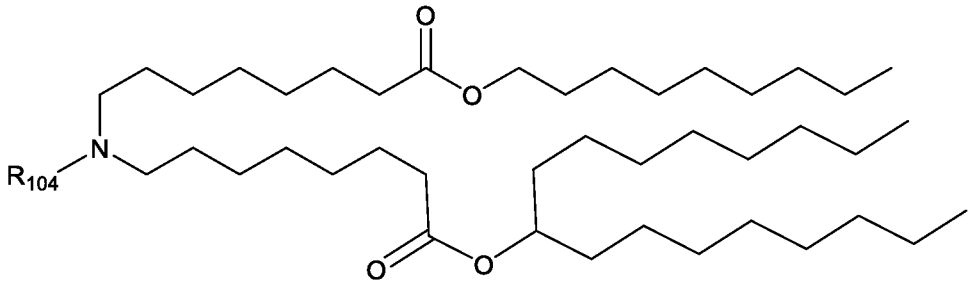
M_1 is a bond or M′;

R_{104} is unsubstituted C_{1-3}alkyl, or $—(CH_2)_nQ$, in which n is 2, 3, or 4, and Q is OH, $—NHC(S)N(R_{100})_2$, $—NHC(O)N(R_{100})_2$, $—N(R_{100})C(O)R_{100}$, $—N(R_{100})S(O)_2R_{100}$, $—N(R_{100})R_{108}$, $—NHC(=NR_{109})N(R_{100})_2$, $—NHC(=CHR_{109})N(R_{100})_2$, $—OC(O)N(R_{100})_2$, $—N(R_{100})C(O)OR_{100}$, heteroaryl or heterocycloalkyl;

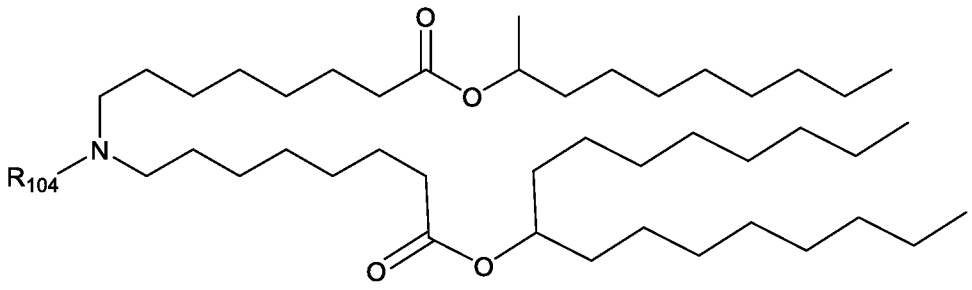
M and M′ are independently selected from —C(O)O—, —OC(O)—, —C(O)N(R′)—, —P(O)(OR′)O—, —S—S—, an aryl group, and a heteroaryl group;

and R_{102} and R_{103} are independently selected from the group consisting of H, C_{1-14}alkyl, and C_{2-14}alkenyl.

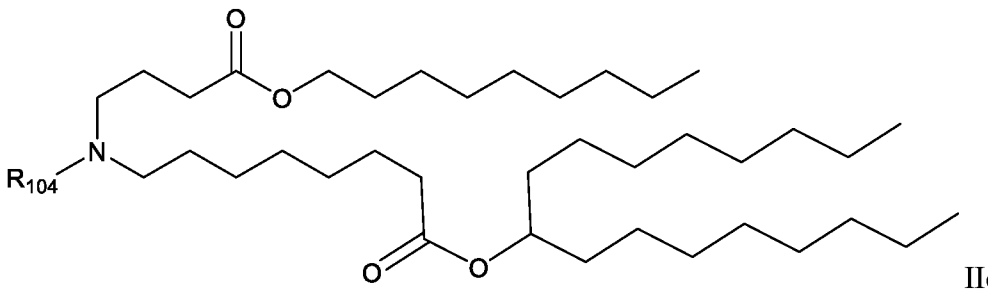
In certain embodiments, a subset of compounds of Formula (I) includes those of Formula (IIa), (IIb), (IIc), or (IIe):



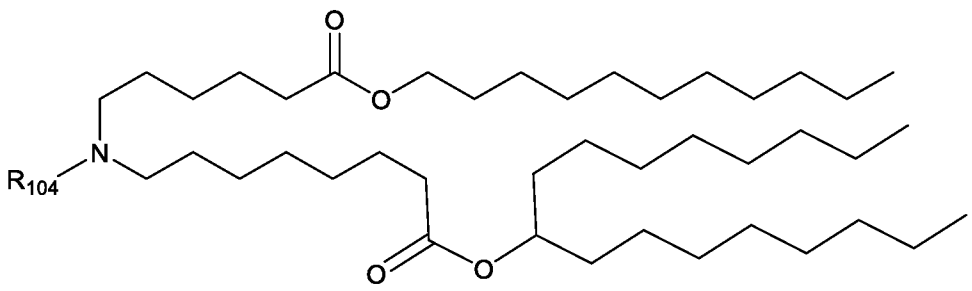
IIa.



IIb



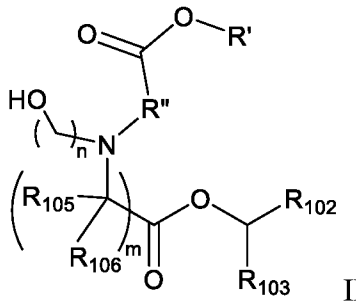
IIc



IIe

or a salt, isotope, or isomer thereof, wherein R_4 is as described herein.

In certain embodiments, a subset of compounds of Formula (I) includes those of Formula (IId):



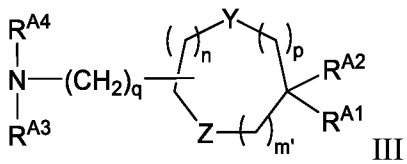
IId

or a salt, isotope, or isomer thereof, wherein n is 2, 3, or 4;

and m, R′, R″, and R_2 through R_6 are as described herein. For example, each of R_{102} and R_{103} may be independently selected from the group consisting of C_{5-14}alkyl and C_{5-14}alkenyl.

In another aspect, the disclosure features a nanoparticle composition including a lipid component comprising a compound as described herein (e.g., a compound according to Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (II)).

In certain embodiments, the ionizable lipid component of the present invention is a compound of Formula (III):



III

or a salt, isotope, or isomer thereof,

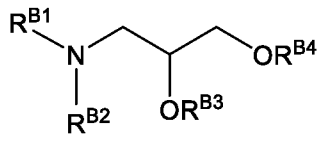
wherein R^{A1} and R^{A2} are either the same or different and independently optionally substituted C_{12}-C_{24}alkyl, optionally substituted C_{12}-C_{24}alkenyl, optionally substituted C_{12}-C_{24}alkynyl, or optionally substituted C_{12}-C_{24}acyl;

R^{A3} and R^{A4} are either the same or different and independently optionally substituted C_1-C_6alkyl, optionally substituted C_1-C_6alkenyl, or optionally substituted C_1-C_6alkynyl or R^{A3} and

R^{A4} may join to form an optionally substituted heterocyclic ring of 4 to 6 carbon atoms and 1 or 2 heteroatoms chosen from nitrogen and oxygen;

m', n, and p are either the same or different and independently either 0 or 1 with the proviso that m', n, and p are not simultaneously 0; q is 0, 1, 2, 3, or 4; and Y and Z are either the same or different and independently O, S, or NH.

In certain embodiments, the ionizable lipid component of the present invention is a compound of Formula (IV):



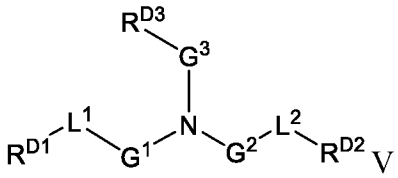
IV

or a salt, isotope, or isomer thereof,

wherein R^{B1} and R^{B2} are independently selected and are H or C_1-C_3 alkyls,

R^{B3} and R^{B4} are independently selected and are alkyl groups having from about 10 to about 20 carbon atoms, and at least one of R^{B3} and R^{B4} comprises at least two sites of unsaturation. In certain instances, R^{B3} and R^{B4} are both the same, i.e., R^{B3} and R^{B4} are both linoleyl (C_{18}), etc. In certain other instances, R^{B3} and R^{B4} are different, i.e., R^{B3} is tetradectrienyl (C_{14}) and R^{B4} is linoleyl (C_{18}). In a preferred embodiment, the cationic lipid of Formula 1 is symmetrical, i.e., R^{B3} and R^{B4} are both the same. In another preferred embodiment, both R^{B3} and R^{B4} comprise at least two sites of unsaturation. In some embodiments, R^{B3} and R^{B4} are independently selected from the group consisting of dodecadienyl, tetradecadienyl, hexadecadienyl, linoleyl, and icosadienyl. In a preferred embodiment, R^{B3} and R^{B4} are both linoleyl. In some embodiments, R^{B3} and R^{B4} comprise at least three sites of unsaturation and are independently selected from, e.g., dodecatrienyl, tetradectrienyl, hexadecatrienyl, linolenyl, and icosatrienyl.

In certain embodiments, the ionizable lipid component of the present invention is a compound of Formula (V):



V

or a salt, isotope, or isomer thereof,

wherein one of L^1 or L^2 is —O(C=O)—, —(C=O)O—, —C(=O)—, —O—, —$S(O)_v$—, —S—S—, —C(=O)S—, SC(=O)—, —$NR^aC(=O)$—, —$C(=O)NR^a$—, $NR^aC(=O)NR^a$—, —$OC(=O)NR^a$— or —$NR^aC(=O)O$—, and the other of L^1 or L^2 is —O(C=O)—, —(C=O)O—, —C(=O)—, —O—, —$S(O)_v$—, —S—S—, —C(=O)S—, SC(=O)—, —$NR^aC(=O)$—, —$C(=O)NR^a$—, $NR^aC(=O)NR^a$—, —$OC(=O)NR^a$— or —$NR^aC(=O)O$— or bond;

G^1 and G^2 are each independently unsubstituted C_1-C_{12}alkylene or C_1-C_{12}alkenylene;

G^3 is C_1-C_{24}alkylene, C_1-C_{24}alkenylene, C_3-C_8cycloalkylene, C_3-C_8cycloalkenylene;

R^a is H or C_1-C_{12}alkyl;

R^{D1} and R^{D2} are each independently C_6-C_{24}alkyl or C_6-C_{24}alkenyl;

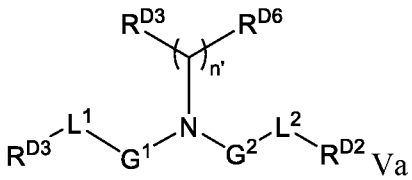
R^{D3} is H, OR^{D5}, CN, —$C(=O)OR^{D4}$, —$OC(=O)R^{D4}$ or —$NR^{D5}C(=O)R^{D4}$;

R^{D4} is C_1-C_{12}alkyl;

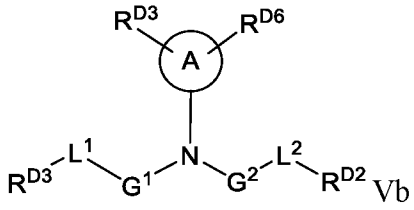
R^{D5} is H or C_1-C_6alkyl; and

v is 0, 1 or 2.

In certain embodiments, the ionizable lipid component of the present invention is a compound of Formula (Va) or (Vb):



Va



Vb

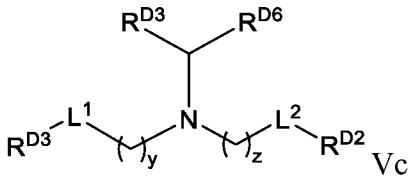
or a salt, isotope, or isomer thereof, wherein

A is a 3 to 8-membered cycloalkyl or cycloalkylene ring;

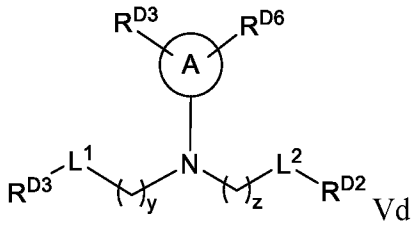
R^{D6} is, at each occurrence, independently H, OH or C_1-C_{24}alkyl;

n' is an integer ranging from 1 to 15.

In certain embodiments, the ionizable lipid component of the present invention is a compound of Formula (Vc) or (Vd):



Vc

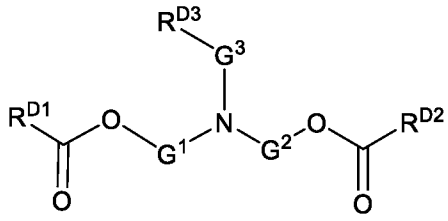


Vd

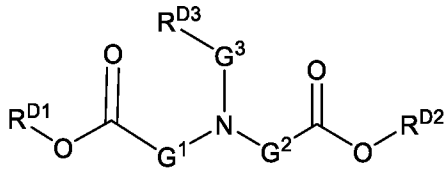
or a salt, isotope, or isomer thereof, wherein

y and z are each independently integers ranging from 1 to 12.

In certain embodiments, the ionizable lipid component of the present invention is a compound of Formula (Ve) or (Vf):



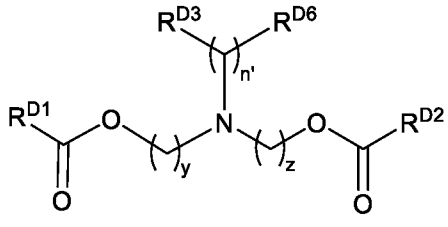
Ve



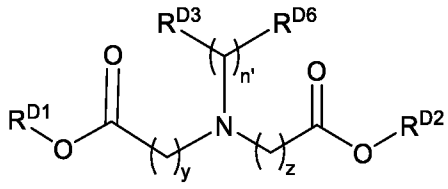
Vf

or a salt, isotope, or isomer thereof.

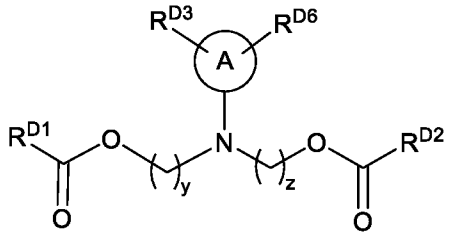
In certain embodiments, the ionizable lipid component of the present invention is a compound of Formula (Vg), (Vh), (Vi), or (Vj):



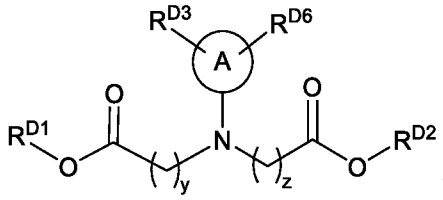
Vg



Vh



Vi



Vj

or a salt, isotope, or isomer thereof.

In some embodiments of Formula (V), n is an integer ranging from 2 to 12, for example from 2 to 8 or from 2 to 4. For example, in some embodiments, n is 3, 4, 5 or 6. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5. In some embodiments, n is 6.

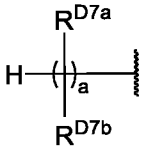
In some embodiments of Formula (V), y and z are each independently an integer ranging from 2 to 10. For example, in some embodiments, y and z are each independently an integer ranging from 4 to 9 or from 4 to 6.

In some embodiments of Formula (V), R^{D6} is H. In other embodiments, R^{D6} is C_1-C_{24}alkyl. In other embodiments, R^{D6} is OH.

In some embodiments of Formula (V), G^3 is unsubstituted. In other embodiments, G^3 is substituted.

In various different embodiments, G^3 is linear C_1-C_{24}alkylene or linear C_1-C_{24}alkenylene.

In some other foregoing embodiments of Formula (V), R^{D1} or R^{D2}, or both, is C_6-C_{24}alkenyl. For example, in some embodiments, R^{D1} and R^{D2} each, independently have the following structure:

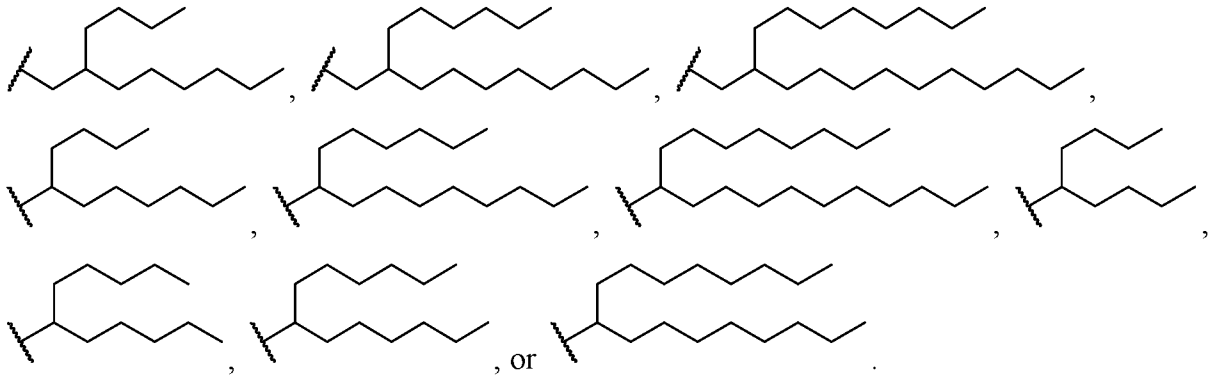


wherein R^{D7a} and R^{D7b} are, at each occurrence, independently H or C_1-C_{12}alkyl; and a is an integer from 2 to 12,

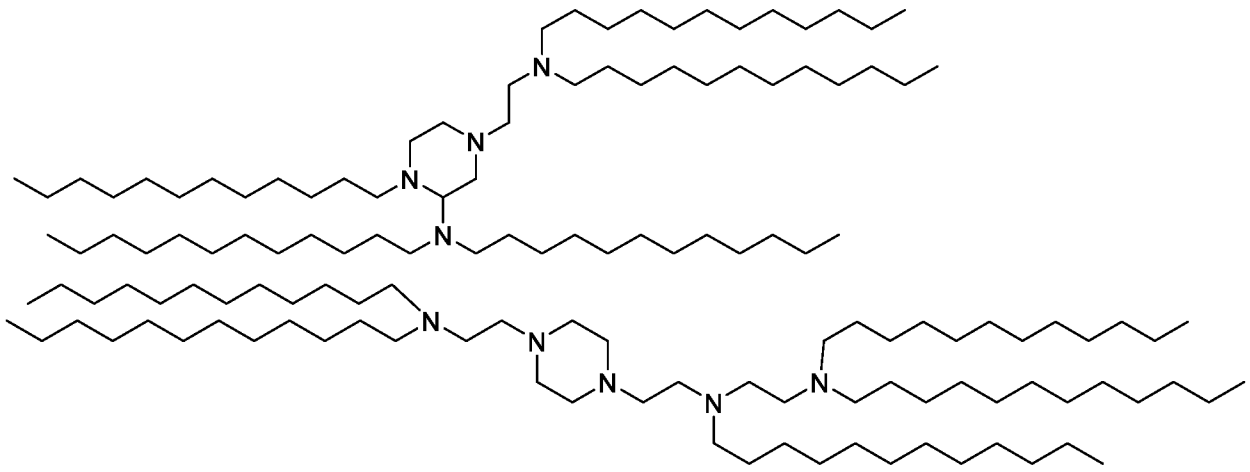
wherein R^{D7a}, R^{D7b} and a are each selected such that R^{D1} and R^{D2} each independently comprise from 6 to 20 carbon atoms. For example, in some embodiments a is an integer ranging from 5 to 9 or from 8 to 12.

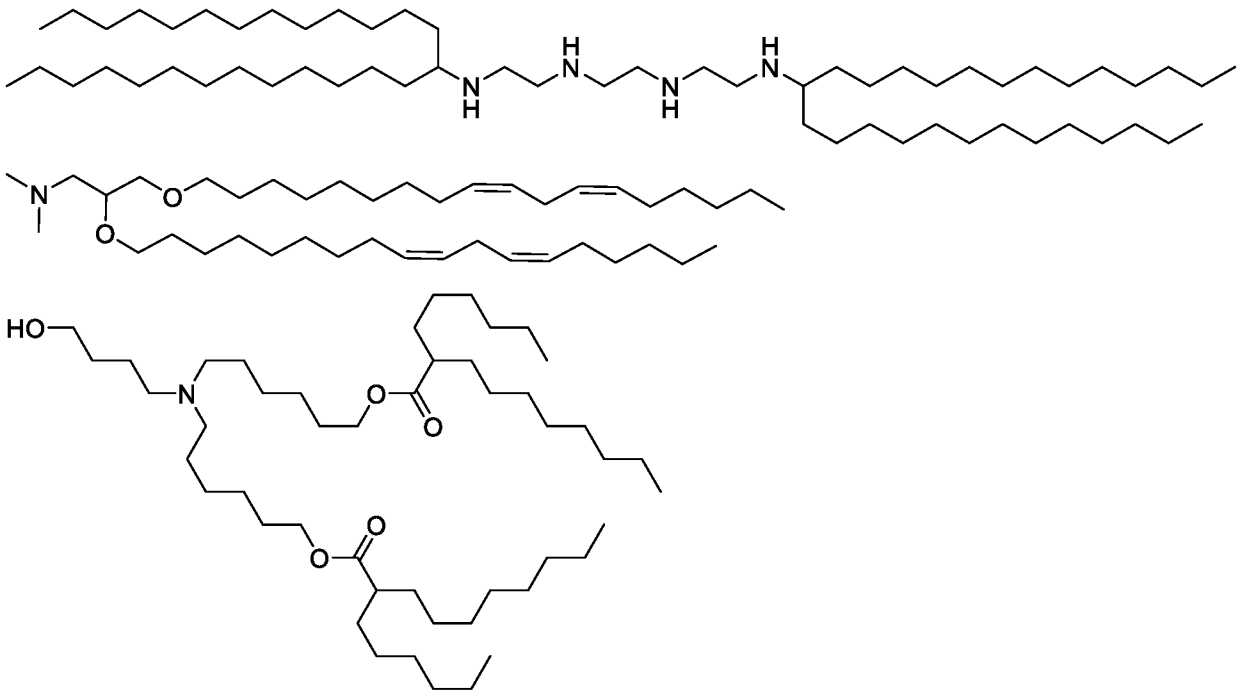
In some embodiments of Formula (V), at least one occurrence of R^{D7a} is H. For example, in some embodiments, R^{D7a} is H at each occurrence. In other embodiments, at least one occurrence of R^{D7b} is C_1-C_8alkyl. For example, in some embodiments, C_1-C_8alkyl is methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, n-hexyl or n-octyl.

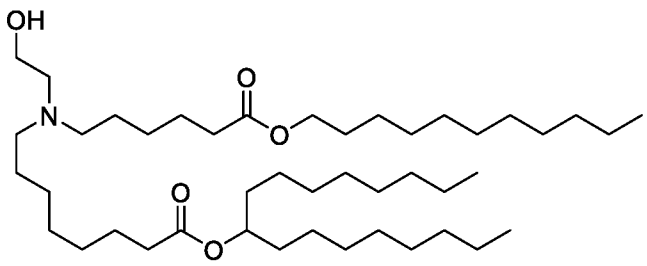
In other embodiments of Formula (V), R^{D1} or R^{D2}, or both, has one of the following structures:

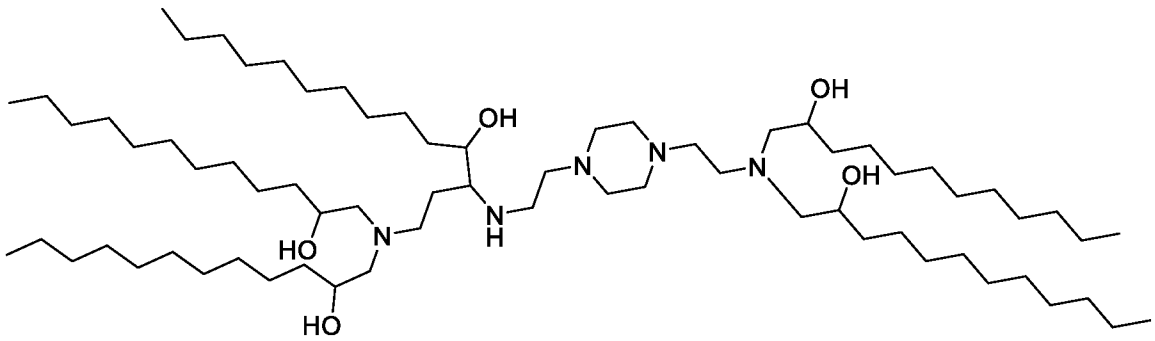


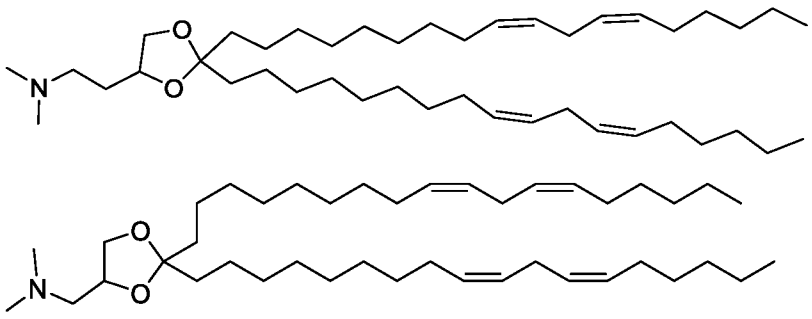
In certain embodiments the ionizable lipid component of the present invention is selected from:

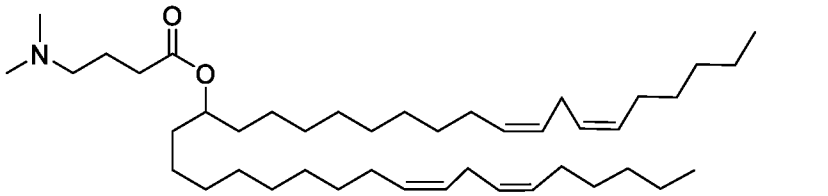


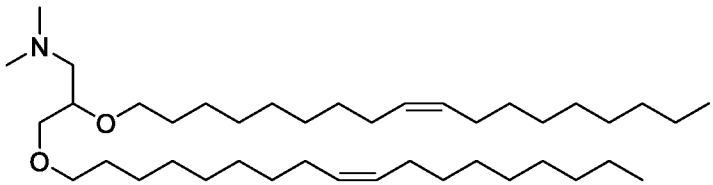


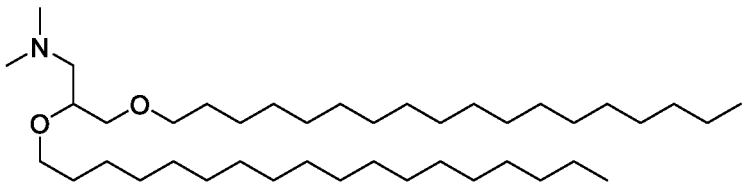


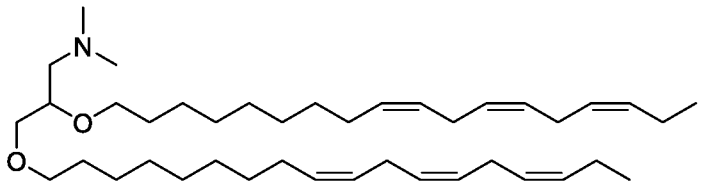


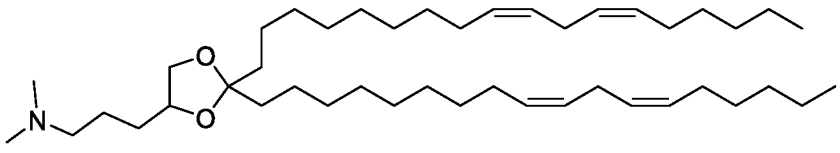


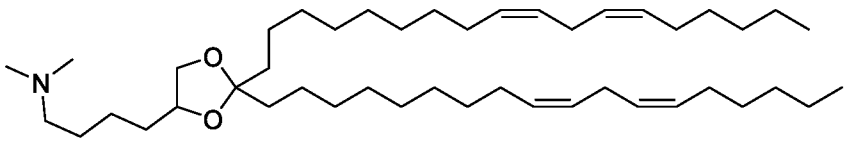


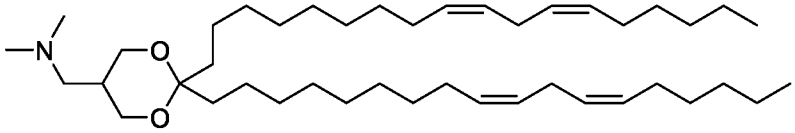


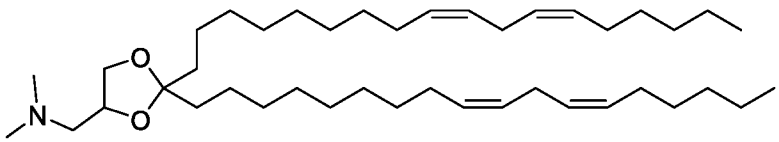


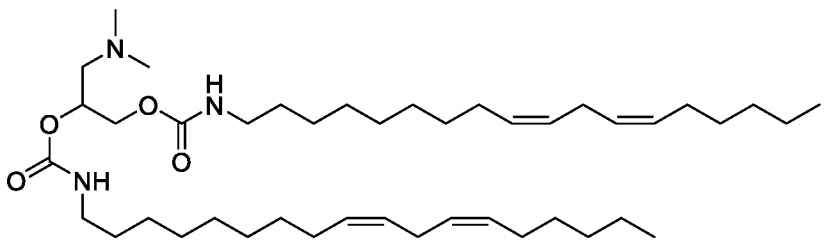


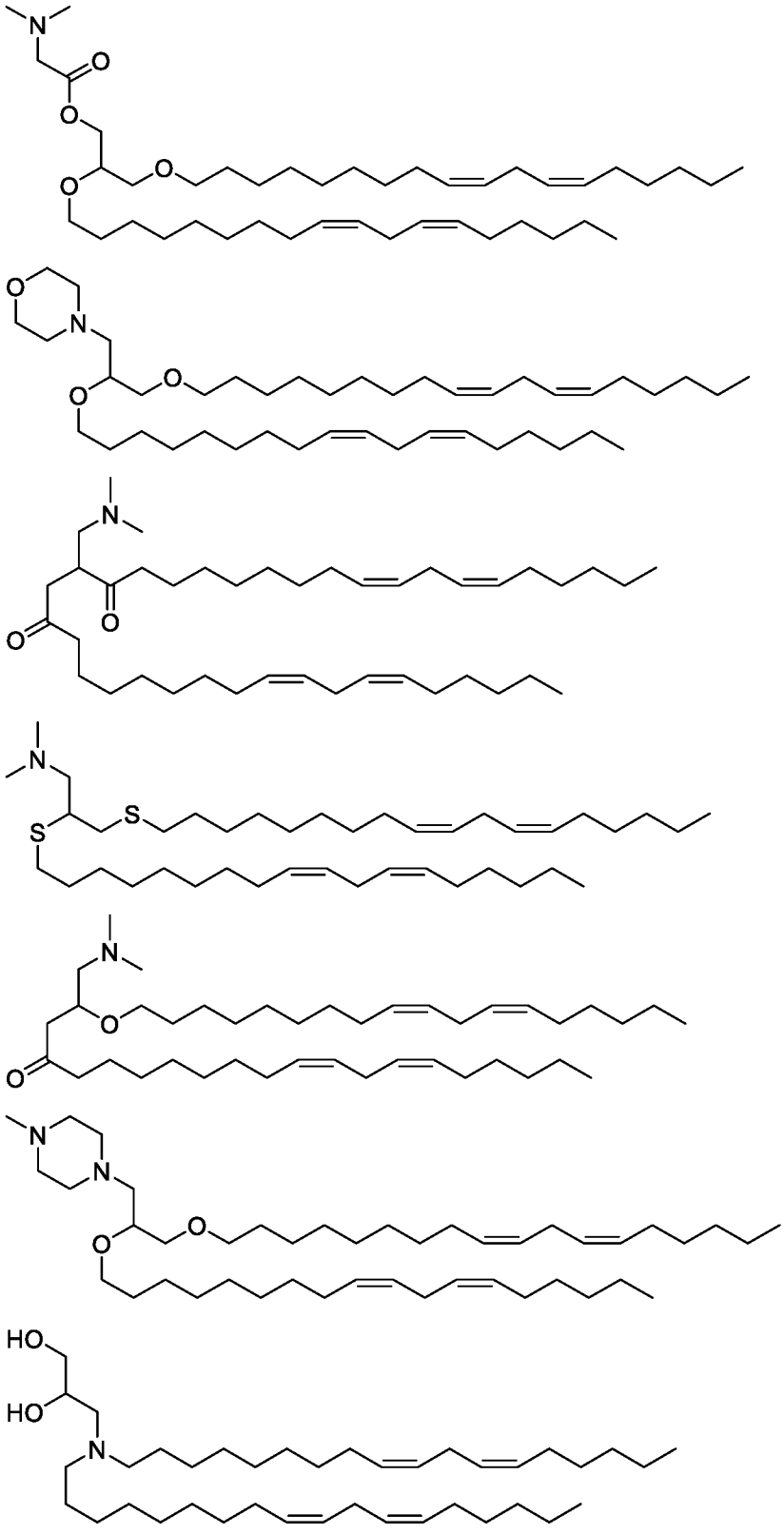


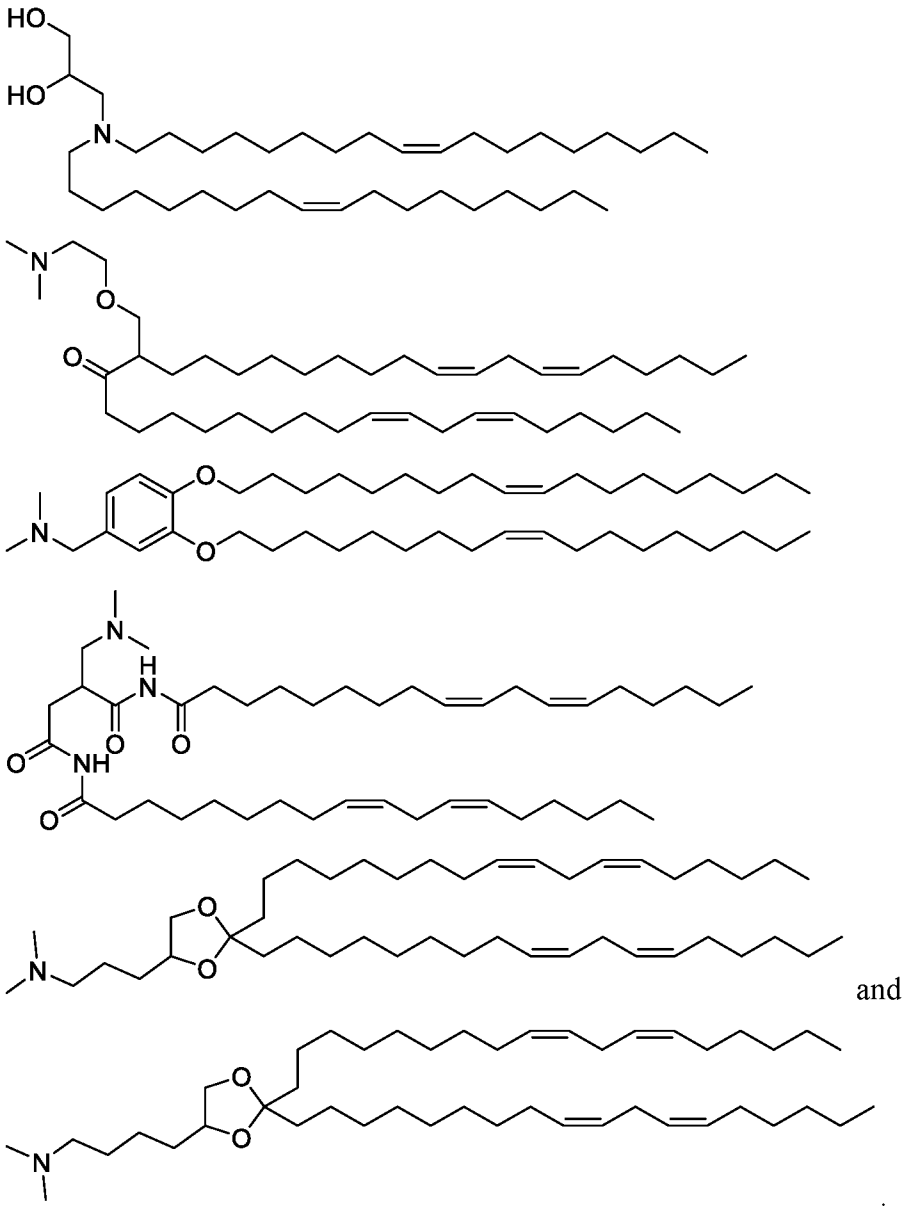












and

In certain embodiments, the ionizable lipid component of the present invention is selected from 3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazineethanamine (KL10), N1-[2-(didodecylamino)ethyl]-N1,N4,N4-tridodecyl-1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3β)-cholest-5-en-3-

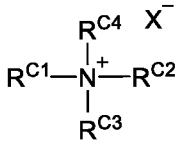
yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and (2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)), 1,2-distearyloxy-N,N-dimethyl-3-aminopropane (DSDMA), 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane (DLenDMA) 2,2-dilinoleyl-4-(3-dimethylaminopropyl)-[1,3]-dioxolane (DLin-K-C3-DMA), 2,2-dilinoleyl-4-(4-dimethylaminobutyl)-[1,3]-dioxolane (DLin-K-C4-DMA), 2,2-dilinoleyl-5-dimethylaminomethyl-[1,3]-dioxane (DLin-K6-DMA), 2,2-dilinoleyl-4-N-methylpepiazino-[1,3]-dioxolane (DLin-K-MPZ), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), 1,2-dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), 3-(N,N-dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-dioleylamino)-1,2-propanediol (DOAP), 1,2-dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 3-(N—(N′,N′-dimethylaminoethane)-carbamoyl)cholesterol (DC-Chol), dioctadecylamidoglycyl spermine (DOGS), 2-[5′-(cholest-5-en-3-beta-oxy)-3′-oxapentoxy)-3-dimethyl-1-(cis,cis-9′,1-2′-octadecadienoxy)propane (CpLinDMA), N,N-dimethyl-3,4-dioleyloxybenzylamine (DMOBA), 1,2-N,N′-dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP), 1,2-Dilinoleoylcarbamyl-3-dimethylaminopropane (DLinCDAP), 2,2-dilinoleyl-4-(3-dimethylaminopropyl)-[1,3]-dioxolane (DLin-K-C3-DMA), 2,2-dilinoleyl-4-(4-dimethylaminobutyl)-[1,3]-dioxolane (DLin-K-C4-DMA), 1,2-N,N′-dilinoleylcarbamyl-3-dimethylaminopropane (DLincarbDAP), and 1,1'-((2-(4-(2-((1-(bis(2-hydroxydodecyl)amino)-4-hydroxytetradecan-3-yl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200).

Permanently Cationic Lipids

As used herein "cationic lipid" refers to a lipid that is charged regardless of pH. Typically, these lipids are positively charged due to a quaternary ammonium moiety, including but not limited to a tetraalkylammonium group.

Permanently cationic lipids as used herein can also include a quaternary zwitterionic lipid, which is defined as a neutral lipid containing both a positive and negative charge. Under certain pH conditions the negatively charged component of the zwitterionic lipid may be partially or completely protonated, resulting in a positively charged molecule. One skilled in the art will appreciate the nature of the equilibrium of protonation under different pH conditions. Thus, an ionizable lipid or zwitterionic cationic lipid can be considered to be protonated when the protonated species is the predominant species in the equilibrium mixture.

In certain embodiments, the cationic lipid component of the present invention is a compound of Formula (VI):

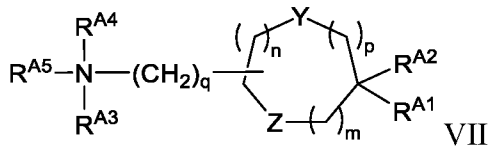


VI

wherein R^{C1} and R^{C2} are independently selected and are H or C_1-C_3alkyls,

R^{C3} and R^{C4} are independently selected and are alkyl groups having from about 10 to about 20 carbon atoms, and at least one of R^{C3} and R^{C4} comprises at least two sites of unsaturation. In certain instances, R^{C3} and R^{C4} are both the same, i.e., R^{C3} and R^{C4} are both linoleyl (C_{18}), etc. In certain other instances, R^{C3} and R^{C4} are different, i.e., R^{C3} is tetradecatrienyl (C_{14}) and R^{C4} is linoleyl (C_{18}). In a preferred embodiment, the cationic lipids of the present invention are symmetrical, i.e., R^{C3} and R^{C4} are both the same. In another preferred embodiment, both R^{C3} and R^{C4} comprise at least two sites of unsaturation. In some embodiments, R^{C3} and R^{C4} are independently selected from the group consisting of dodecadienyl, tetradecadienyl, hexadecadienyl, linoleyl, and icosadienyl. In a preferred embodiment, R^{C3} and R^{C4} are both linoleyl. In some embodiments, R^{C3} and R^{C4} comprise at least three sites of unsaturation and are independently selected from, e.g., dodecatrienyl, tetradecatrienyl, hexadecatrienyl, linolenyl, and icosatrienyl.

In certain embodiments, the cationic lipid component of the present invention is a compound of Formula (VII):



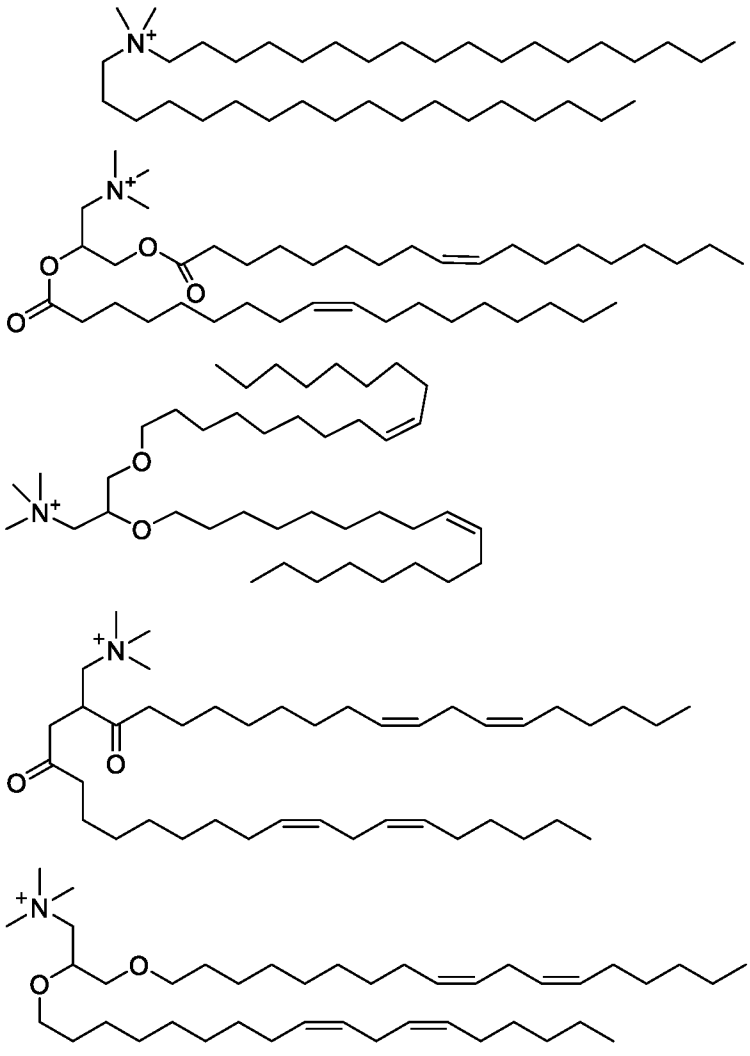
VII

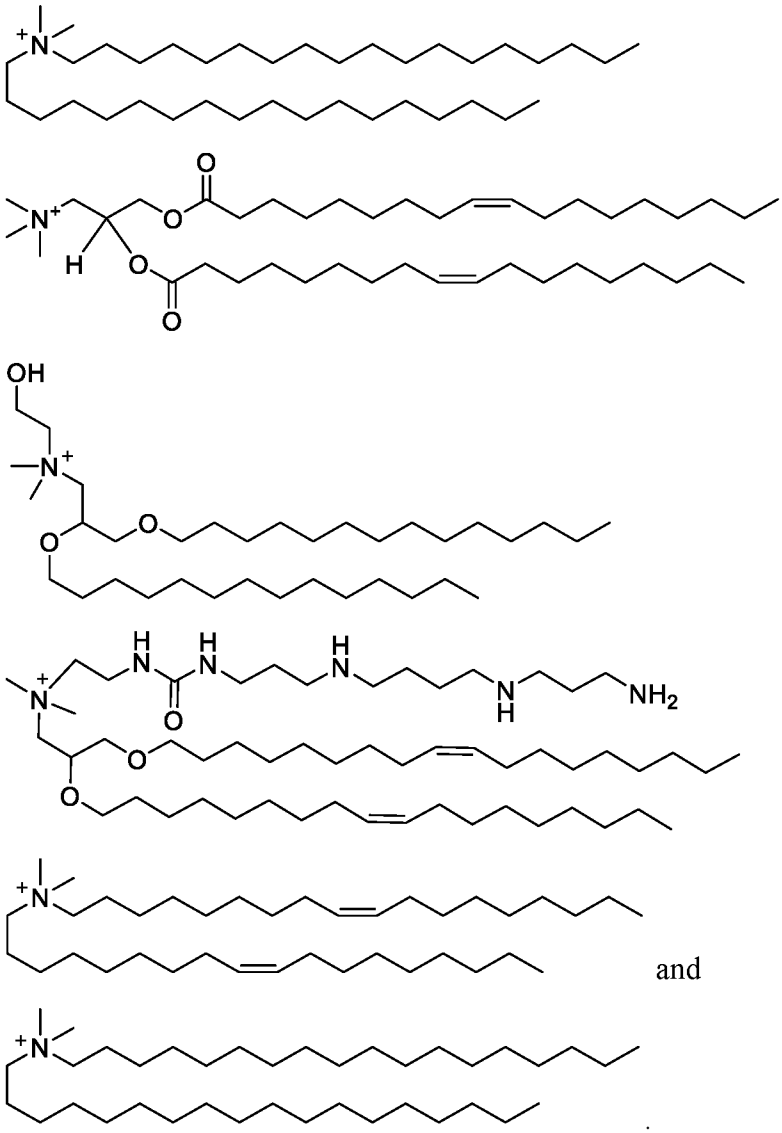
or a salt, isotope, or isomer thereof,

wherein R^{A5} is C_1-C_6alkyl to provide a quaternary amine; and

all other variables are as defined herein.

In certain embodiments, the cationic lipid component of the present invention is selected from:





and

In certain embodiments, the cationic lipid component of the present invention is selected from 1,2-dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC),

dimethyldioctadecylammonium chloride (DDA.Cl), dimethyldioctadecylammonium bromide (DDA.Br),

A number of exemplary cationic and ionizable lipids and related analogs have been described in patents and publications. Examples include but are not limited to: U.S. Patent Publication Nos. 20060083780 and 20060240554; U.S. Pat. Nos. 5,208,036; 5,264,618; 5,279,833; 5,283,185; 5,753,613; and 5,785,992; and PCT Publication No. WO 96/10390, the disclosures of which are each herein incorporated by reference in their entirety for all purposes. Additionally, a number of commercial preparations of cationic lipids are available and can be used in the present invention. These include, e.g., LIPOFECTIN® (commercially available cationic liposomes comprising DOTMA and DOPE, from GIBCO/BRL, Grand Island, N.Y., USA); LIPOFECTAMINE® (commercially available cationic liposomes comprising DOSPA and DOPE, from GIBCO/BRL); and TRANSFECTAM® (commercially available cationic liposomes comprising DOGS from Promega Corp., Madison, Wis., USA).

In certain embodiments the lipid formulation of the present invention is presented as a liposome. Liposomes are usually divided into three groups :multilamellar vesicles (MLV) ; small unilamellar vesicles(SUV) ; and large unilamellar vesicles (LUV). MLVs have multiple bilayers in each vesicle, forming several separatea queous compartments . SUVs and LUVs have a single bilayer encapsulating an aqueous core ; SUVs typically have a diameter 550 nm , and LUVs have a diameter > 50 nm .Liposomes of the invention are ideally LUVs with a diameter in the range of 60-180 nm , and preferably in the range of 80-160 nm.

Targeting Lipids

In certain embodiments the lipid used to form the lipid carrier helps direct the carrier to a specific target organ. Nonlimiting examples of organs that may be selectively targeted include liver, lungs, spleen, kidney, brain, and heart. Several publications disclose the use of targeting lipid carriers and are included by reference herein (Cheng, Q. et al. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing *Nature Nanotechnology*, **15**, 313 (2020); Wei, T., et al. Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing. *Nat Commun* **11,** 3232 (2020); Miller, J.B. et al. Non-viral CRISPR/Cas gene editing in virto and in vivo enabled by synthetic nanoparticle co-delivery of Cas9 mRNA and sgRNA, *Angew. Chem. Int. Ed.* **56**, 1059 (2017))

In certain non-limiting embodiments, a permanently cationic lipid is included in the lipid carrier formulation, and the lipid carrier may selectively target the lungs. In certain non-limiting embodiments, the lung targeting lipid carrier is composed of about 50% DOTAP, about 12% 5A2-SC8, about 12% DOPE, about 24% cholesterol, and/or about 2% DMG-PEG. In certain non-limiting embodiments the lipid carrier is targets the lungs by addition of GALA-cholesterol (cholesterol functionalized with the Glu-Ala-Leu-Ala peptide), Nebulized lipid nanoparticle formulations allow for delivery directly to lungs (Lokugamage, et al. Optimization of lipid nanoparticles for the delivery of nebulized therapeutic mRNA to the lungs 2021 *Nature Biomedical Engineering*. **5** 1059-1068. In certain non-limiting embodiments, the lung targeting lipid carrier contains an amount of PEG lipid for an effective delivery via nebulization. In certain non-limiting embodiments, the nebulized lung targeting lipid carrier comprises from about 5% PEG lipid to about 25% PEG lipid, in addition to one, two, three, four, or five other components.

In certain non-limiting embodiments, the targeting lipid carrier selectively targets the spleen (as described in Khalil et al. Lipid nanoparticle for cell-specific in vivo targeted delivery of nucleic acids. *Biol. Pharm. Bull.* 2020 **43**, 584-595). In certain non-limiting embodiments, the targeting lipid is an anionic lipid, and the carrier selectively targets the spleen. In certain embodiments the spleen targeting lipid carrier is composed of about 17% 5A2-SC8, about 17% DOPE, about 33% cholesterol, about 4% DMG-PEG, and about 30% 18PA. In certain non-limiting embodiments, the lipid carrier is optimized for splenic delivery and is at least or greater than 200 nm in size. In certain non-limiting embodiments, the spleen targeting lipid carrier contains one or more components known to enhance splenic exposure, such as but not limited to, phophatidylserine functionalized lipids.

In certain non-limiting embodiments, the targeting lipid is an ionizable lipid and the carrier selectively targets the liver. In certain embodiments the liver targeting lipid carrier is composed of about 20% 5A2-SC8, about 20% DOPE, about 4% cholesterol, and about 20% DODAP.

Modifications to the lipid carrier can be used to selectively target certain liver cell types, described in Kim et al. Engineered ionizable lipid nanoparticles for targeted delivery of RNA therapeutics into different types of cells in the liver, *Sci. Adv.* 2021; 7; eabf4398, Witzigmann,, D. et al. Lipid nanoparticle technology for therapeutic gene regulation in the liver, *Advanced Drug Delivery Reviews*, 2020, **159**, 344-363, Khalil et al. Lipid nanoparticle for cell-specific in vivo

targeted delivery of nucleic acids. *Biol. Pharm. Bull.* 2020 **43**, 584-595, incorporated by reference herein.

The liver contains two major cell types, hepatocytes and liver sinusoidal endothelial cells (LSECs). The LSECs contain pores known as fenestrae which have approximately 125 nm diameter. Lipid carriers which are smaller than the fenestrae are able to reach the hepatocytes, while larger carriers are effectively blocked out.

Modulation of the size of the lipid carrier is possible through changes in the composition of the carrier, for example changing the structure of the ionizable lipid or changing the quantity of the PEG lipid. In certain embodiments, the liver targeting lipid carrier is optimized for delivery to LSEC cells. In certain embodiments, the liver targeting lipid carrier is optimized for delivery to hepatocytes. In certain embodiments the hepatocyte targeting lipid carrier is optimized to be from about 40 nm to about 100 nm in size. In certain embodiments the hepatocyte targeting lipid carrier is optimized to be from about 60 nm to about 90 nm in size. In certain embodiments the hepatocyte targeting lipid carrier is optimized to be from about 65 nm to about 80 nm in size. In certain embodiments the LSEC targeting lipid carrier is optimized to be from about 120 to about 200 nm in size.

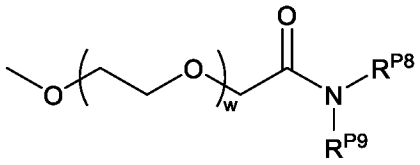
In certain non-limiting embodiments, the liver targeting lipid carrier contains one or more components known to facilitate liver exposure, for example, DOTAP, GL-67, DOPE, STR-R8, DLin-DMA, KC2, MC3, YSK05, YSK13-C3, YSK12-C4, N-acetyl-D-galactosamine (GalNAc) modified lipids, C12-200, and/or CL4H6.

Lipid carrier can also be targeted to the pancreas (described in Kokkinos et al. Targeting the undruggable in pancreatic cancer using nano-based gene silencing drugs, *Biomaterials* **240**, 2020, 119742). Due to the thick collagen layers that surround the pancreas, diffusion of lipid carriers is challenging. Through formulation of smaller lipid carriers, it is possible to administer the contents of the lipid carrier to pancreatic cells. In certain non-limiting embodiments, the lipid carrier is optimized to target pancreatic cells. In certain non-limiting embodiments, the lipid carrier is optimized to target pancreatic cells and is from about 10 nm to about 100 nm in size. In certain non-limiting embodiments, the lipid carrier is optimized to target pancreatic cells and is from about 10 nm to about 60 nm in size. In certain non-limiting embodiments, the lipid carrier is optimized to target pancreatic cells and is from about 20 nm to about 50 nm in size.

PEG Lipids

One or more lipid components of the lipid nanoparticle of the present invention may have or be modified to have one or multiple poly(ethylene glycol) units. Such lipids are referred to herein as "PEG Lipids." In certain embodiments, the PEG moiety includes 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16, 18, 20, 22, 24, 26, 28 or even 30 or more ethylene glycol moieties.

A nonlimiting example of a lipid component of the lipid particle of the present invention is a compound of Formula (VIII):



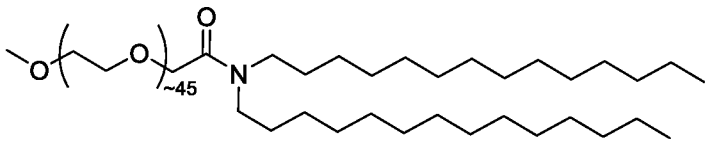
or a salt, isotope, or isomer thereof,

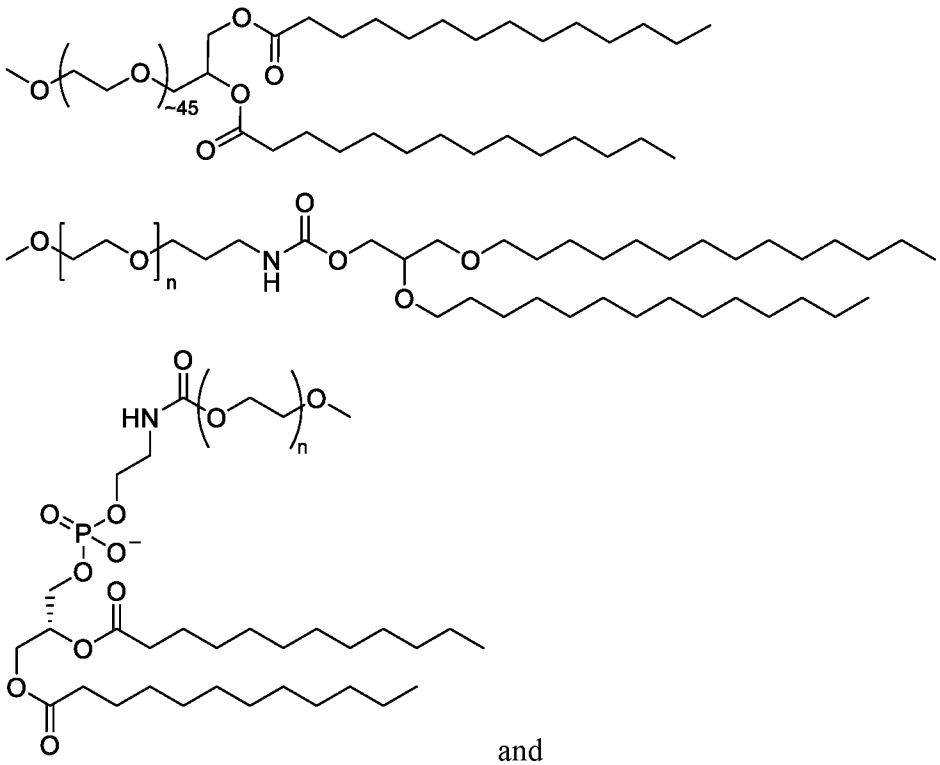
wherein R^{P8} and R^{P9} are each independently a straight or branched, saturated or unsaturated alkyl chain containing from 10 to 30 carbon atoms, wherein the alkyl chain is optionally interrupted by one or more ester bonds; and w has a mean value ranging from that provided above or even up to 4, 50 or 60 or more.

In some embodiments, R^{P8} and R^{P9} are each independently straight, saturated alkyl chains containing from 12 to 16 carbon atoms. In other embodiments, the average w ranges from 42 to 55, for example, the average w is 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54 or 55. In some specific embodiments, the average w is about 49.

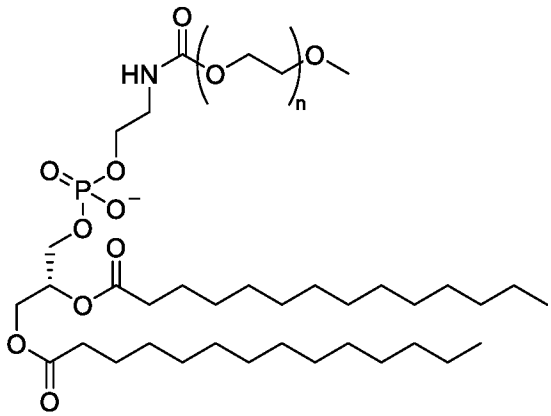
A PEG lipid component may be selected from the non-limiting group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and mixtures thereof. For example, a PEG lipid may be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, or a PEG-DSPE lipid.

In certain embodiments, the PEG lipid component of the present invention is selected from:





and

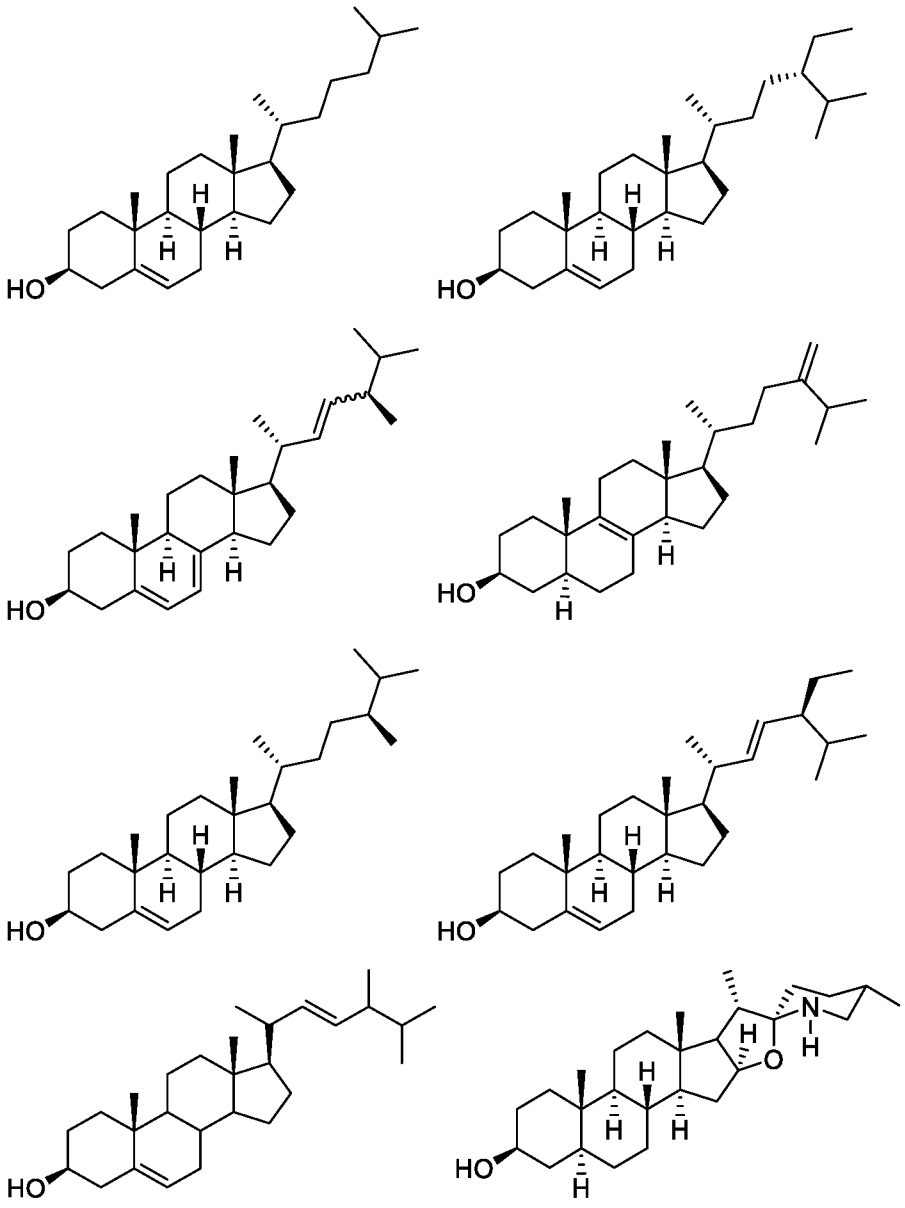


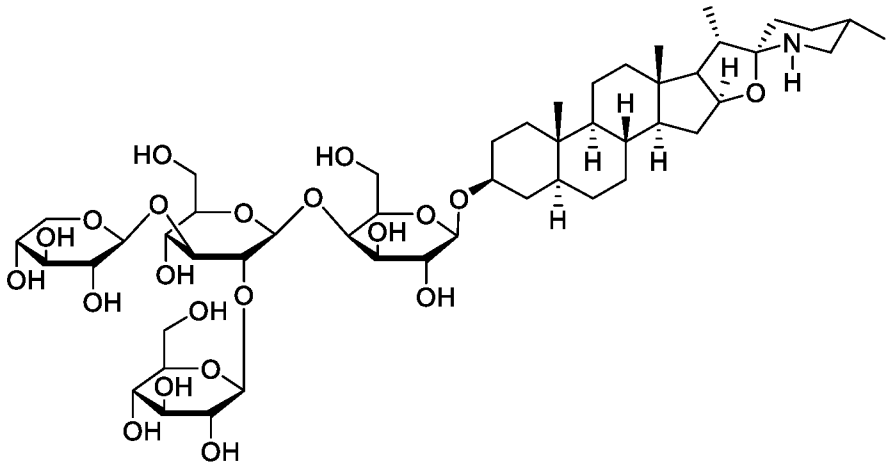
Structural Lipids

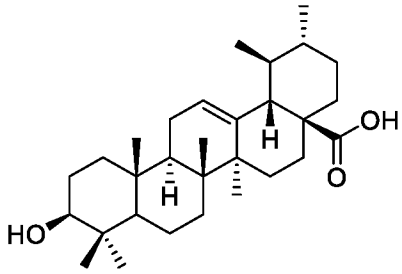
"Structural Lipids," as defined herein, are those lipids that modulate the physical properties of a lipid particle such as a liposome. Examples of such properties are membrane fluidity, melting temperature, and average particle diameter. These lipids typically take the form of sterols or sterol derivatives. Non-limiting examples of structural lipids for use in the lipid particle (which can be a liposome) of the present invention include cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, cholestanol, cholestanone, cholestenone, coprostanol, cholesteryl-2′-hydroxyethyl ether, cholesteryl-4′-hydroxybutyl ether, and mixtures thereof. In some embodiments, the structural lipid

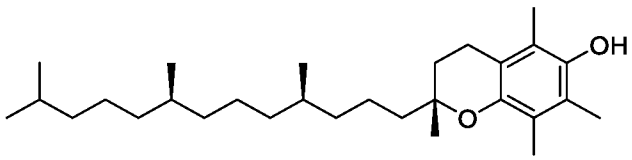
is cholesterol. In some embodiments, the structural lipid includes cholesterol and a corticosteroid (such as prednisolone, dexamethasone, prednisone, and hydrocortisone), or a combination thereof.

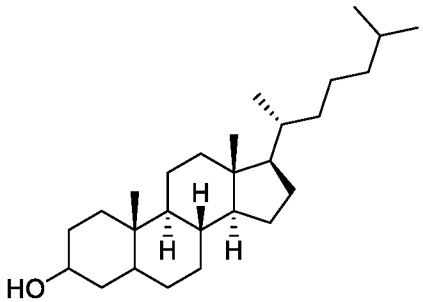
In certain embodiments, the structural lipid component of the present invention is selected from:

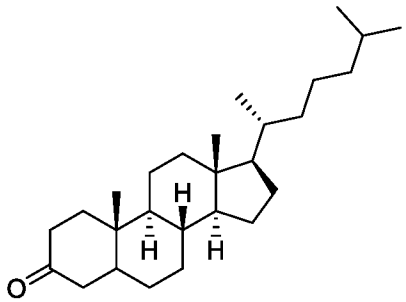


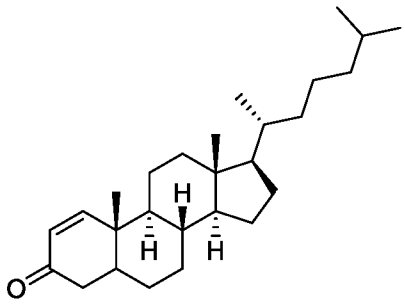


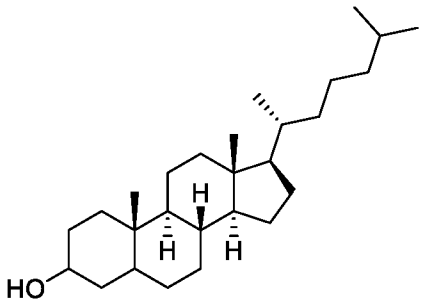


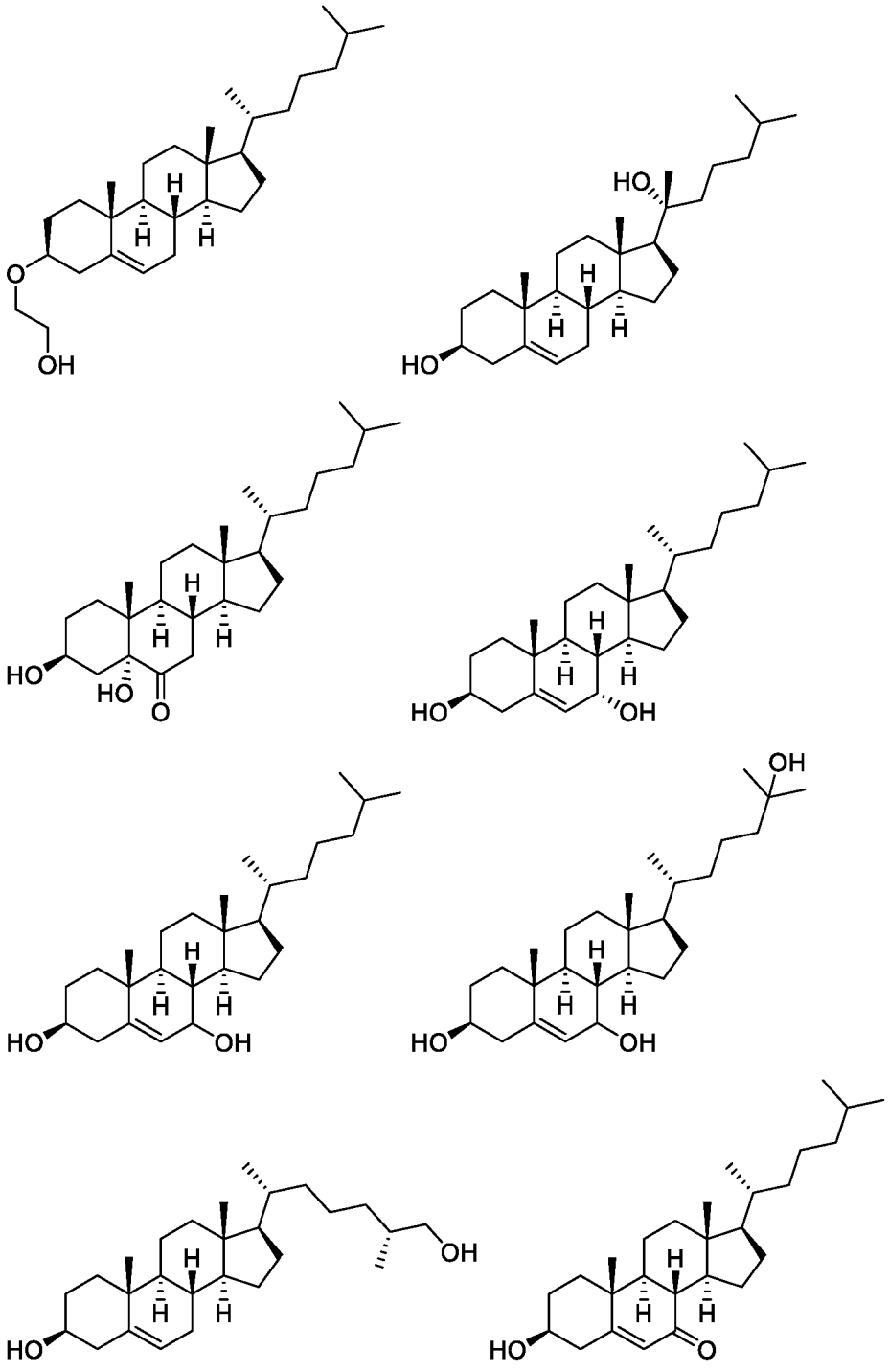


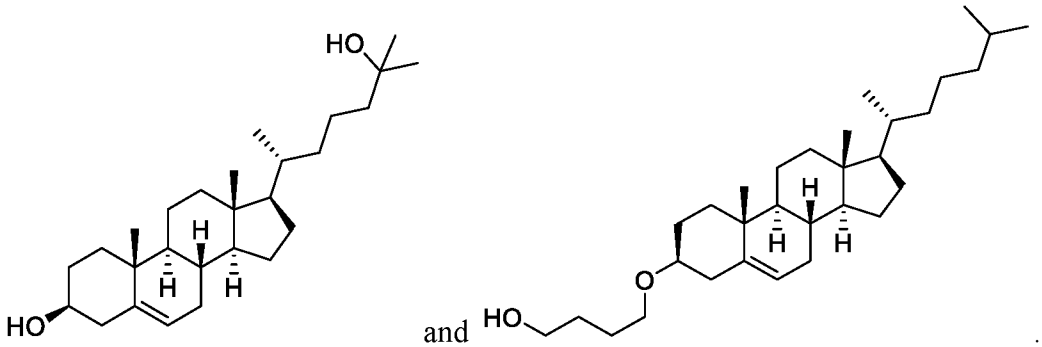












In certain embodiments the structural lipid is an alkyl resorcinol. In certain embodiments the structural lipid is a resorcinol substituted with a 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon alkyl for example 5-hepatadecyl resorcinol. In certain embodiments the structural lipid is a resorcinol substituted with a 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon alkyl.

In certain embodiments the structural lipid is 5-hepatadecylresorcinol.

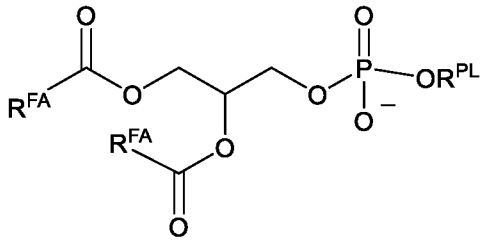
In certain embodiments the structural lipid is a cholesterol hemisuccinate.

In certain embodiments the structural lipid is a bile acid. Non-limiting examples of bile acids include taurocholic acid, glycocholic acid, taurochenodeoxycholic acid, glycochenodeoxycholic acid, deoxycholic acid, and lithocholic acid.

Phospholipids

As used herein, a "phospholipid" is a lipid that includes a phosphate moiety and one or more carbon chains, such as unsaturated fatty acid chains. A phospholipid may include one or more multiple (e.g., double or triple) bonds (e.g., one or more unsaturations). Particular phospholipids may facilitate fusion to a membrane. For example, a cationic phospholipid may interact with one or more negatively charged phospholipids of a membrane (e.g., a cellular or intracellular membrane). Fusion of a phospholipid to a membrane may allow one or more elements of a lipid-containing composition to pass through the membrane permitting, e.g., delivery of the one or more elements to a cell.

In certain embodiments, the phospholipid component of the liposome of the present invention is a compound of formula:



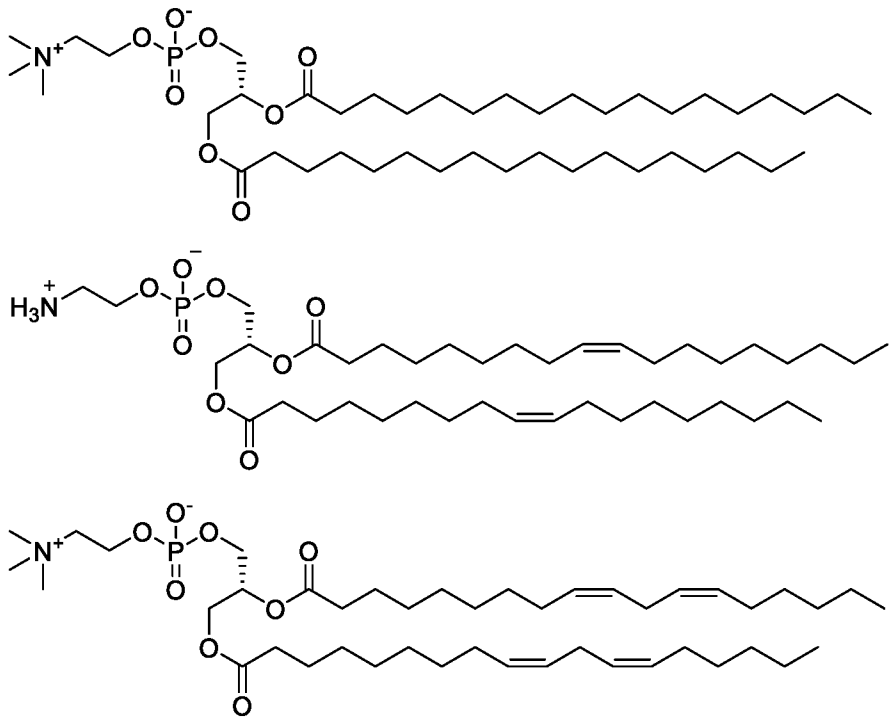
or a salt, isotope, or isomer thereof,

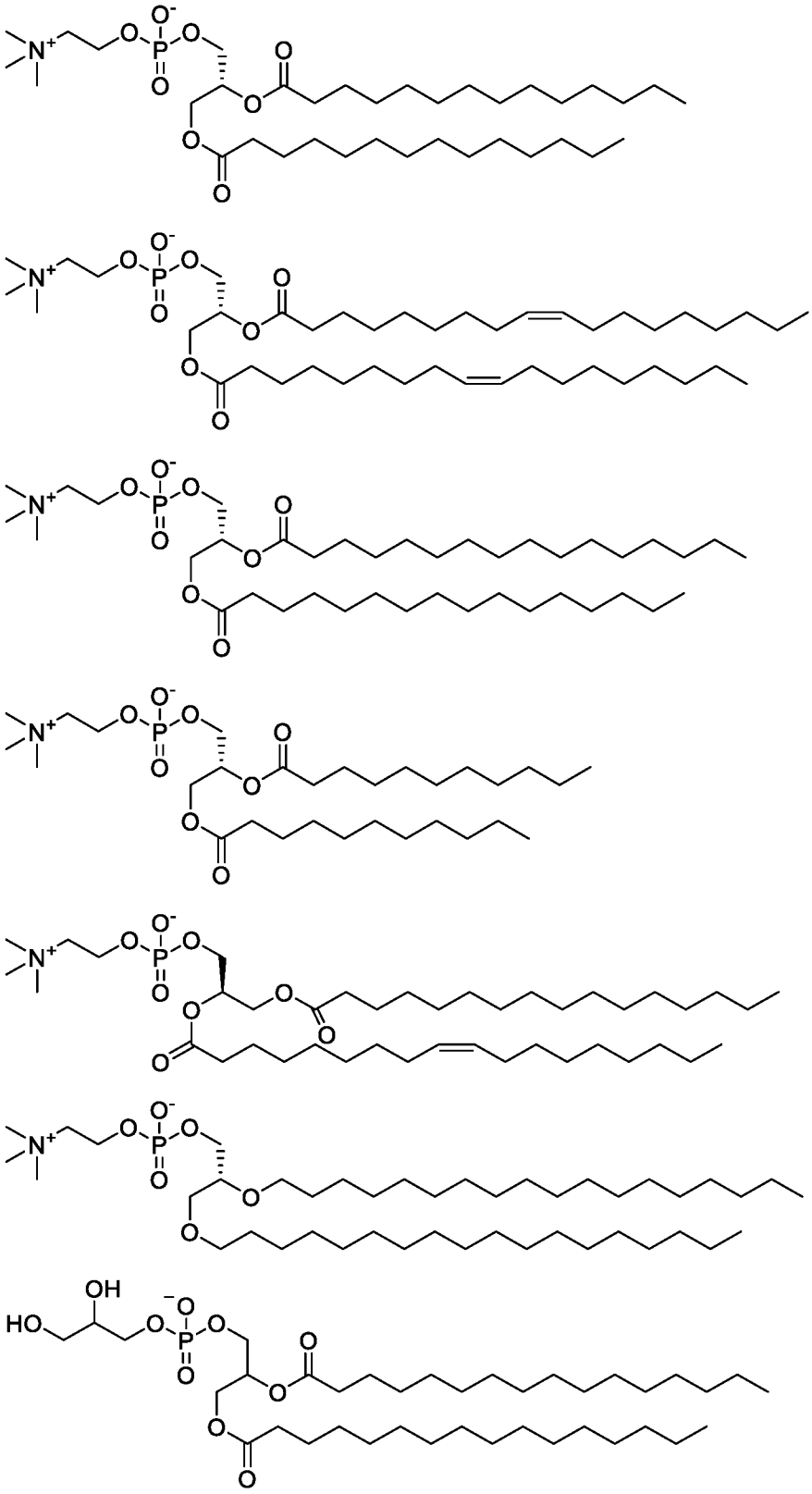
wherein R^{PL} represents a phospholipid moiety and each R^{FA} is a fatty acid moiety with or without unsaturation. R^{PL} can be a phospholipid moiety selected from the non-limiting group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl inositol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and sphingomyelin. Each fatty acid moiety for R^{FA} is independently selected from the non-limiting group consisting of lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, phytanoic acid, arachidic acid, arachidonic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid. Non-natural species including natural species with modifications and substitutions including branching, oxidation, cyclization, and alkynes are also considered. For example, a phospholipid may be functionalized with or cross-linked to one or more alkynes (e.g., an alkenyl group in which one or more double bonds is replaced with a triple bond). Under appropriate reaction conditions, an alkyne group may undergo a copper-catalyzed cycloaddition upon exposure to an azide. Such reactions may be useful in functionalizing a lipid bilayer of a nanoparticle composition to facilitate membrane permeation or cellular recognition or in conjugating a nanoparticle composition to a useful component such as a targeting or imaging moiety (e.g., a dye).

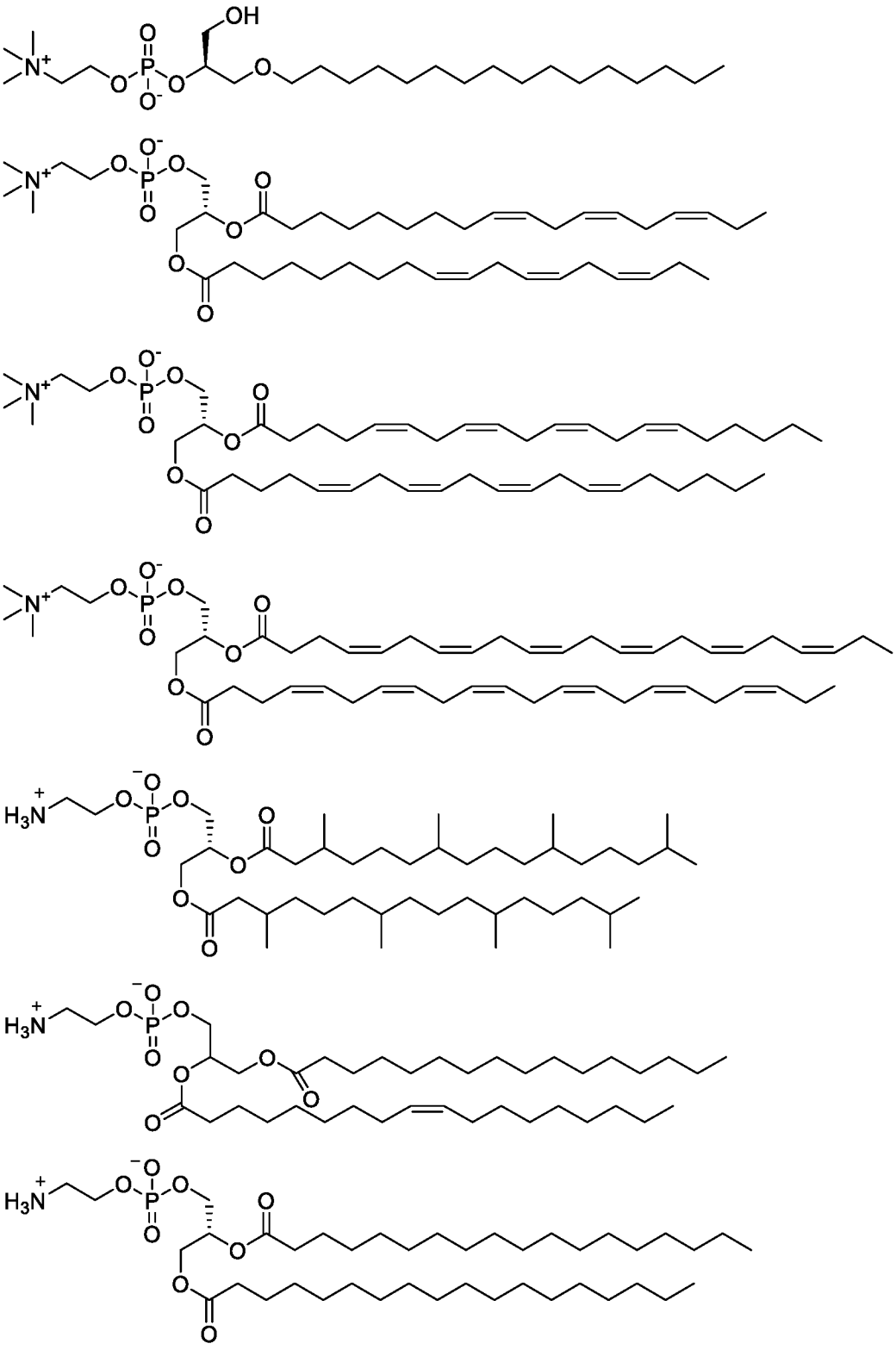
In certain embodiments the phospholipid component of the liposome of the present invention is selected from: 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), dioleoylphosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-

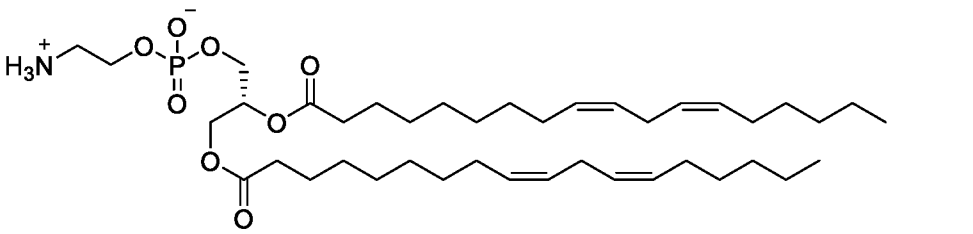
phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), dipalmitoylphosphatidylglycerol (DPPG), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), palmitoyloleoyl-phosphatidylethanolamine (POPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), palmitoyloleyol-phosphatidylglycerol (POPG), dipalmitoyl-phosphatidylethanolamine (DPPE), dimyristoyl-phosphatidylethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), dielaidoyl-phosphatidylethanolamine (DEPE), stearoyloleoyl-phosphatidylethanolamine (SOPE), egg phosphatidylcholine (EPC), and sphingomyelin.

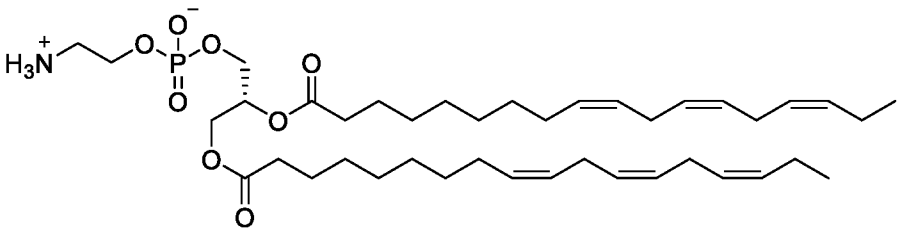
In certain embodiments the phospholipid component of the liposome of the present invention is selected from:

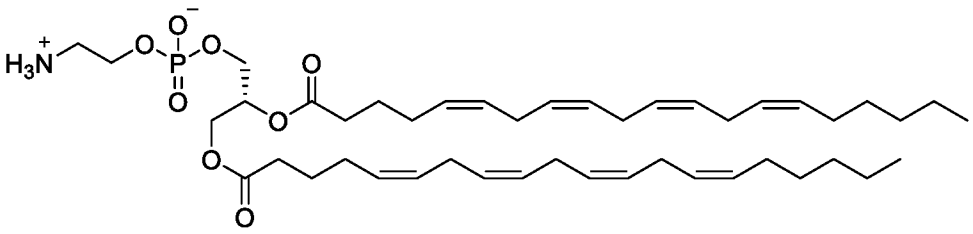


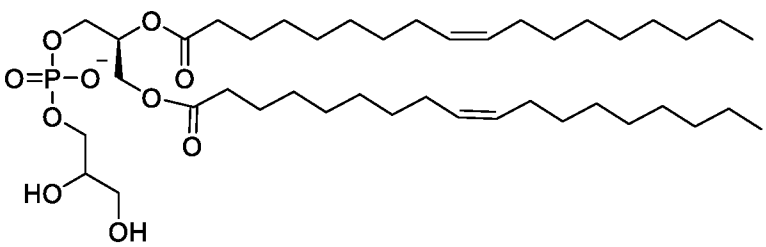


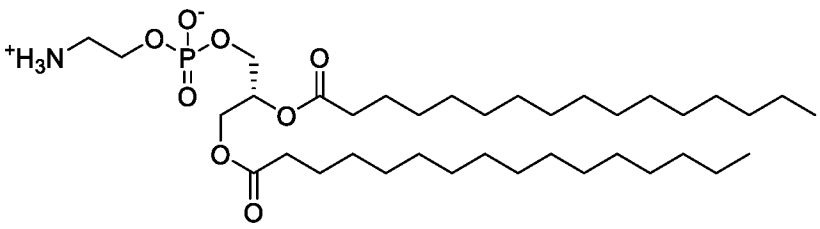


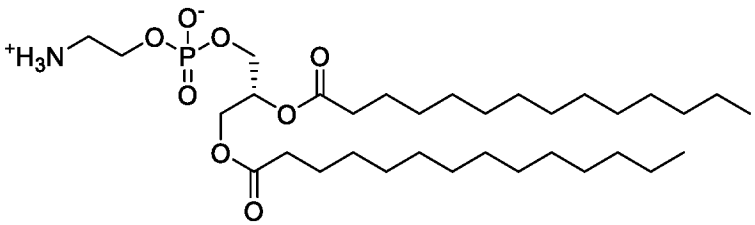




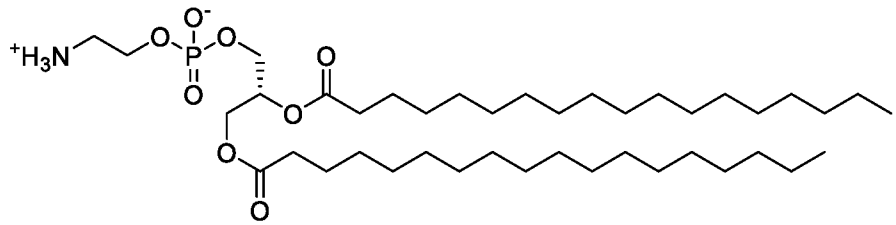


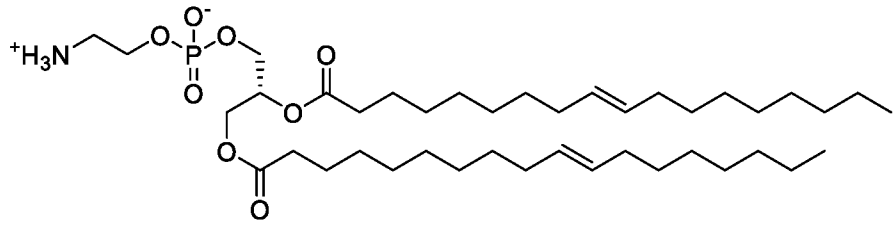


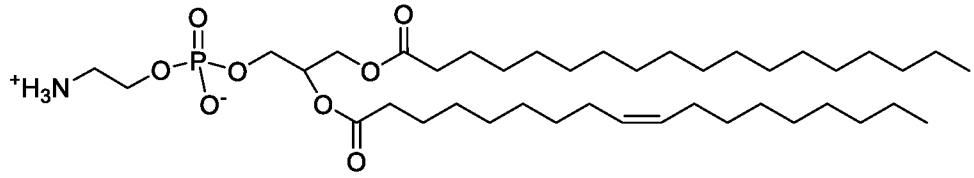




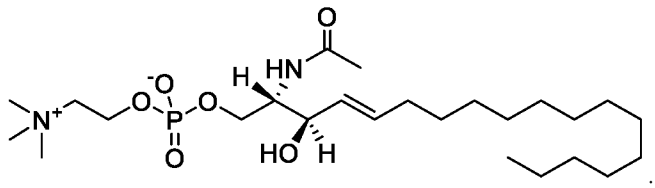
5







and



In certain embodiments the one or more phospholipids are selected from

1,2-Didecanoy 1-sn-G lycero-3-phosphatidylcholine
1,2-Dierucoyl-sn-G lycero-3-Phosphate
1,2-Erucoyl-sn-Glycero-3-phosphatidylcholine
1,2-Dierucoyl-sn-Glycero-3-phosphatidylethanolamine
1,2-Dierucoyl-sn-G lycero-3 [Phosphatidy 1-rac-(I -glycerol. ..) 1,2-Linoleoyl-sn-Glycero-3-phosphatidylcholine
1,2-Dilauroyl-sn-G lycero-3-Phosphate
1,2-Dilauroyl-sn-Glycero-3-phosphatidylcholine
1,2-Dilauroyl-sn-Glycero-3-phosphatidylethanolamine
1,2-Dilauroyl-sn-Glycero-3 [Phosphatidyl-rac-(I-glycerol. ..) 1,2-Dilauroyl-sn-Glycero-3-phosphatidylserine
1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine
1,2-Dimyristoyl-sn-G lycero-3-Phosphate

1,2-Dimyristoyl-sn-Glycero-3-phosphatidylcholine
1,2-Dimyristoyl-sn-G lycero-3-phosphatidylethanolamine 1,2-M yristoyl-sn-Glycero-3 [Phosphatidy 1-rac-(I -glycerol. ..) 1,2-Dimyristoyl-sn-Glycero-3-phosphatidylserine
1,2-Dioleoy 1-sn-G lycero-3-Phosphate
1,2-Dioleoyl-sn-Glycero-3-phosphatidylcholine
1,2-Dioleoyl-sn-Glycero-3-phosphatidylethanolamine
1,2-Dioleoy l-sn-Glycero-3 [Phosphatidy 1-rac-(I -glycerol. ..) 1,2-Dioleoyl-sn-Glycero-3-phosphatidylserine
1,2-Dipalmitoy 1-sn-G lycero-3-Phosphate
1,2-Dipalmitoyl-sn-Glycero-3-phosphatidylcholine
1,2-Dipalmitoyl-sn-G lycero-3-phosphatidylethanolamine 1,2-Dipalmitoyl-sn-Glycero-3 [Phosphatidyl-rac-(I-glycerol. ..) 1,2-Dipalmitoyl-sn-G lycero-3-phosphatidy lserine
1,2-diphytanoy l-sn-glycero-3-phosphoethanolamine
1,2-Distearoyl-sn-G lycero-3-Phosphate
1,2-Didecanoy 1-sn-G lycero-3-phosphatidylcholine
1,2-Dierucoyl-sn-G lycero-3-Phosphate
1,2-Erucoyl-sn-Glycero-3-phosphatidylcholine
1,2-Dierucoyl-sn-Glycero-3-phosphatidylethanolamine
1,2-Dierucoyl-sn-G lycero-3 [Phosphatidy 1-rac-(I -glycerol. ..) 1,2-Linoleoyl-sn-Glycero-3-phosphatidylcholine
1,2-Dilauroyl-sn-G lycero-3-Phosphate
1,2-Dilauroyl-sn-Glycero-3-phosphatidylcholine
1,2-Dilauroyl-sn-Glycero-3-phosphatidylethanolamine
1,2-Dilauroyl-sn-Glycero-3 [Phosphatidyl-rac-(I-glycerol. ..) 1,2-Dilauroyl-sn-Glycero-3-phosphatidylserine
1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine
1,2-Dimyristoyl-sn-G lycero-3-Phosphate
1,2-Distearoyl-sn-G lycero-3-phosphatidy lcholine

1,2-Diostearpyl-sn-G lycero-3-phosphatidylethanolamine
Egg-PC
Hydrogenated Egg PC
High purity Hydrogenated Soy PC
Hydrogenated Soy PC
1,2-Distearoyl-sn-Glycero-3 [Phosphatidyl-rac-(I-glycerol. ..)
1,2-Distearoyl-sn-G lycero-3-phosphatidy lserine
1-M yristoyl-sn-G lycero-3-phosphatidylcholine
l-Palmitoyl-sn-Glycero-3-phosphatidylcholine
l-Stearoyl-sn-Glycero-3-phosphatidylcholine
1-M yristoy 1,2-palmitoyl-sn-Glycero 3-phosphatidylcholine
1-M yristoyl,2-stearoyl-sn-G lycero-3-phosphatidy lcholine
1-Palmitoyl,2-myristoy l-sn-Glycero-3-phosphatidylcholin
1-Palmitoyl,2-oleoy l-sn-Glycero-3-phosphatidylcholine
1-Palmitoyl-2-oleoyl-sn-Glycero-3-phosphatidylethanolam
1,2-Dioleoyl-sn-Glycero-3 [Phosphatidyl-rac-(1-glycerol) .
1-Palmitoyl,2-stearoyl-sn-G lycero-3-phosphatidylcholine
1-Stearoyl,2-myristoyl-sn-Glycero-3-phosphatidylcholine
1-Stearoyl,2-oleoyl-sn-G lycero-3-phosphatidy lcholine
1-Stearoyl,2-palmitoyl-sn-Glycero-3-phosphatidylcholine

In certain embodiments the one or more phospholipids are selected from DDPC, DEPA, DEPC, DEPE, DEPG, DLOPC, DLPA, DLPC, DLPE, DLPG, DLPS, DMG, DMPA, DMPC, DMPE, DMPG, DMPS, DOPA, DOPC, DOPE, DOPG, DOPS, DPPA, DPPC, DPPE, DPPG, DPPS, DPyPE, DSPA, DSPC, DSPE, DSPG, DSPS, EPC, HEPC, HSPC, HSPC, L YSO PC MYRIS TIC, L YSOPC PALMITIC, L YSOPC STEARIC, Milk Sphingomyelin MPPC, MSPC, PMPC, POPC, POPE, POPG, PSPC, SMPC, SOPC, and SPPC.

IV. Lipid Formulation

The lipid delivery system of the present invention can be comprised of any ratio of one or multiple lipids selected from: ionizable lipids, cationic lipids, PEG lipids, non-charged lipids, structural lipids, and phospholipids along with any other excipient that provides the desired results.

In certain embodiments the lipid formulation is about 30 to about 70 mol % cationic and/or ionizable lipid, about 5 to about 30 mol % phospholipid, about 5 to about 50 mol % structural lipid, and about 0.5 to about 3 mol % PEG lipid, such that the combined total mol % is 100.

In certain embodiments the lipid formulation is about 35 to about 65 mol % cationic and/or ionizable lipid, about 10 to about 25 mol % phospholipid, about 10 to about 45 mol % structural lipid, and about 1 to about 2.5 mol % PEG lipid, such that the combined total mol % is 100.

In certain embodiments the lipid formulation is about 40 to about 60 mol % cationic and/or ionizable lipid, about 15 to about 20 mol % phospholipid, about 15 to about 40 mol % structural lipid, and about 1.5 to about 2 mol % PEG lipid, such that the combined total mol % is 100.

In certain embodiments the lipid formulation is about 45 to about 55 mol % cationic and/or ionizable lipid, about 16 to about 19 mol % phospholipid, about 20 to about 35 mol % structural lipid, and about 1.75 mol % PEG lipid, such that the combined total mol % is 100.

In certain embodiments the lipid formulation is about 52 mol % cationic and/or ionizable lipid, about 17.5 mol % phospholipid, about 28.75 mol % structural lipid, and about 1.75 mol % PEG lipid, such that the combined total mol % is 100.

In certain embodiments the lipid formulation is about 50 to about 65 mol % cationic and/or ionizable lipid, about 4 to about 10 mol % phospholipid, about 30 to about 40 mol % structural lipid, and about 0.5 to about 2 mol % PEG lipid, such that the combined total mol % is 100.

In certain embodiments the lipid formulation is about 57.1 mol % cationic and/or ionizable lipid, about 7.1 mol % phospholipid, about 34.3 mol % structural lipid, and about 1.5 mol % PEG lipid, such that the combined total mol % is 100.

Lipid Particle Structure

In certain embodiments of the invention, the particle encapsulating the active antiviral nucleoside 5'-(mono-, di- or tri-)phosphate or 5'-phosphate is a liposome.

In certain embodiments of the invention, the particle encapsulating the active antiviral nucleoside 5'-(mono-, di- or tri-)phosphate or 5'-phosphate is a solid lipid particle.

In certain embodiments of the invention, the particle encapsulating the active antiviral nucleoside 5'-(mono-, di- or tri-)phosphate or 5'-phosphate is a microparticle.

In certain embodiments of the invention, the particle encapsulating the active antiviral nucleoside 5'-(mono-, di- or tri-)phosphate or 5'-phosphate is a nanoparticle.

In certain embodiments of the invention, the particle encapsulating the active antiviral nucleoside 5'-(mono-, di- or tri-)phosphate or 5'-phosphate is a solid lipid nanoparticle.

In certain embodiments of the invention, the particle encapsulating the active antiviral nucleoside 5'-(mono-, di- or tri-)phosphate or 5'-phosphate is a solid lipid microparticle.

In certain embodiments of the invention, the particle encapsulating the active antiviral nucleoside 5'-(mono-, di- or tri-)phosphate or 5'-phosphate is a nanoliposome.

In certain embodiments of the invention, the particle encapsulating the active antiviral nucleoside 5'-(mono-, di- or tri-)phosphate or 5'-phosphate is a microliposome.

The lipid particle of the present invention can be prepared by well known methods widely reported in patents and literature.

In certain nonlimiting embodiments, it is prepared via ethanol drop nano or micro precipitation. This process has been described in Sabnis, S., et al., "A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates" *Mol. Ther.* **26**, 1509-1519 (2018). Briefly, the lipids are mixed in the desired ratio and dissolved in ethanol. The lipid solution is then combined with a 6.25 mM sodium acetate buffer (pH 5) containing the nucleoside triphosphate(s) at a 1:3 ratio (ethanol:aqueous) using a microfluidic mixer. Formulations are then dialyzed against PBS (pH 7.4) in dialysis cassettes for at least 18 hr. Formulations are then concentrated using Amicon ultra centrifugal filters (EMD Millipore, Billerica, MA), passed through a 0.22-μm filter, then stored at 4°C until use.

Additionally, nano or micro particles can be made with mixing processes such as microfluidics and T-junction mixing of two fluid streams, one of which contains the nucleoside triphosphate(s) and the other has the lipid components in solution at the desired ratio.

Lipid compositions may be for example prepared by combining an ionizable and/or cationic lipid, a phospholipid (such as DOPE or DSPC, obtainable from Avanti Polar Lipids, Alabaster, Ala.), a PEG lipid (such as 1,2-dimyristoyl-sn-glycerol methoxypolyethylene glycol, also known as PEG-DMG, obtainable from Avanti Polar Lipids, Alabaster, Ala.), and a structural lipid (such as cholesterol, obtainable from Sigma-Aldrich, Taufkirchen, Germany) at

concentrations of about 50 mM in ethanol. Solutions should be cooled while in storage at, for example, −20° C. Lipids are combined to yield desired molar ratios and diluted with water and ethanol to a final lipid concentration of between about 5.5 mM and about 25 mM.

Nano or micro particle compositions including a nucleoside 5'-phosphate(s) and a lipid component may for example be prepared by combining the lipid solution with a solution including the nucleoside 5'-phosphate(s) at lipid component to nucleoside 5'-phosphate(s) wt:wt ratios between about 5:1 and about 50:1. The lipid solution is rapidly injected using a NanoAssemblr microfluidic based system at flow rates between about 10 ml/min and about 18 ml/min into the nucleoside 5'-phosphate(s) solution to produce a suspension with a water to ethanol ratio between about 1:1 and about 4:1.

Nano or micro particle compositions can be processed by dialysis to remove ethanol and achieve buffer exchange. Formulations are dialyzed twice against phosphate buffered saline (PBS), pH 7.4, at volumes 200 times that of the primary product using Slide-A-Lyzer cassettes (Thermo Fisher Scientific Inc., Rockford, Ill.) with a molecular weight cutoff of 10 kD. The first dialysis is carried out at room temperature for 3 hours. The formulations are then dialyzed overnight at 4° C. The resulting nano or micro particle suspension is filtered through 0.2 μm sterile filters (Sarstedt, Nümbrecht, Germany) into glass vials and sealed with crimp closures. Nano or micro particle composition solutions of 0.01 mg/ml to 0.10 mg/ml are generally obtained.

The method described above induces nanoprecipitation and particle formation. Alternative processes including, but not limited to, T-junction and direct injection, may be used to achieve the same nanoprecipitation. Numerous other processes are also available to make microparticles, which are well known in the art.

In certain non-limiting illustrative embodiments the formulation comprises two or more lipids selected from the table below.

Table 1 Lipids for use in the present invention

#		IUPAC name	Class
1	KL10	3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazineethanamine	Ionizable lipid
2	KL22	N1-[2-(didodecylamino)ethyl]-N1,N4,N4-tridodecyl-1,4-piperazinediethanamine	Ionizable lipid
3	KL25	14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane	Ionizable lipid
4	DLin-DMA	1,2-dilinoleyloxy-N,N-dimethylaminopropane	Ionizable lipid

#		IUPAC name	Class
5	DLin-K-DMA	2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane	Ionizable lipid
6	DLin-MC3-DMA	heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate	Ionizable lipid
7	DLin-KC2-DMA	2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane	Ionizable lipid
8	DODMA	1,2-dioleyloxy-N,N-dimethylaminopropane	Ionizable lipid
9	Octyl-CLinDMA	2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine	Ionizable lipid
10	Octyl-CLinDMA (2R)	(2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine	Ionizable lipid
11	(Octyl-CLinDMA (2S))	(2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine	Ionizable lipid
12	(DSDMA),	1,2-distearyloxy-N,N-dimethyl-3-aminopropane	Ionizable lipid
13	(DLenDMA)	1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane	Ionizable lipid
14	DLin-K-C3-DMA	2,2-dilinoleyl-4-(3-dimethylaminopropyl)-[1,3]-dioxolane	Ionizable lipid
15	DLin-K-C4-DMA	2,2-dilinoleyl-4-(4-dimethylaminobutyl)-[1,3]-dioxolane	Ionizable lipid
16	DLin-K6-DMA	2,2-dilinoleyl-5-dimethylaminomethyl-[1,3]-dioxane	Ionizable lipid
17	DLin-K-DMA	2,2-dilinoleyl-4-N-methylpepiazino-[1,3]-dioxolane (DLin-K-MPZ), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane	Ionizable lipid
18	DLin-C-DAP	1,2-dilinoleylcarbamoyloxy-3-dimethylaminopropane	Ionizable lipid
19	(DLin-DAC	1,2-dilinoleyoxy-3-(dimethylamino)acetoxypropane	Ionizable lipid
20	DLin-MA	1,2-dilinoleyoxy-3-morpholinopropane	Ionizable lipid
21	DLinDAP	1,2-dilinoleoyl-3-dimethylaminopropane	Ionizable lipid
22	DLin-S-DMA	1,2-dilinoleylthio-3-dimethylaminopropane	Ionizable lipid
23	DLin-2-DMAP	1-linoleoyl-2-linoleyloxy-3-dimethylaminopropane	Ionizable lipid
24	DLin-MPZ	1,2-dilinoleyloxy-3-(N-methylpiperazino)propane	Ionizable lipid
25	DLinAP	3-(N,N-dilinoleylamino)-1,2-propanediol	Ionizable lipid

#		IUPAC name	Class
26	DOAP	3-(N,N-dioleylamino)-1,2-propanediol	Ionizable lipid
27	DLin-EG-DMA	1,2-dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane	Ionizable lipid
28	DC-Chol	3-(N—(N′,N′-dimethylaminoethane)-carbamoyl)cholesterol	Ionizable lipid
29	DOGS	dioctadecylamidoglycyl spermine	Ionizable lipid
30	CpLinDMA	2-[5′-(cholest-5-en-3-beta-oxy)-3′-oxapentoxy)-3-dimethyl-1-(cis,cis-9′,1-2′-octadecadienoxy)propane	Ionizable lipid
31	DMOBA	N,N-dimethyl-3,4-dioleyloxybenzylamine	Ionizable lipid
32	DOcarbDAP	1,2-N,N′-dioleylcarbamyl-3-dimethylaminopropane	Ionizable lipid
33	DLinCDAP	1,2-Dilinoleoylcarbamyl-3-dimethylaminopropane	Ionizable lipid
34	DLin-K-C3-DMA	2,2-dilinoleyl-4-(3-dimethylaminopropyl)-[1,3]-dioxolane	Ionizable lipid
35	DLin-K-C4-DMA	2,2-dilinoleyl-4-(4-dimethylaminobutyl)-[1,3]-dioxolane	Ionizable lipid
36	DLincarbDAP	1,2-N,N′-dilinoleylcarbamyl-3-dimethylaminopropane	Ionizable lipid
37	C12-200	1,1'-((2-(4-(2-((1-(bis(2-hydroxydodecyl)amino)-4-hydroxytetradecan-3-yl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol)	Ionizable lipid
38	C14-113	1,1',1",1'"-(((methylazanediyl)bis(ethane-2,1-diyl))bis(azanetriyl))tetrakis(tetradecan-2-ol)	Ionizable lipid
39	C12-113	1,1',1",1'"-(((methylazanediyl)bis(ethane-2,1-diyl))bis(azanetriyl))tetrakis(dodecan-2-ol)	Ionizable lipid
40	C14-120	15,24-bis(2-hydroxytetradecyl)-18,21-dioxa-15,24-diazaoctatriacontane-13,26-diol	Ionizable lipid
41	C14-96	1,1'-((3-((2-hydroxytetradecyl)(methyl)amino)propyl)azanediyl)bis(tetradecan-2-ol)	Ionizable lipid
42	C12-120	13,22-bis(2-hydroxydodecyl)-16,19-dioxa-13,22-diazatetratriacontane-11,24-diol	Ionizable lipid
43	C18-96	1,1'-((3-((2-hydroxyoctadecyl)(methyl)amino)propyl)azanediyl)bis(octadecan-2-ol)	Ionizable lipid
44	C14-100	1,1',1",1'"-(propane-1,3-diylbis(azanetriyl))tetrakis(tetradecan-2-ol)	Ionizable lipid
45	C14-110	1,1',1",1'",1"",1""-((nitrilotris(ethane-2,1-diyl))tris(azanetriyl))hexakis(tetradecan-2-ol)	Ionizable lipid
46	C18-63	1,1'-(propane-1,3-diylbis(methylazanediyl))bis(octadecan-2-ol)	Ionizable lipid
47	C18-120	19,28-bis(2-hydroxyoctadecyl)-22,25-dioxa-19,28-diazahexatetracontane-17,30-diol	Ionizable lipid
48	C16-113	1,1',1",1'"-(((methylazanediyl)bis(ethane-2,1-diyl))bis(azanetriyl))tetrakis(hexadecan-2-ol)	Ionizable lipid

#		IUPAC name	Class
49	C12-112	1,1'-((3-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)propyl)azanediyl)bis(dodecan-2-ol)	Ionizable lipid
50	C14-112	1,1'-((3-((2-(bis(2-hydroxytetradecyl)amino)ethyl)(2-hydroxytetradecyl)amino)propyl)azanediyl)bis(tetradecan-2-ol)	Ionizable lipid
51	YSK05	1-methyl-4,4-bis[9Z,12Z]-ocatadeca-9,12-dien-1-yloxy]piperidine	Ionizable Lipid
52	YSK13-C13	3-(dimethylamino)propyl (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trienoate	Ionizable lipid
53	YSK12-C4	(6Z,9Z,28Z,31Z)-19-(4-(dimethylamino)butyl)heptatriaconta-6,9,28,31-tetraen-19-ol	Ionizable lipid
54	GL-67	N^4-cholesteryl-spermine HCl	Ionizable lipid
55	CL4H6	7-(4-(dipropylamino)butyl)-7-hydroxytridecane-1,13-diyl dioleate	Ionizable Lipid
56	DLin-TMA.Cl	1,2-dilinoleyloxy-3-trimethylaminopropane chloride salt	Cationic lipid
57	DLin-TAP.Cl	1,2-dilinoleoyl-3-trimethylaminopropane chloride salt	Cationic lipid
58	DOTMA	N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride	Cationic lipid
59	DDAB	N,N-distearyl-N,N-dimethylammonium bromide	Cationic lipid
60	DOTAP	N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride	Cationic lipid
61	DMRIE	N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide	Cationic lipid
62	DOSPA	2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate	Cationic lipid
63	DODAC	N,N-dioleyl-N,N-dimethylammonium chloride	Cationic lipid
64	DDA.Cl	dimethyldioctadecylammonium chloride	Cationic lipid
65	DDA.Br	dimethyldioctadecylammonium bromide	Cationic lipid
66	STR-R8	stearylated-octaarginine	Cationic Lipid
67	PEG-DMG	PEG-1,2-dimyristoyl-sn-glycerol	PEG Lipid
68	PEG-DLPE	PEG-1,2-Dilauroyl-sn-Glycero-3-Phosphoethanolamine	PEG Lipid
69	PEG-DMPE	PEG-N-(Methylpolyoxyethylene oxycarbonyl)-1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine	PEG Lipid
70	PEG-DPPC	PEG- dipalmitoyl phosphatidyl choline	PEG Lipid
71	PEG-DSPE	PEG-1,2-distearoyl-sn-glycero-3-phosphoethanolamine	PEG Lipid
72	DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine	Phospholipid

#		IUPAC name	Class
73	DOPE	1,2-dioleoyl-sn-glycero-3-phosphoethanolamine	Phospholipid
74	DOPE-mal	dioleoylphosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate	Phospholipid
75	DLPC	1,2-dilinoleoyl-sn-glycero-3-phosphocholine	Phospholipid
76	DMPC	1,2-dimyristoyl-sn-glycero-phosphocholine	Phospholipid
77	DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine	Phospholipid
78	DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine	Phospholipid
79	DUPC	1,2-diundecanoyl-sn-glycero-phosphocholine	Phospholipid
80	POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine	Phospholipid
81	18:0 Diether PC	1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine	Phospholipid
82	OChems PC	1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine	Phospholipid
83	DPPG	dipalmitoylphosphatidylglycerol	Phospholipid
84	C16 Lyso PC	1-hexadecyl-sn-glycero-3-phosphocholine	Phospholipid
85		1,2-dilinolenoyl-sn-glycero-3-phosphocholine	Phospholipid
86		1,2-diarachidonoyl-sn-glycero-3-phosphocholine,	Phospholipid
87		1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine	Phospholipid
88	ME 16.0 PE	1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine	Phospholipid
89	POPE	palmitoyloleoyl-phosphatidylethanolamine	Phospholipid
90		1,2-distearoyl-sn-glycero-3-phosphoethanolamine	Phospholipid
91		1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine,	Phospholipid
92		1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine	Phospholipid
93		1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine,	Phospholipid
94		1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine	Phospholipid
95	DOPG	1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt	Phospholipid
96	POPG	palmitoyloleyol-phosphatidylglycerol	Phospholipid
97	DPPE	dipalmitoyl-phosphatidylethanolamine	Phospholipid
98	DMPE	dimyristoyl-phosphatidylethanolamine	Phospholipid
99	DSPE	distearoyl-phosphatidylethanolamine	Phospholipid
100	(DEPE	dielaidoyl-phosphatidylethanolamine	Phospholipid
101	(SOPE	stearoyloleoyl-phosphatidylethanolamine	Phospholipid
102	(EPC)	egg phosphatidylcholine	Phospholipid
103		Sphingomyelin	Phospholipid
104		1,2-Didecanoy 1-sn-G lycero-3-phosphatidylcholine	Phospholipid
105		1,2-Dierucoyl-sn-G lycero-3-Phosphate	Phospholipid
106		1,2-Erucoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
107		1,2-Dierucoyl-sn-Glycero-3-phosphatidylethanolamine	Phospholipid
108		1,2-Dierucoyl-sn-G lycero-3 [Phosphatidy 1-rac-(I -glycerol)	Phospholipid
109		1,2-Linoleoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
110		1,2-Dilauroyl-sn-G lycero-3-Phosphate	Phospholipid

#		IUPAC name	Class
111		1,2-Dilauroyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
112		1,2-Dilauroyl-sn-Glycero-3-phosphatidylethanolamine	Phospholipid
113		1,2-Dilauroyl-sn-Glycero-3 [Phosphatidyl-rac-(I-glycerol)	Phospholipid
114		1,2-Dilauroyl-sn-Glycero-3-phosphatidylserine	Phospholipid
115		1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine	Phospholipid
116		1,2-Dimyristoyl-sn-G lycero-3-Phosphate	Phospholipid
117		1,2-Dimyristoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
118		1,2-Dimyristoyl-sn-G lycero-3-phosphatidylethanolamine	Phospholipid
119		1,2-M yristoyl-sn-Glycero-3 [Phosphatidy 1-rac-(I -glycerol)	Phospholipid
120		1,2-Dimyristoyl-sn-Glycero-3-phosphatidylserine	Phospholipid
121		1,2-Dioleoy 1-sn-G lycero-3-Phosphate	Phospholipid
122		1,2-Dioleoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
123		1,2-Dioleoyl-sn-Glycero-3-phosphatidylethanolamine	Phospholipid
124		1,2-Dioleoy l-sn-Glycero-3 [Phosphatidy 1-rac-(I -glycerol)	Phospholipid
125		1,2-Dioleoyl-sn-Glycero-3-phosphatidylserine	Phospholipid
126		1,2-Dipalmitoy 1-sn-G lycero-3-Phosphate	Phospholipid
127		1,2-Dipalmitoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
128		1,2-Dipalmitoyl-sn-Glycero-3 [Phosphatidyl-rac-(I-glycerol)	Phospholipid
129		1,2-Dipalmitoyl-sn-G lycero-3-phosphatidylethanolamine	Phospholipid
130		1,2-Dipalmitoyl-sn-G lycero-3-phosphatidylserine	Phospholipid
131		1,2-diphytanoy l-sn-glycero-3-phosphoethanolamine	Phospholipid
132		1,2-Distearoyl-sn-G lycero-3-Phosphate	Phospholipid
133		1,2-Didecanoy 1-sn-G lycero-3-phosphatidylcholine	Phospholipid
134		1,2-Dierucoyl-sn-G lycero-3-Phosphate	Phospholipid
135		1,2-Erucoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
136		1,2-Dierucoyl-sn-Glycero-3-phosphatidylethanolamine	Phospholipid
137		1,2-Dierucoyl-sn-G lycero-3 [Phosphatidy 1-rac-(I -glycerol)	Phospholipid
138		1,2-Linoleoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
139		1,2-Dilauroyl-sn-G lycero-3-Phosphate	Phospholipid
140		1,2-Dilauroyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
141		1,2-Dilauroyl-sn-Glycero-3-phosphatidylethanolamine	Phospholipid
142		1,2-Dilauroyl-sn-Glycero-3 [Phosphatidyl-rac-(I-glycerol)	Phospholipid
143		1,2-Dilauroyl-sn-Glycero-3-phosphatidylserine	Phospholipid
144		1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine	Phospholipid
145		1,2-Dimyristoyl-sn-G lycero-3-Phosphate	Phospholipid
146		1,2-Distearoyl-sn-G lycero-3-phosphatidy lcholine	Phospholipid
147		1,2-Diostearpyl-sn-G lycero-3-phosphatidylethanolamine	Phospholipid
148		Egg-Phosphatidylcholine	Phospholipid
149		Hydrogenated Egg Phosphatidylcholine	Phospholipid
150		High purity Hydrogenated Soy Phosphatidylcholine	Phospholipid
151		Hydrogenated Soy Phosphatidylcholine	Phospholipid
152		1,2-Distearoyl-sn-Glycero-3 [Phosphatidyl-rac-(I-glycerol. ..)	Phospholipid
153		1,2-Distearoyl-sn-G lycero-3-phosphatidy lserine	Phospholipid
154		1-M yristoyl-sn-G lycero-3-phosphatidylcholine	Phospholipid

#		IUPAC name	Class
155		l-Palmitoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
156		l-Stearoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
157		1-M yristoy 1,2-palmitoyl-sn-Glycero 3-phosphatidylcholine	Phospholipid
158		1-M yristoyl,2-stearoyl-sn-G lycero-3-phosphatidy lcholine	Phospholipid
159		1-Palmitoyl,2-myristoy l-sn-Glycero-3-phosphatidylcholin	Phospholipid
160		1-Palmitoyl,2-oleoy l-sn-Glycero-3-phosphatidylcholine	Phospholipid
161		1-Palmitoyl-2-oleoyl-sn-Glycero-3-phosphatidylethanolam	Phospholipid
162		1,2-Dioleoyl-sn-Glycero-3 [Phosphatidyl-rac-(1-glycerol) .	Phospholipid
163		1-Palmitoyl,2-stearoyl-sn-G lycero-3-phosphatidylcholine	Phospholipid
164		1-Stearoyl,2-myristoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
165		1-Stearoyl,2-oleoyl-sn-G lycero-3-phosphatidy lcholine	Phospholipid
166		1-Stearoyl,2-palmitoyl-sn-Glycero-3-phosphatidylcholine	Phospholipid
167		(R)-2,3-bis(stearoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
168		2-ammonioethyl ((R)-2,3-bis(oleoyloxy)propyl) phosphate	Biodegradable Lipid
169		(R)-2,3-bis(((9Z,12Z)-octadeca-9,12-dienoyl)oxy)propyl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
170		(R)-2,3-bis(tetradecanoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
171		(R)-2,3-bis(oleoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
172		(R)-2,3-bis(palmitoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
173		(R)-2,3-bis(undecanoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
174		(R)-2-(oleoyloxy)-3-(palmitoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
175		(R)-2,3-bis(octadecyloxy)propyl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
176		2,3-bis(palmitoyloxy)propyl (2,3-dihydroxypropyl) phosphate	Biodegradable Lipid
177		(S)-1-(hexadecyloxy)-3-hydroxypropan-2-yl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
178		(R)-2,3-bis(((9Z,12Z,15Z)-octadeca-9,12,15-trienoyl)oxy)propyl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
179		2-ammonioethyl ((2R)-2,3-bis((3,7,11,15-tetramethylhexadecanoyl)oxy)propyl) phosphate	Biodegradable Lipid
180		(2S,3R,E)-2-acetamido-3-hydroxyoctadec-4-en-1-yl (2-(trimethylammonio)ethyl) phosphate	Biodegradable Lipid
181		Cholesterol	Structural Lipid

#		IUPAC name	Class
182		(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5S)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
183		(3S,9S,10R,13R,14R,17R)-17-((R)-5,6-dimethylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
184		(3S,5S,10S,13R,14R,17R)-10,13-dimethyl-17-((R)-6-methyl-5-methyleneheptan-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
185		(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5S)-5,6-dimethylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
186		(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5S,E)-5-ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
187		(3S,10R,13R,17R)-17-((2R,E)-5,6-dimethylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
188		(2aS,4S,5'S,6aS,6bS,8aS,9S,10S,11aS,12aS,12bR)-5',6a,8a,9-tetramethyloctadecahydrospiro[naphtho[2',1':4,5]indeno[2,1-b]furan-10,2'-piperidin]-4-ol	Structural Lipid
189		(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylic acid	Structural Lipid
190		(R)-2,5,7,8-tetramethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman-6-ol	Structural Lipid
191		(8R,9S,10S,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
192		(8R,9S,10S,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)hexadecahydro-3H-cyclopenta[a]phenanthren-3-one	Structural Lipid
193		(8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-4,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3H-cyclopenta[a]phenanthren-3-one	Structural Lipid
194		(8R,9S,10S,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
195		2-(((3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)ethan-1-ol	Structural Lipid

#		IUPAC name	Class
196		(3S,8S,9S,10R,13S,14S,17S)-17-((R)-2-hydroxy-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
197		(3S,5R,8S,9S,10R,13R,14S,17R)-3,5-dihydroxy-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)hexadecahydro-6H-cyclopenta[a]phenanthren-6-one	Structural Lipid
198		(3S,7S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,7-diol	Structural Lipid
199		(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,7-diol	Structural Lipid
200		(3S,8S,9S,10R,13R,14S,17R)-17-((R)-6-hydroxy-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,7-diol	Structural Lipid
201		(3S,8S,9S,10R,13R,14S,17R)-17-((2R,6R)-7-hydroxy-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
202		(3S,8S,9S,10R,13R,14S,17R)-3-hydroxy-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-1,2,3,4,8,9,10,11,12,13,14,15,16,17-tetradecahydro-7H-cyclopenta[a]phenanthren-7-one	Structural Lipid
203		(3S,8S,9S,10R,13R,14S,17R)-17-((R)-6-hydroxy-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	Structural Lipid
204		4-(((3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)butan-1-ol	Structural Lipid
205		taurocholic acid	Structural Lipid
206		glycocholic acid	Structural Lipid
207		taurochenodeoxycholic acid	Structural Lipid
208		glycochenodeoxycholic acid	Structural Lipid

In certain embodiments the formulation comprises an ionizable lipid and one or more lipids selected from a cationic lipid, a PEG lipid, phospholipid, biodegradable lipid, and or a structural lipid.

In certain embodiments the formulation comprises a cationic lipid, and one or more lipids selected from an ionizable lipid, a PEG lipid, phospholipid, biodegradable lipid, and or a structural lipid.

In certain embodiments the formulation comprises a PEG lipid, and one or more lipids selected from an ionizable lipid, a cationic lipid, phospholipid, biodegradable lipid, and or a structural lipid.

In certain embodiments the formulation comprises a phospholipid, and one or more lipids selected from an ionizable lipid, a cationic lipid, PEG lipid, biodegradable lipid, and or a structural lipid.

In certain embodiments the formulation comprises a biodegradable lipid, and one or more lipids selected from an ionizable lipid, a cationic lipid, PEG lipid, phospholipid, and or a structural lipid.

In certain embodiments the formulation comprises a structural lipid, and one or more lipids selected from an ionizable lipid, a cationic lipid, PEG lipid, phospholipid, and or a biodegradable lipid.

In certain embodiments one or more lipids is mannosylated.

In certain embodiments one or more lipids is glycosylated.

In certain embodiments the ionizable lipid is a lipid listed in Table 1.

In certain embodiments the cationic lipid is a lipid listed in Table 1.

In certain embodiments the PEG lipid is a lipid listed in Table 1.

In certain embodiments the phospholipid is a lipid listed in Table 1.

In certain embodiments the biodegradable lipid is a lipid listed in Table 1.

In certain embodiments the structural lipid is a lipid listed in Table 1.

V. Pharmaceutical Compositions and Dosage Forms

The 5'-monophosphate, 5'-diphopshate, or 5'-triphosphate of an active antiviral nucleoside in a cationic lipid nano or micro particle as described herein can be administered in an effective amount to a host in need thereof to treat any of the disorders described herein using any suitable

approach that achieves the desired therapeutic result. The amount and timing of the lipid nano or micro particle administration will, of course, be dependent on the host being treated, the instructions of the supervising medical specialist, on the time course of the exposure, on the manner of administration, on the pharmacokinetic properties of the particular active compound, and on the judgment of the prescribing physician. Thus, because of host-to-host variability, the dosages given below are a guideline and the physician can selected appropriate doses of the compound to achieve the treatment that the physician considers appropriate for the host. In considering the degree of treatment desired, the physician can balance a variety of factors such as age and weight of the host, presence of preexisting disease, as well as presence of other diseases.

In a typical embodiment, the 5'-monophosphate, 5'-diphosphate, or 5'-triphosphate of an active antiviral nucleoside in a cationic lipid nano or micro particle is administered orally, intravenously, topically, systemically, intravitreal, parenterally, by inhalation, injection, implant, or in suppository form, intranasally, for example as a nasal spray, via the inhaled pulmonary route, transdermally, rectally, intramuscular, inhalation, intra-aortal, intracranial, subdermal, intraperitioneal, subcutaneous, transnasal, sublingual, or rectal or by other means, in dosage unit formulations optionally containing conventional pharmaceutically acceptable carriers. The nano or micro particles are not usually administered orally because the lipid breaks down under acidic conditions and will not survive the acidity in the stomach, however, they can be administered orally if suitably protected from this degradation via an enteric coating or other means.

The pharmaceutical composition may be formulated as any pharmaceutically useful form, e.g., a transdermal patch, a subcutaneous patch, a dry powder, an inhalation formulation, in a medical device, or suppository.

The therapeutically effective dosage of the 5'-monophosphate, 5'-diphopshate, or 5'-triphosphate of an active antiviral nucleoside in a cationic lipid nano or microparticle as described herein will be determined by the health care practitioner depending on the condition, size and age of the patient as well as the route of delivery. In general, a therapeutically effective amount in a pharmaceutical dosage form may, in certain embodiments 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. The 5'-monophosphate, 5'-diphosphate, or 5'-triphosphate of an active antiviral nucleoside in a cationic lipid nano or micro

particle 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.

In certain embodiments, the 5'-monophosphate, 5'-diphosphate, or 5'-triphosphate of an active antiviral nucleoside in a cationic lipid nano or micro particle will be administered in an amount ranging from about 250 micrograms up to about 800 milligrams or more, 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.

In certain embodiments, the 5'-monophosphate, 5'-diphosphate, or 5'-triphosphate of an active antiviral nucleoside in a cationic lipid nano or micro particle is administered at least once, twice or three times a day for up to 12 weeks. In certain embodiments, the cationic lipid nano or micro particle is administered at least once a day for up to 10 weeks. In certain embodiments, the cationic lipid nano or micro particle is administered at least once a day for up to 8 weeks. In certain embodiments, the cationic lipid nano or micro particle is administered at least once a day for up to 6 weeks. In certain embodiments, the cationic lipid nano or micro particle is administered at least once a day for up to 4 weeks. In certain embodiments, the cationic lipid nano or micro particle is administered at least once a day for at least 4 weeks. In certain embodiments, the cationic lipid nano or micro particle is administered at least once a day for at least 6 weeks. In certain embodiments, the cationic lipid nano or micro particle is administered at least once a day for at least 8 weeks. In certain embodiments, the cationic lipid nano or micro particle is administered at least once a day for at least 10 weeks. In certain embodiments, the cationic lipid nano or micro particle is administered at least once a day for at least 12 weeks. In certain embodiments, the cationic lipid nano or micro particle 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, the cationic lipid nano or micro particle 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.

The pharmaceutical formulations can comprise the 5'-triphosphate, 5'-diphosphate, or 5'-monophosphate of an active antiviral nucleoside in a cationic lipid nanoparticle in any pharmaceutically acceptable carrier.

Carriers include excipients and diluents and must be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the patient being treated. The carrier can be inert or it can possess pharmaceutical benefits of its own. The amount of carrier employed in conjunction with the compound is sufficient to provide a practical quantity of material for administration per unit dose of the compound.

Classes of carriers include, but are not limited to binders, buffering agents, coloring agents, diluents, disintegrants, emulsifiers, flavorants, glidents, lubricants, preservatives, stabilizers, surfactants, tableting agents, and wetting agents. Some carriers may be listed in more than one class, for example vegetable oil may be used as a lubricant in some formulations and a diluent in others. Exemplary pharmaceutically acceptable carriers include sugars, starches, celluloses, powdered tragacanth, malt, gelatin; talc, and vegetable oils. Optional active agents may be included in a pharmaceutical composition, which do not substantially interfere with the activity of the compound of the present invention.

Depending on the intended mode of administration, the pharmaceutical compositions can be in the form of solid form or a semi-solid dosage form that the 5'-monophosphate, 5'-diphosphate, or 5'-triphosphate of an active antiviral nucleoside in a cationic lipid nanoparticle is stable in, such as, for example, suppositories, or the like, preferably in unit dosage form suitable for single administration of a precise dosage. The compositions will include an effective amount of the selected drug in combination with a pharmaceutically acceptable carrier and, in addition, can include other pharmaceutical agents, adjuvants, diluents, buffers, and the like.

Thus, the compositions of the disclosure can be administered as pharmaceutical formulations including those suitable for rectal, nasal, topical, pulmonary, vaginal administration or in a form suitable for administration by inhalation or insufflation.

In addition to the active compounds or their salts, the pharmaceutical formulations can contain other additives, such as pH-adjusting additives. In particular, useful pH-adjusting agents include acids, such as hydrochloric acid, bases or buffers, such as sodium lactate, sodium acetate, sodium phosphate, sodium citrate, sodium borate, or sodium gluconate. Further, the formulations can contain antimicrobial preservatives. Useful antimicrobial preservatives include methylparaben, propylparaben, and benzyl alcohol. An antimicrobial preservative is typically employed when the formulations is placed in a vial designed for multi-dose use. The

pharmaceutical formulations described herein can be lyophilized using techniques well known in the art.

Pharmaceutical formulations also are provided which provide a controlled release of a compound described herein, including through the use of a degradable polymer, as known in the art.

Formulations suitable for rectal administration are typically presented as unit dose suppositories. These may be prepared by admixing the active disclosed compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Formulations suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil, which maintain the stability of the isolated morphic form. Carriers which may be used include petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

Formulations suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. In certain embodiments, microneedle patches or devices are provided for delivery of drugs across or into biological tissue, particularly the skin. The microneedle patches or devices permit drug delivery at clinically relevant rates across or into skin or other tissue barriers, with minimal or no damage, pain, or irritation to the tissue.

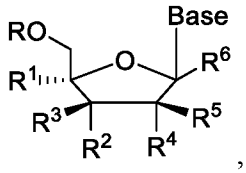
Formulations suitable for administration to the lungs can be delivered by a wide range of passive breath driven and active power driven single/-multiple dose dry powder inhalers (DPI). The devices most commonly used for respiratory delivery include nebulizers, metered-dose inhalers, and dry powder inhalers. Several types of nebulizers are available, including jet nebulizers, ultrasonic nebulizers, and vibrating mesh nebulizers. Selection of a suitable lung delivery device depends on parameters, such as nature of the drug and its formulation, the site of action, and pathophysiology of the lung.

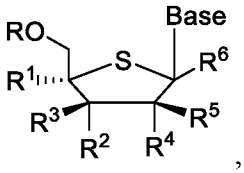
For parenteral formulations, the carrier will usually comprise sterile water or aqueous sodium chloride solution, though other ingredients, including those which aid dispersion, also may be included. Of course, where sterile water is to be used and maintained as sterile, the compositions and carriers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

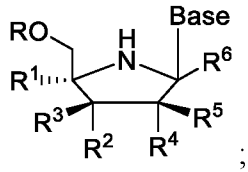
Liposomal suspensions (including liposomes targeted to viral antigens) may also be prepared by conventional methods to produce pharmaceutically acceptable carriers. This may be appropriate for the delivery of free nucleosides, acyl/alkyl nucleosides or phosphate ester pro-drug forms of the nucleoside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

In certain embodiments the present invention provides:

1. A 5'-phosphorylated antiviral monomeric nucleotide in a cationic lipid nano or microparticle composition.
2. The composition of embodiment 1, wherein the cationic lipid is permanently cationic.
3. The composition of embodiment 1, wherein the cationic lipid ionizable.
4. The composition of any one of embodiments 1-3, wherein the 5'-phosphorylated antiviral monomeric nucleotide is a 5'-monophosphate.
5. The composition of any one of embodiments 1-3, wherein the 5'-phosphorylated antiviral monomeric nucleotide is a 5'-diphosphate.
6. The composition of any one of embodiments 1-3, wherein the 5'-phosphorylated antiviral monomeric nucleotide is a 5'-triphosphate.
7. The composition of any one of embodiments 1-6, that also comprises a non-ionizable lipid.
8. The composition of any one of embodiments 1-7, that also comprises a sterol.
9. The composition of any one of embodiments 1-8, that also comprises a PEG lipid
10. The composition of any one of embodiments 1-9, that also comprises a structural lipid.
11. The composition of any one of embodiments 1-10, that also comprises a phospholipid.
12. A composition comprising a cationic lipid and an active compound of Formula



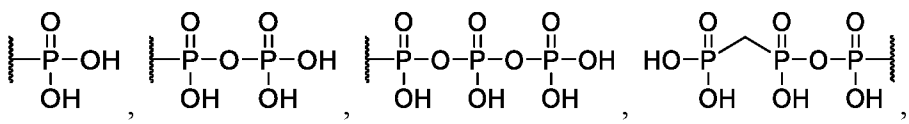
,


, or


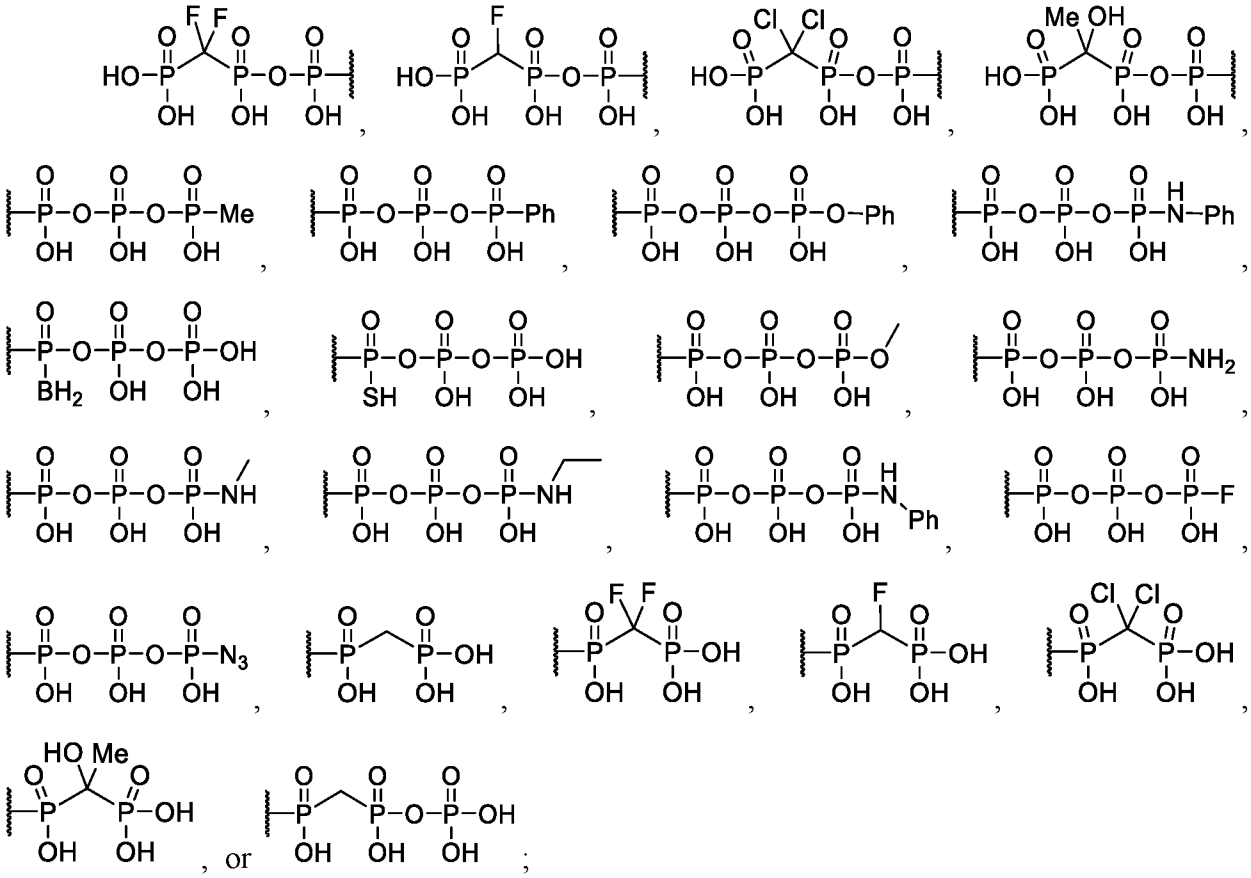
;

or a pharmaceutically acceptable salt thereof;

wherein:

R is


,



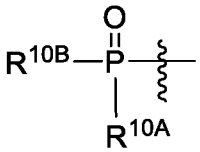
R^1, R^2, R^3, R^4, R^5 and R^6 are independently selected from the group consisting of H, NR^7R^8, $-C(O)NR^7R^8$, $-OC(O)NR^7R^8$, $-C(O)OR^7$, $-OC(O)OR^7$, $-S(O)_nR^9$, $-S(O)_2NR^7R^8$, N_3, $-OR^{10}$, CN, halogen, C_1-C_8alkyl, C_1-C_8haloalkyl, $C_{1\text{-}8}$alkoxy, $-(C_0$-C_2alkyl)(cycloalkyl), $-C_2$-C_8alkenyl, $-C_2$-C_8alkynyl, and aryl(C_1-C_8alkyl)-;

or any two of R^1, R^2, R^3, R^4, R^5 and R^6 on adjacent carbons when taken together are -OC(O)O- or $-O(CR^7R^8)O$- or when taken together with the ring carbon atoms to which are attached form a double bond;

R^7 and R^8 are independently selected from the group consisting of H, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, $-(C_0$-C_2alkyl)(cycloalkyl), aryl(C_1-C_8alkyl)-, $-(C_0$-C_2alkyl)(heterocyclo), aryl, heteroaryl, $-C(O)R^{10C}$, and $-S(O)_n(C_1$-$C_8)$alkyl or R^7 and R^8 taken together with a nitrogen to which they are both attached form a 3 to 7 membered heterocyclic ring;

R^9 is selected from the group consisting of C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, $-(C_0$-C_2alkyl)(cycloalkyl), aryl(C_1-C_8alkyl)-, $-(C_0$-C_2alkyl)(heterocyclo), aryl, and heteroaryl;

R^{10} is selected from the group consisting of H, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, $-(C_0$-C_2alkyl)(cycloalkyl), aryl(C_1-C_8alkyl)-, $-(C_0$-C_2alkyl)(heterocyclo), aryl, heteroaryl, -



$C(O)R^{10C}$, $-S(O)_n(C_1-C_8)$alkyl, , diphosphate, triphosphate, an optionally substituted carbonyl linked amino acid, or $-C(O)R^{10C}$;

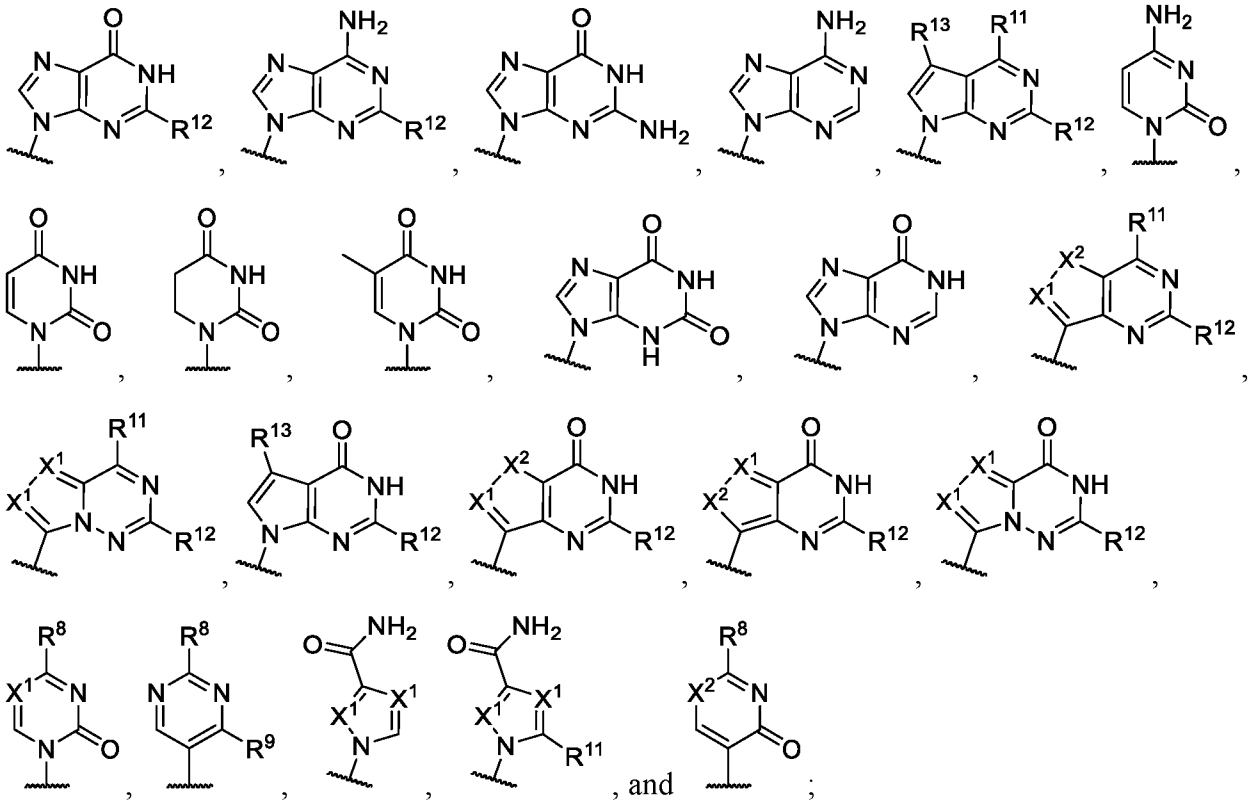
R^{10A} is selected from the group consisting of OH, an –O-optionally substituted aryl, an –O-optionally substituted heteroaryl, and an optionally substituted heterocyclyl;

R^{10B} is selected from the group consisting of OH, an optionally substituted N-linked amino acid, and an optionally substituted N-linked amino acid ester; and

R^{10C} is alkyl, alkenyl, alkynyl, $-(C_0-C_2$alkyl$)$(cycloalkyl), $-(C_0-C_2$alkyl$)$(heterocyclo), $-(C_0-C_2$alkyl$)$(aryl), $-(C_0-C_2$alkyl$)$(heteroaryl), -O-alkyl, -O-alkenyl, -O-alkynyl, $-O-(C_0-C_2$alkyl$)$(cycloalkyl), $-O-(C_0-C_2$alkyl$)$(heterocyclo), $-O-(C_0-C_2$alkyl$)$(aryl), or $-O-(C_0-C_2$alkyl$)$(heteroaryl);

n is independently selected at each instance from 0, 1, 2, and 3;

Base is selected from:



each X^1 is independently N or CR^{13};

X^2 is independently NR^7, O, or $S(O)_n$;

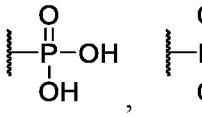
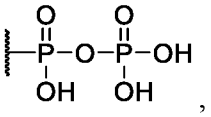
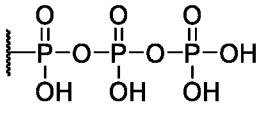
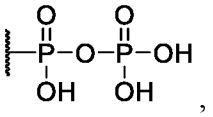
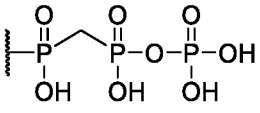
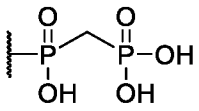
R^{11} is independently halogen, $-NR^7R^8$, N_3, NO, NO_2, $C(O)R^{10C}$, CN, —CH(=NR^7), —CH=NNHR^7, —CH=N(OR^7), —CH(OR^7)$_2$, —C(=O)NR^7R^8, -C(=S)NR^7R^8, -C(=O)OR^7, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, -(C_0-C_2alkyl)(cycloalkyl), aryl, -(C_0-C_2alkyl)(heterocyclo), heteroaryl, —$C(O)R^{10C}$, —$S(O)_n$(C_1-C_8)alkyl, aryl(C_1-C_8alkyl)-, or $OR^{10;}$ and

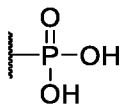
R^{12} and R^{13} are independently selected at each instance from the group consisting of H, halogen, $-NR^7R^8$, N_3, NO, NO_2, $C(O)R^{10C}$, CN, —CH(=NR^7), —CH=NHNR^7, —CH=N(OR^7), —CH(OR^7)$_2$, —C(=O)NR^7R^8, -C(=S)NR^7R^8, —C(=O)OR^7, R^7, OR^{10}, and SR^8.

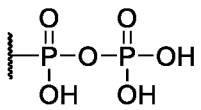
13. The composition of embodiment 12, wherein the composition comprises nanoparticles.

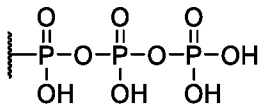
14. The composition of embodiment 12, wherein the composition comprises microparticles.

15. The composition of any one of embodiments 12-14, wherein R is


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, or 
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16. The composition of any one of embodiments 12-14, wherein R is 
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17. The composition of any one of embodiments 12-14, wherein R is 
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18. The composition of any one of embodiments 12-14, wherein R is 
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19. The composition of any one of embodiments 12-18, wherein R^1 is hydrogen.

20. The composition of any one of embodiments 12-18, wherein R^1 is methyl.

21. The composition of any one of embodiments 12-18, wherein R^1 is F.

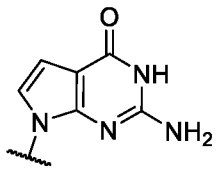
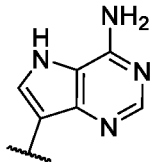
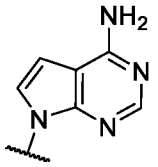
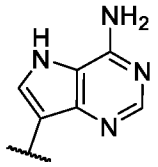
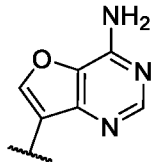
22. The composition of any one of embodiments 12-21, wherein R^2 is hydrogen.

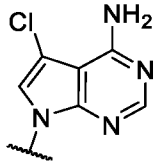
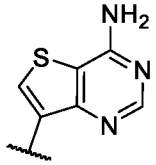
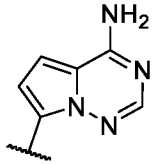
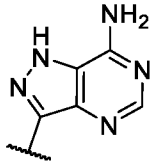
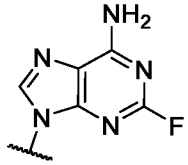
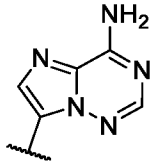
23. The composition of any one of embodiments 12-21, wherein R^2 is methyl.

24. The composition of any one of embodiments 12-21, wherein R^2 is F.

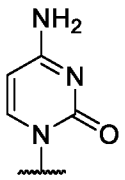
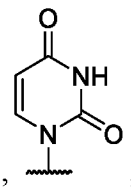
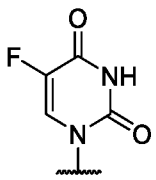
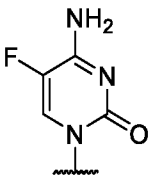
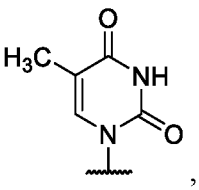
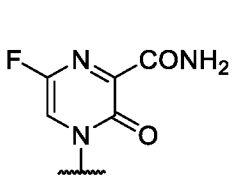
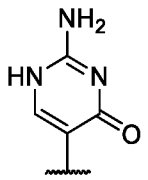
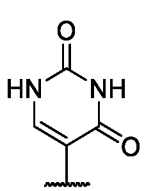
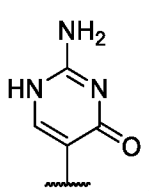
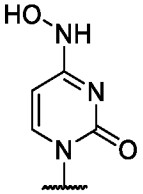
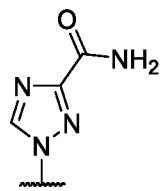
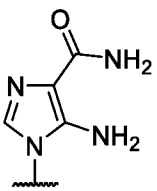
25. The composition of any one of embodiments 12-24, wherein R^3 is hydrogen.

26. The composition of any one of embodiments 12-24, wherein R^3 is methyl.
27. The composition of any one of embodiments 12-24, wherein R^3 is F.
28. The composition of any one of embodiments 12-27, wherein R^4 is hydrogen.
29. The composition of any one of embodiments 12-27, wherein R^4 is methyl.
30. The composition of any one of embodiments 12-27, wherein R^4 is F.
31. The composition of any one of embodiments 12-30, wherein R^5 is hydrogen.
32. The composition of any one of embodiments 12-30, wherein R^5 is methyl.
33. The composition of any one of embodiments 12-30, wherein R^5 is F.
34. The composition of any one of embodiments 12-33, wherein R^6 is hydrogen.
35. The composition of any one of embodiments 12-34, wherein Base is selected from the group consisting of:

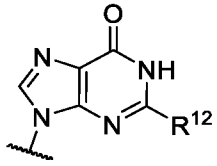
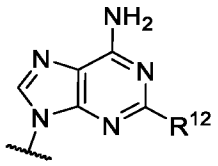
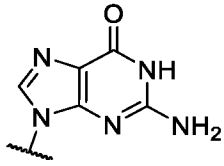
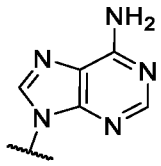
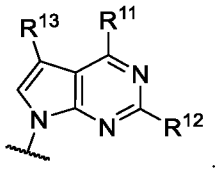

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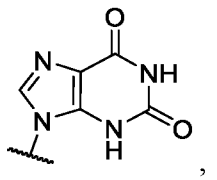
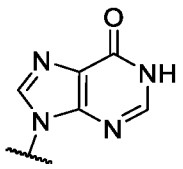
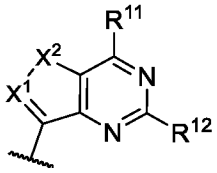
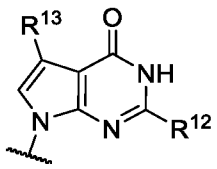
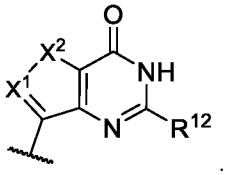
36. The composition of any one of embodiments 12-34, wherein Base is selected from the group consisting of:


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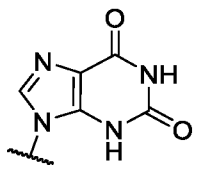
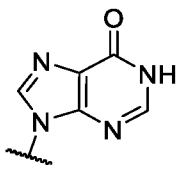
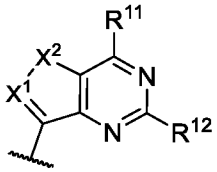
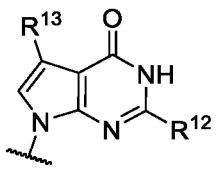
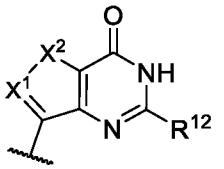
37. The composition of any one of embodiments 12-34, wherein Base is selected from the group consisting of:





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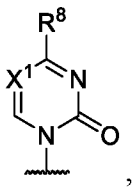
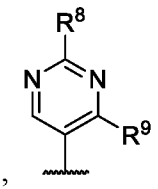
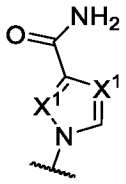
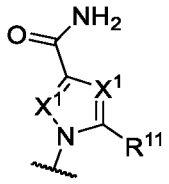
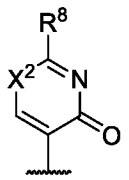
38. The composition of any one of embodiments 12-34, wherein Base is selected from the group consisting of:





, and .

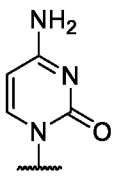
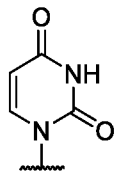
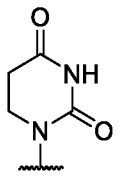
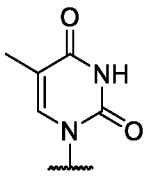
39. The composition of any one of embodiments 12-34, wherein Base is selected from the group consisting of:





, and .

40. The composition of any one of embodiments 12-34, wherein Base is selected from the group consisting of:





, and .

41. The composition of any one of embodiments 12-34, wherein Base is selected from the group consisting of:




, and .

42. The composition of any one of embodiments 12-41, wherein X^1 is N.

43. The composition of any one of embodiments 12-41, wherein X^1 is CR^{13}.

44. The composition of any one of embodiments 12-43, wherein X^2 is NR^7.

45. The composition of any one of embodiments 12-43, wherein X^2 is O.

46. The composition of any one of embodiments 12-45, wherein R^{11} is halogen.

47. The composition of any one of embodiments 12-45, wherein R^{11} is $-NR^7R^8$.

48. The composition of any one of embodiments 12-45, wherein R^{11} is halogen.

49. The composition of any one of embodiments 12-45, wherein R^{11} is F.

50. The composition of any one of embodiments 12-45, wherein R^{11} is Cl.

51. The composition of any one of embodiments 12-45, wherein R^{11} is cyano.

52. The composition of any one of embodiments 12-51, wherein R^{12} is halogen.

53. The composition of any one of embodiments 12-51, wherein R^{12} is $-NR^7R^8$.

54. The composition of any one of embodiments 12-51, wherein R^{12} is halogen.

55. The composition of any one of embodiments 12-51, wherein R^{12} is F.

56. The composition of any one of embodiments 12-51, wherein R^{12} is Cl.

57. The composition of any one of embodiments 12-51, wherein R^{12} is cyano.

58. The composition of any one of embodiments 12-57, wherein R^{13} is halogen.

59. The composition of any one of embodiments 12-57, wherein R^{13} is $-NR^7R^8$.

60. The composition of any one of embodiments 12-57, wherein R^{13} is halogen.

61. The composition of any one of embodiments 12-57, wherein R^{13} is F.

62. The composition of any one of embodiments 12-57, wherein R^{13} is Cl.

63. The composition of any one of embodiments 12-57, wherein R^{13} is cyano.

64. The composition of any one of embodiments 12-63, wherein R^7 is hydrogen.

65. The composition of any one of embodiments 12-63, wherein R^7 is methyl.

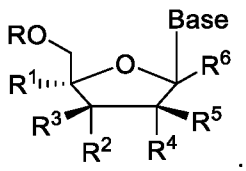
66. The composition of any one of embodiments 12-63, wherein R^7 is ethyl.

67. The composition of any one of embodiments 12-66, wherein R^8 is hydrogen.

68. The composition of any one of embodiments 12-66, wherein R^8 is methyl.

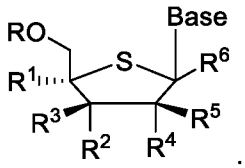
69. The composition of any one of embodiments 12-66, wherein R^8 is ethyl.

70. The composition of any one of embodiments 12-69, wherein the compound is of Formula



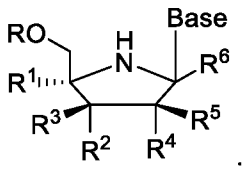
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71. The composition of any one of embodiments 12-69, wherein the compound is of Formula



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72. The composition of any one of embodiments 12-69, wherein the compound is of Formula



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73. The composition of any one of embodiments 1-72, wherein the cationic lipid is selected from the group consisting of PEG-DMG, PEG-DSG, DODMA, DODAP, DOTMA, DOTAP, KC2 and MC3.

74. The composition of any one of embodiments 1-72, wherein the cationic lipid is DODAP.

75. The composition of any one of embodiments 1-72, wherein the cationic lipid is DODMA.

76. The composition of any one of embodiments 1-72, wherein the cationic lipid is DOTAP.

77. The composition of any one of embodiments 1-72, wherein the cationic lipid is KC2.

78. The composition of any one of embodiments 1-72, wherein the cationic lipid is MC3.

79. The composition of any one of embodiments 1-78, comprising a second cationic lipid.

80. The composition of any one of embodiments 1-79, comprising a phospholipid.

81. The composition of embodiment 80, wherein the phospholipid is selected from DDPC, DEPA, DEPC, DEPE, DEPG, DLOPC, DLPA, DLPC, DLPE, DLPG, DLPS, DMG, DMPA, DMPC, DMPE, DMPG, DMPS, DOPA, DOPC, DOPE, DOPG, DOPS, DPPA, DPPC, DPPE, DPPG, DPPS, DPyPE, DSPA, DSPC, DSPE, DSPG, DSPS, EPC, HEPC, HSPC, HSPC, STEARIC, Milk Sphingomyelin MPPC, MSPC, PMPC, POPC, POPE, POPG, PSPC, SMPC, SOPC, and SPPC.

82. The composition of embodiment 80, wherein the phospholipid is selected from DDPC, DEPA, DEPC, DEPE, DEPG, DLOPC, DLPA, DLPC, DLPE, DLPG, DLPS, DMG, and DMPA.

83. The composition of embodiment 80, wherein the phospholipid is selected from DMPC, DMPE, DMPG, DMPS, DOPA, DOPC, DOPE, DOPG, DOPS, DPPA, DPPC, DPPE, and DPPG.

84. The composition of embodiment 80, wherein the phospholipid is selected from DPPS, DPyPE, DSPA, DSPC, DSPE, DSPG, DSPS, EPC, HEPC, HSPC, and HSPC.
85. The composition of embodiment 80, wherein the phospholipid is selected from MPPC, MSPC, PMPC, POPC, POPE, POPG, PSPC, SMPC, SOPC, and SPPC.
86. The composition of embodiment 80, wherein the phospholipid is DSPC.
87. The composition of any one of embodiments 1-86 comprising a structural lipid.
88. The composition of embodiment 87 comprising cholesterol.
89. A pharmaceutical composition comprising a composition of any one of embodiments 1-88, wherein the composition is suitable for pharmaceutical administration.
90. A method of treating a virus comprising administering an effective amount of a composition of any one of embodiments 1-89 to a patient in need thereof.
91. The method of embodiment 90, wherein the patient is a human.
92. The method of embodiment 90 or 91, wherein the virus is a DNA virus.
93. The method of embodiment 90 or 91, wherein the virus is a RNA virus.
94. The method of embodiment 90 or 91, wherein the virus is selected from the group consisting of HIV, HBV, HCV, SARS-CoV-2, EV-68, EV-71, Respiratory Syncytial virus, Dengue Fever virus, Yellow Fever virus, West Nile virus, Chikungunya virus, Tick-borne encephalitis virus, Zika virus, Japanese encephalitis virus, Semliki Forest virus, Venezuelan equine encephalitis virus, Sindbis virus, Hanta virus, Rift valley fever virus, Crimean Congo Hemorrhagic fever virus, ebola virus, Marburg virus, California encephalitis, Eastern equine encephalitis virus, Western equine encephalitis virus, St. Louis encephalitis virus, Nipah virus, Tickborne encephalitis viruses, Omsk hemorrhagic fever virus, Alkhurma virus, Kyasanur Forest virus, John Cunningham (JC) virus, Norovirus and Norwalk virus, HHV-6, HHV-8, Junin virus, Guanarito, Chopare, Lujo, Hepatitis A, Rotavirus, Papilloma Virus, Lacrosse, Metapneumovirus, Parainfluenza virus, Influenza A virus, Influenza B virus, Coxsackie virus, and Poliovirus.
95. The method of embodiment 90 or 91, wherein the virus is SARS-CoV-2.
96. The method of embodiment 90 or 91, wherein the virus is HCV.
97. The method of embodiment 90 or 91, wherein the virus is HIV.
98. Use of a composition of any one of embodiments 1-89 in the manufacture of a medicament to treat a virus.

99. The composition of any one of embodiments 1-89 for use in the treatment of a virus.

VI. Combination and Alternation Therapy

It is well recognized that drug-resistant variants of viruses can emerge after prolonged treatment with an antiviral agent. Drug resistance sometimes occurs by mutation of a gene that encodes for an enzyme used in viral replication. The efficacy of a drug against a viral 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 principal drug. Alternatively, the pharmacokinetic, bio distribution, half-life, or other parameter of the drug can be altered by such combination therapy. It is anticipated for example that (i) more than one active antiviral 5'-(mono, di or tri)phosphate or 5'-phosphate nucleotides can be included in the cationic lipid nanoparticle of the present invention to be delivered, or (ii) one active antiviral 5'-(mono, di or tri)phosphate or 5'-phosphate nucleotides can be included in the cationic lipid nano micro particle of the present invention and one prodrug of an active nucleos(t)ide can be included or (iii) one active antiviral 5'-(mono, di or tri)phosphate or 5'-phosphate nucleotides can be included in the cationic lipid nano or micro particle of the present invention to be delivered and another active nucleotide can be administered through a different route of delivery, for example, oral.

VII. Viral Infections to be treated or prevented via Cationic Lipid Particle Delivery of Active Antiviral Agent

According to the invention, the 5'-(mono, di or tri)phosphate, or a 5'-phosphate of an active antiviral can be stabilized and directly administered in an effective amount in a cationic lipid nano or micro particle to a host in need of treatment or prophylaxis against a virus such as a Coronavirus, Hepacivirus, or Flavivirus, and more particularly, SARS-CoV, including SARS-CoV-2, HIV, HBV, HCV, Ebola, Dengue virus 2, West Nile virus (WNV), Zika, or Yellow fever virus.

More generally, the 5'-(mono, di or tri)phosphate or 5'-phosphate of an active antiviral nucleos(t)ide can be directly administered in an effective amount in a cationic lipid nano or micro particle to a host in need of treatment or prophylaxis against any of the viruses below.

In certain embodiments the active pharmaceutical composition of the present invention is used to treat a virus selected from HIV, HBV, HCV, SARS-CoV-2, EV-68, EV-71, Respiratory

Syncytial virus, Dengue Fever virus, Yellow Fever virus, West Nile virus, Chikungunya virus, Tick-borne encephalitis virus, Zika virus, Japanese encephalitis virus, Semliki Forest virus, Venezuelan equine encephalitis virus, Sindbis virus, Hanta virus, Rift valley fever virus, Crimean Congo Hemorrhagic fever virus, ebola virus, Marburg virus, California encephalitis, Eastern equine encephalitis virus, Western equine encephalitis virus, St. Louis encephalitis virus, Nipah virus, Tickborne encephalitis viruses, Omsk hemorrhagic fever virus, Alkhurma virus, Kyasanur Forest virus, John Cunningham (JC) virus, Norovirus and Norwalk virus, HHV-6, HHV-8, Junin virus, Guanarito, Chopare, Lujo, Hepatitis A, Rotavirus, Papilloma Virus, Lacrosse, Metapneumovirus, Parainfluenza virus, Influenza A virus, Influenza B virus, Coxsackie virus, and Poliovirus.

The Baltimore classification system sorts viruses into Groups, labeled I-VII, according to their genome. DNA viruses belong to Groups I, II, and VII, while RNA viruses belong to Groups III-VI. RNA viruses use ribonucleic acid as their genetic material. An RNA virus can have double-stranded (ds) RNA or single stranded RNA and can also be positive-stranded or negative-stranded. Group III viruses are double-stranded RNA viruses. Groups IV and V are both single-stranded RNA viruses, but Groups IV viruses are positive-sense and Groups V are negative-sense. Group VI are positive-sense single-stranded RNA viruses that replicate through a DNA intermediate. Treatment or prophylaxis of any of these viruses can be accomplished according to the present invention.

DNA Viruses

In certain aspects, the 5'-(mono, di or tri)phosphate or 5'-phosphate of an active antiviral nucleoside of the present invention is administered to a host in need thereof in the cationic lipid delivery system as described herein, including a human, to treat a Group I dsDNA virus, including, but not limited to a virus from the *Adenoviridae*, *Herpesviridae*, *Papovaviridae*, and *Poxviridae* family. Adenoviruses usually cause respiratory illnesses or conjunctivitis. Adenovirus types 3, 4, 7, and 14 are most commonly associated with acute respiratory illness, while Adenovirus types 8, 19, 37, 53, and 54, can cause epidemic keratoconjunctivitis. In certain embodiments, an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat a virus from the *Adenoviridae* family.

Viruses of the *Herpesviridae* family include Herpes simplex virus, varicella-zoster virus, cytomegalovirus, and Epstein–Barr virus. In certain embodiments, an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat Herpes simplex virus. In certain embodiments, the Herpes simplex virus is Herpes simplex virus-1 (HSV-1). In certain embodiments, the Herpes simplex virus is Herpes simplex virus-2 (HSV-2). In certain embodiments, an active antiviral nucleoside of the present invention is administered to a host in need thereof to treat Kaposi's sarcoma-associated herpesvirus (KSHV).

In certain embodiments, an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat a virus of the *Papovaviridae* family, including, but not limited to JC virus and HPV. In an alternative embodiment, an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to trat a virus of the *Poxviridae* family, including, but not limited to, cowpox, myxoma virus, monkeypox, vaccinia virus.

In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat a Group II ssDNA virus, including, but not limited to a virus from the *Parvoviridae* or the *Anelloviridae* virus. In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof to treat a virus of the *Parvoviridae* family, including *Parvovirus B19* which causes fifth disease in humans. In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof to treat a virus of the *Anelloviridae* family, including Torque teno virus.

Group VII viruses have partial dsDNA genomes and make ssRNA intermediates that act as mRNA, but are also converted back into dsDNA genomes by reverse transcriptase, necessary for genome replication. The only Group VII class of viruses that is pathogenic in humans is the Class *Hepadnaviridae* and Class Hepadnaviridae only contains one viral species that is pathogenic in humans: hepatitis B virus (HBV). In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof to treat HBV.

RNA viruses

In certain aspects, the 5'-(mono, di or tri)phosphate or 5'-phosphate of an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof in the cationic lipid delivery system as described herein, including a human, to treat a Group III dsRNA virus selected from the *Amalgaviridae* family, *Birnaviridae* family, *Chrysoviridae* family, *Cystoviridae* family, *Endornaviridae* family, *Hypoviridae* family, *Megabirnaviridae* family, *Partitiviridae* family, *Picobirnaviridae* family, *Quadriviridae* family, *Reoviridae* family and *Totiviridae* family.

In some embodiments, the 5'-(mono, di or tri)phosphate or 5'-phosphate of an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat a Group IV positive-sense ssRNA virus. The order Nidovirales includes the following families: *Arteviridae, Coronaviridae, Mesoniviridae*, and *Roniviridae*. The order Picornavirales includes the following families: *Dicistroviridae, Ifaviridae, Marnaviridae, Picornaviridae* and *Secoviridae*. The order Tymovirales includes the following families: *Alphaflexiviridae, Betaflexiviridae, Gammaflexiviridae* and *Tymoviridae*. The following positive-sense ssRNA viruses include viruses from the following unassigned families: *Alphatetraviridae*, *Alvernaviridae*, *Astroviridae*, *Barnaviridae*, *Benyviridae*, *Bromoviridae*, *Caliciviridae*, *Carmotetraviridae*, *Closteroviridae*, *Flaviviridae, Fusariviridae, Hepeviridae, Leviviridae, Luteoviridae, Narnaviridae, Nodaviridae, Permutotetraviridae, Potyviridae, Togaviridae, Tombusviridae* and *Virgaviridae*.

In some embodiments, the 5'-(mono, di or tri)phosphate or 5'-phosphate of an active antiviral nucleoside of the present invention is administered in the cationic lipid delivery system to a host in need thereof, including a human, to treat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In other embodiments, an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat other *Coronaviridae* viral infections.

In certain aspects, the 5'-(mono, di or tri)triphosphate or 5'-phosphate of an active antiviral nucleoside of the present invention is administered in a cationic lipid particle to a host in need thereof, including a human, to treat a *Flaviviridae* viral infection including, but not limited to, infections with viruses of the genera *Hepacivirus*, *Flavivirus* and *Pestivirus*. Species of the *Hepacivirus* genera include *Hepacivirus* A – *Hepacivirus* N. The hepatitis C virus (HCV) is caused

by *Hepatovirus* C and In certain embodiments, an active antiviral nucleoside of the present invention is administered to treat HCV.

Flavivirus infections include Dengue fever, Kyasanur Forest disease, Powassan disease, Wesselsbron disease, West Nile fever, yellow fever, Zika virus, Rio bravo, Rocio, Negishi, and the encephalitises including: Japanese B encephalitis, Montana myotis leukoencephalitis virus, central European encephalitis (tick-borne encephalitis), Ilheus virus, Murray Valley encephalitis, St. Louis encephalitis, Louping ill, and Russian spring-rodents summer encephalitis. In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat an infection of the genera *Flavivirus*. In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat Dengue Fever. In certain embodiments, the Dengue Fever is Type 2. In certain embodiments, the Dengue Fever is Type 3. In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat West Nile fever. In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat Yellow Fever. In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat Japanese B encephalitis.

Pestivirus infections include primarily livestock diseases, including swine fever in pigs, BVDV (bovine viral diarrhea virus) in cattle, and Border Disease virus infections.

In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat a *Picornavirus* infections including, but not limited to infections with viruses of the genuses *Aphthovirus, Aquamavirus, Avihepatovirus, Cardiovirus, Cosavirus, Dicipivirus, Enterovirus, Erbovirus, Hepatovirus, Kobuvirus, Megrivirus, Parechovirus, Salivirus, Sapelovirus, Senecavirus, Teschovirus,* and *Tremovirus.*

In some embodiments, the 5'-(mono, di, tri)phosphate or 5'-phosphate of an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat a *Togaviridae* family virus. The *Togaviridae* family comprises four genera: *Alphavirus, Arterivirus, Rubivirus* and *Pestivirus*. The alphavirus genus

contains four viruses that produce encephalitis: Eastern equine encephalitis (EEE) virus, Venezuelan equine encephalitis (VEE) virus, Western equine encephalitis (WEE) virus and the Everglades virus. In addition, the Alphavirus genus includes the Chikungunya virus, Mayaro virus, Ockelbo virus, O'nyong-nyong virus, Ross River virus, Semliki Forest virus and Sindbis virus (SINV). The Arterivirus genus contains a single member: the equine arteritis virus. The pestivirus genus contains three viruses of veterinary importance, namely the bovine viral diarrhea virus (BVDV), hog cholera virus and border disease virus. The only member of the Rubivirus genus is the rubella virus.

In some embodiments the 5'-(mono, di and tri)phosphate or 5'-phosphate of an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered in the cationic lipid material to a host in need thereof, including a human, to treat a Group V negative-sense ssRNA viruses. In certain embodiments, the virus is from the order Mononegavirales. The Mononegavirales order includes, but is not limited to, the following families: *Artoviridae, Bornaviridae, Filoviridae, Lispiviridae, Mymonaviridae, Nyamiviridae, Paramyxoviridae, Pneumoviridae, Rhabdoviridae, Sunviridae, and Xinmoviridae.* Viruses of *Filoviridae* include Ebola virus and Marburg virus. Viruses of *Pneumoviridae* include human respiratory syncytial virus B1 (HRSV-B1) and human respiratory syncytial virus A2 (HRSV-A2). In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof, including a human, to treat human respiratory syncytial virus B1 or human respiratory syncytial virus A2.

Paramyxoviridae viruses include Measles virus, Mumps virus, Nipah virus, Hendra virus, and Newcastle disease virus (NDV) and viruses of *Rhabdoviridae* include Rabies virus and *Nyamiviridae*, Nyavirus. Unassigned families and viruses include, but are not limited to: *Arenaviridae*, Lassa virus; *Bunyaviridae*, Hantavirus, Crimean-Congo hemorrhagic fever; *Ophioviridae* and *Orthomyxoviridae*, influenza virus.

In some embodiments, the 5'-(mono, di or tri)phosphate or 5'-phosphate of the active antiviral nucleoside of the present invention is administered in the cationic lipid delivery system to a host in need thereof, including a human, to treat a *Bunyaviridae* family virus. The *Bunyaviridae* family comprises more than two hundred named viruses and the family is divided into five genera: Hantavirus, Nairovirus, Orthobunyavirus, Phlebovirus and Tospovirus. The Hantavirus genus includes the Hantaan virus. The Nairovirus genus includes the Crimean-Congo

Hemorrhagic Fever virus and Dugbe viruses. The Orthobunyavirus genus is comprised of approximately one hundred seventy viruses that have been divided into multiple serogroups. The Serogroups include Anopheles A serogroup, Anopheles B serogroup, Bakau serogroup, Bunyamwera serogroup, Bwamba serogroup, California serogroup, Capim serogroup, Gamboa serogroup, Group C serogroup, Guama serogroup, Koongol serogroup, Mapputta serogroup, Minatitlan serogroup, Nyando serogroup, Olifanstlei serogroup, Patois serogroup, Simbu serogroup, Tete serogroup, Turlock serogroup, Wyeomyia serogroup and the Unclassified group. The Anopheles A serogroup includes the Anopheles A virus, Tacaiuma virus, Virgin River virus, Trombetas complex, Arumateua virus, Caraipe virus, Trombetas virus and the Tucurui virus. The Anopheles B serogroup includes the Anopheles B virus and the Boraceia virus. The Bakau serogroup includes the Bakau virus and the Nola virus. The Bunyamwera serogroup includes the Birao virus, Bozo virus, Bunyamwera virus, Cache Valley virus, Fort Sherman virus, Germiston virus, Guaroa virus, Ilesha virus, Kairi virus, Main Drain virus, Northway virus, Playas virus, Potosi virus, Shokwe virus, Stanfield virus, Tensaw virus, Xingu virus, Batai virus, Calovo virus, Chittoor virus, Garissa virus, KV-141 virus, and Ngari virus. The Bwamba serogroup includes the Bwamba and Pongola viruses. The California serogroup includes the California encephalitis virus, Chatanga virus, Inkoo virus, Jamestown Canyon virus, Jerry Slough virus, Keystone virus, Khatanga virus, La Crosse virus, Lumbo virus, Melao virus, Morro Bay virus, San Angelo virus, Serra do Navio virus, Snowshoe hare virus, South River virus, Tahyna virus, and the Trivittatus virus. The Capim serogroup includes the Acara virus, Benevides virus and the Capim virus. The Gamboa serogroup includes the Alajuela virus, Gamboa virus, Pueblo Viejo virus and San Juan virus. The Group C serogroup includes, but is not limited to, Bruconha virus, Ossa virus, Apeu virus, Brunconha virus, Caraparu virus, Vinces virus, Madrid virus, Gumbo limbo virus, Marituba virus, Murutucu virus, Nepuyo virus, Restan virus, Itaqui virus and Oriboca virus. The Guama serogroup includes, but is not limited to, the Bertioga virus, Bimiti virus, Cananeia virus, Guama virus, Guaratuba virus, Itimirim virus and Mirim virus. The Koongol serogroup includes, but is not limited to, the Koongol virus and Wongal virus. The Mapputta serogroup includes, but is not limited to, the Buffalo Creek virus, Mapputta virus, Maprik virus, Murrumbidgee virus and Salt Ash virus. The Minatitlan serogroup includes, but is not limited to, Minatitlan virus and Palestina virus. The Nyando serogroup includes, but is not limited to, Eretmapodites virus and Nyamdo virus. The Olifanstlei serogroup includes, but is not limited to, Botambi virus and Olifanstlei virus.

The Patois serogroup includes, but is not limited to, Abras virus, Babahoyo virus, Pahayokee virus, Patois virus and Shark River virus. The Simbu serogroup includes, but is not limited to, Iquitos virus, Jatobal virus, Leanyer virus, Madre de Dios virus, Oropouche virus, Oya virus, Thimiri virus, Akabane virus, Tinaroo virus, Douglas virus, Sathuperi virus, Aino virus, Shuni virus, Peaton virus, Shamonda virus, Schmallenberg virus and Simbu virus. The Tete serogroup includes, but is not limited to, Batama virus and Tete virus. The Turlock serogroup includes, but is not limited to, M'Poko virus, Turlock virus and Umbre virus. The Wyeomyia serogroup includes, but is not limited to, Anhembi virus, Cachoeira Porteira virus, Iaco virus, Macaua virus, Sororoca virus, Taiassui virus, Tucunduba virus and Wyeomyia virus. The Unclassified serogroup includes, but is not limited to, Batama virus, Belmont virus, Enseada virus, Estero Real virus, Jurona virus, Kaeng Khei virus and Kowanyama virus. The Phlebovirus genus includes, but is not limited to, the Naples and Sicilian Sandfly Fever viruses and Rift Valley Fever virus. The Tospovirus genus includes, but is not limited to, the type species Tomato spotted wilt virus and the following species: Bean necrotic mosaic virus, Capsicum chlorosis virus, Groundnut bud necrosis virus, Groundnut ringspot virus, Groundnut yellow spot virus, Impatiens necrotic spot virus, Iris yellow spot virus, Melon yellow spot virus, Peanut bud necrosis virus, Peanut yellow spot virus, Soybean vein necrosis-associated virus, Tomato chlorotic spot virus, Tomato necrotic ringspot virus, Tomato yellow ring virus, Tomato zonate spot virus, Watermelon bud necrosis virus, Watermelon silver mottle virus and Zucchini lethal chlorosis virus.

Group VI viruses are RNA viruses that have diploid (two copies) ssRNA genomes that must be converted, using the enzyme reverse transcriptase, to dsDNA. The dsDNA is then transported to the nucleus of the host cell and inserted into the host genome. The Group VI human pathogens are restricted to one class of retroviruses, Class *Retroviridae*, and to two genera within this class: *Deltaretrovirus* and *Lentivirus*. In certain embodiments an active antiviral in the cationic lipid delivery system as described herein is administered to a host in need thereof to treat a virus from the *Deltaretrovirus* genus, including, but not limited to, HTLV-1, which increases the risk of developing adult T-cell leukemia/lymphoma. In certain embodiments an active antiviral nucleoside in the cationic lipid delivery system as described herein is administered to a host in need thereof to treat a virus from the *Lentivirus* genus, including, but not limited to, HIV.

VIII. Examples

Example 1. Preparation of Liposome-Encapsulated Nucleoside Triphosphate

Dioleoyl-3-trimethylammonium propane (DOTAP, 18:1TAP) solubilized in chloroform (1 mg DOTAP per 1 ml of $CHCl_3$) was purchased from Avanti Polar Lipids (Alabaster, AL). Solid samples of Remdesivir triphosphate (GS-443902, **Formulation 1**) and Sofosbuvir triphosphate (PSI-7409, **Formulation 2**) were purchased from MedChemExpress (Monmouth Junction, NJ) and solubilized in deionized water to a final concentration of 10 mM. In a glass vial, 3.3 ml of the DOTAP/$CHCl_3$ solution was added, and $CHCl_3$ was evaporated first by a stream of air followed by vacuum for 2 hours leaving a thin film of DOTAP. The 10 mM nucleotide-triphosphate solution was added to the DOTAP film and swirled at room temperature until an emulsion formed. Both samples were then subjected to three rounds of freeze-thaw to homogenize the particles. This process yields 10 mM nucleotide triphosphate in 50 mM lipid concentration of DOTAP liposomes. The resulting particles with remdesivir triphosphate are denoted **Formulation 1** and the resulting particles with sofosbuvir triphosphate are denoted **Formulation 2**.

A representative example of the formulation of the present invention can be made in the following manner:

The four component lipids heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate (36.93 mg, 52 mol %), DMG-PEG2000 (4.39 mg, 1.75 mol %), DSPC (13.85 mg, 17.5 mol %), and cholesterol (11.12 mg, 28.75 mol %) are combined together and dissolved in ethanol (3 ml). The resulting ethanolic solution is mixed 9 ml of 6.25 mM sodium acetate buffer (pH 5) containing about 6.5 mg of fully dissolved active nucleoside triphosphate. The mixed solution is then dialyzed against PBS (pH 7.4) in dialysis cassettes for at least 18 hr. Formulations are concentrated using Amicon ultra centrifugal filters (EMD Millipore, Billerica, MA), passed through a 0.22-mm filter, and stored at 4 °C until use.

Example 2. Cytopathic Effect Assay in Vero Cells Against SARS-CoV-2

Experiment Description

Full-dose antiviral testing was conducted on two test-items. Assays against live SARS-CoV-2 were performed against the MEX-BC1/2020 strain. Test-items as 10mM distilled water stocks and were kept at 4°C until their use. At the time of use, sample volumes were measured, distilled water was added to 100µL final volume (65µL and 14µL were added to **Formulation 1**

and **Formulation 2**, respectively), samples were heated to 50°C for 5 minutes, then allowed to cool to room temperature before use. All the test-items were assessed in parallel for antiviral and viability assays. Vero E6 cells were utilized to evaluate the antiviral activity of the formulation test-items against SARS-CoV-2. Test-items were pre-incubated first with target cells for 1h at 37°C before infection with SARS-CoV-2. Following pre-incubation, cells were challenged with viral inoculum. Putative inhibitors were present in the cell culture for the duration of the infection (96 hours), at which time a Neutral Red uptake assay was performed to determine the extent of the virus-induced cytopathic effect (CPE). Prevention of the virus-induced CPE was used as a surrogate marker to determine the antiviral activity of the test-items against SARS-CoV-2. Control wells also included a known inhibitor of SARS-CoV-2: GS-441524, the main plasma metabolite of the polymerase inhibitor remdesivir (GS-5734). A cell viability assay with Vero E6 uninfected cells was set up in parallel in a separate plate and for the same duration of the infectivity assay (96h). Cell viability was also determined with the Neutral Red method. Eight dilutions of the tested samples were tested in triplicates for the antiviral assay and duplicates for the viability assay. Two-fold serial dilutions started at 200μM. When possible, IC50 (antiviral) and CC50 (inhibition of viability) values of the test-items were determined using GraphPad Prism software.

Antiviral Activity of **Formulation 1** and **Formulation 2**

Of the two test-items evaluated against live SARS-CoV-2, **Formulation 1** completely prevented the virus-induced cytopathic effect (CPE) at 50μM. The protective effect was observed in a dose-dependent manner starting at the lowest concentration evaluated, and then declined or completely disappeared at concentrations of 100μM or 200μM, respectively. These findings suggest that **Formulation 1** inhibits the replication of SARS-CoV-2 in infected cells. The cytotoxicity displayed by the test-item at the higher concentrations may have played a role in the reduction of the antiviral effect seen at 100μM and 200μM. The estimated IC50 for **Formulation 1** against SARS-CoV-2 was 7.4μM. Microscopic evaluation of the monolayers after 96h of infection also confirmed the prevention of the virus-induced CPE exerted by **Formulation 1**. **Formulation 2** did not prevent the virus-induced CPE at any concentration tested, and concentrations above 25μM resulted in a profound decrease of cell viability. The CC50 value for **Formulation 2** was 18.7μM. By comparison, the control inhibitor GS-441524 at concentrations of 6.7μM or greater, completely prevented the virus-induced. Microscopic evaluation of the

monolayers also confirmed the prevention of the virus-induced CPE exerted by GS-441524. Final results are displayed in FIG. 1 and FIG. 2.

Control Inhibitors and Quality Controls

Quality controls for the infectivity assays were performed on every plate to determine: i) signal to background (S/B) values; ii) inhibition by known inhibitors of SARS-CoV-2 (for antiviral assay), and iii) variation of the assay, as measured by the coefficient of variation (C.V.) of all data points. All controls worked as anticipated for each assay. GS-441524, known inhibitor of SARS-CoV-2 infection, prevented completely the virus-induced CPE of the infected cells. The IC50 obtained for GS-441524 was 0.67 μM, with no significant loss of viability in uninfected cells observed at 20μM. Overall variation of duplicates in the antiviral assay was 9.3%, and overall variation in the viability assays was 12.2%. The ratio of signal-to-background (S/B) for the antiviral assay was 2.0-fold, determined by comparing the A540nm values in uninfected ("mock") cells with that observed in cells challenged with SARS-CoV-2 in the presence of vehicle alone. When comparing the signal in uninfected cells to the signal in "no-cells" background wells, the S/B ratio of the antiviral assay was 23.9-fold. For the viability assay the signal to background ("no cells" value) was 31.9 - fold.

Experimental Procedure

To evaluate antiviral activity against SARS-CoV-2 (MEX-BC1/2020), a CPE-based antiviral assay was performed by infecting Vero E6 cells in the presence or absence of test-items. Infection of cells leads to significant cytopathic effect and cell death after 4 days of infection. In this assay, reduction of CPE in the presence of inhibitors was used as a surrogate marker to determine the antiviral activity of the tested items. Viability assays to determine test-item-induced loss of cell viability was monitored in parallel using the same readout (Neutral Red), but utilizing uninfected cells incubated with the test-items.

Vero E6 cells were maintained in DMEM with 10% fetal bovine serum (FBS), hereby called DMEM10. Twenty-four hours after cell seeding, test samples were submitted to serial dilutions with DMEM2 in a different plate. Then, media was removed from cells, and serial dilutions of test-items were added to the cells and incubated for 1h at 37°C in a humidified incubator. After cells were pre-incubated with test-items, then cultures were challenged with

SARS-CoV-2 resuspended in DMEM with 2% FBS (DMEM2). The amount of viral inoculum was previously titrated to result in a linear response inhibited by antivirals with known activity against SARS-CoV-2. Cell culture media with the virus inoculum was not removed after virus adsorption, and test-items and virus were maintained in the media for the duration of the assay (96h). After this period, the extent of cell viability was monitored with the neutral red (NR) uptake assay.

The virus-induced CPE was monitored under the microscope after 3 days of infection. After 4 days cells were stained with neutral red to monitor cell viability. Viable cells incorporate neutral red in their lysosomes. The uptake of neutral red relies on the ability of live cells to maintain the pH inside the lysosomes lower than in the cytoplasm, a process that requires ATP. Inside the lysosome the dye becomes charged and is retained. After a 3h incubation with neutral red (0.033%), the extra dye is washed away, and the neutral red is extracted from lysosomes by incubating cells for 15 minutes with a solution containing 50% ethanol and 1% acetic acid. The amount of neutral red is estimated by measuring absorbance at 540nm in a plate reader.

Test-items were evaluated in duplicates (viability) or triplicates (CPE) using serial 2-fold dilutions. Controls included uninfected cells ("mock-infected"), and infected cells to which only vehicle was added. Some cells were treated with GS-441524 in a full dose-response curve starting at 20μM. GS-441524 is the main metabolite of remdesivir, a broad-spectrum antiviral that blocks the RNA polymerase of SARS-CoV-2.

Data Analysis of CPE-based Antiviral Assay

The average absorbance at 540nm (A540) observed in infected cells (in the presence of vehicle alone) was calculated, and then subtracted from all samples to determine the inhibition of the virus induced CPE. Data points were then normalized to the average A540 signal observed in uninfected cells ("mock") after subtraction of the absorbance signal observed in infected cells. In the neutral red CPE-based assay, uninfected cells remained viable and uptake the dye at higher levels than non-viable cells. In the absence of antiviral agents the virus-induced CPE kills infected cells and leads to lower A540 (this value equals 0% inhibition). By contrast, incubation with the antiviral agent (GS-441524) prevents the virus induced CPE and leads to absorbance levels similar to those observed in uninfected cells. Full recovery of cell viability in infected cells represent 100% inhibition of virus replication. Final results are displayed in FIG. 1 and FIG. 2.

Example 3. Viability Assay (Neutral Red Uptake Method)

Uninfected Vero E6 cells were incubated with eight concentrations of test-items or control inhibitors dilutions using the same starting doses used for the antiviral assay. The incubation temperature and duration of the incubation period mirrored the conditions of the prevention of virus-induced CPE assay, and cell viability was evaluated with the neutral red uptake method but this time utilizing uninfected cells, otherwise the procedure was the same as the one used for the antiviral assays. The extent of viability was monitored by measuring absorbance at 540nm. When analyzing the data, background levels obtained from wells with no cells were subtracted from all data-points. Absorbance readout values were given as a percentage of the average signal observed in uninfected cells treated with vehicle alone. Final results are displayed in FIG. 1 and FIG. 2.

QC and Analysis of Cytotoxicity data

The average signal obtained in wells with no cells was subtracted from all samples. Readout values were given as a percentage of the average signal observed in uninfected cells treated with vehicle alone (DMEM2). The signal-to-background (S/B) obtained was 31.9-fold. DMSO was used as a cytotoxic compound control in the viability assays. DMSO blocked cell viability by more than 99% when tested at 10%. Final results are displayed in FIG. 1 and FIG. 2.

Example 4. HCV Antiviral Assay

Sample Preparation

Each liposomal particle composition was prepared in chloroform in a glass vial. The chloroform was evaporated overnight in a vacuumed desiccator. The prewarmed solution of sofosbuvir triphosphate (RS1) and sofosbuvir monophosphate (RS2) were separately added at 200 μL per vial to the liposomal particle compositions being tested. Each test article was heated for 60 minutes at 65°C with vortexing of the vial every 10 minutes. Each test article was then placed at 4°C for 15 minutes, then heated to 65°C for 15 minutes for a total of three cycles prior to assay set up. Each test article concentration was treated as 10 mM stock.

Liposomal Particle Composition

Liposomal Particle 3	3.9 mg DODAP 4.8 mg DSPC
Liposomal Particle 4	2.5 mg DODAP 6.5 mg DSPC 0.5 mg cholesterol
Liposomal Particle 5	2.5 mg DODAP 6.5 mg DSPC 2.4 mg cholesterol

Antiviral Lipid Nanoparticle

Formulation 3	0.68 mg of sofosbuvir monophosphate 3.9 mg DODAP 4.8 mg DSPC
Formulation 4	0.68 mg of sofosbuvir monophosphate 2.5 mg DODAP 6.5 mg DSPC 0.5 mg cholesterol
Formulation 5	1 mg of sofosbuvir triphosphate 2.5 mg DODAP 6.5 mg DSPC 2.4 mg cholesterol

Each test article was diluted to 2 µM (2x in-well concentration; 20 µL of intermediate 0.1 mM stock in assay medium) in a drug dilution tube containing 980 µL of assay medium. One hundred micro liters (100 µL) of the 2 µM solution was transferred to 900 µL of assay medium (log dilution) and again for a third serial dilution. One hundred microliters of each concentration were added in triplicate wells for efficacy on one set of microtiter plates containing 100 µL per well of fresh assay medium and in triplicate wells on a second set of microtiter plates for cytotoxicity evaluation. sofosbuvir was purchased from Sigma Aldrich (St. Louis, MO) and evaluated as a positive control compound in the antiviral assays.

Hepatitis C Virus Assay

Cell Culture - The reporter cell line Huh-luc/neo-ET was obtained from Dr. Ralf Bartenschlager (Department of Molecular Virology, Hygiene Institute, University of Heidelberg, Germany) by lmQuest BioSciences through a specific licensing agreement. This cell line harbors the persistently replicating 1Jagluc-ubi-neo/NS3-3'/ET replicon containing the firefly luciferase gene-ubiquitin-neomycin phosphotransferase fusion protein and EMCV IRES driven NS3-5B HCV coding sequences containing the ET tissue culture adaptive mutations (E1202G, T1208I, and K1846T). A stock culture of the Huh-luc/neo•ET was expanded by culture in DMEM supplemented with 10% FCS, 2 mM glutamine, penicillin (100 IU/ml)/streptomycin (100 µg/ml) and 1 X nonessential amino acids plus 1 mg/ml G418. The cells were split 1 :4 and cultured for two passages in the same media plus 250 µg/ml G418. The cells were treated with trypsin and enumerated by staining with trypan blue and seeded into 96-well tissue culture plates at a cell culture density 5 x 103 cells per well and incubated at 37'C 5% CO_2 for 24 hours.

Compound Addition - Following the 24 hours incubation, media was removed and replaced with the same media minus the G418 plus the diluted test compounds in triplicate. The cells were incubated an additional 72 hours at 37"C 5% CO_2 then anti-HCV activity was measured by luciferase endpoint. Duplicate plates were treated and incubated in parallel for assessment of cellular toxicity by XTT staining.

Cellular Viability - The cell culture monolayers from treated cells were stained with the tetrazolium dye XTT following 72 hours incubation to evaluate the cellular viability of the Huh-luc/neo-ET reporter cell line in the presence of the compounds.

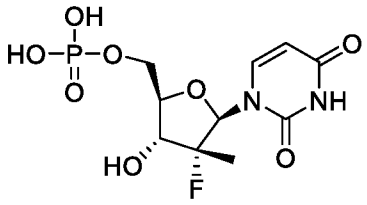
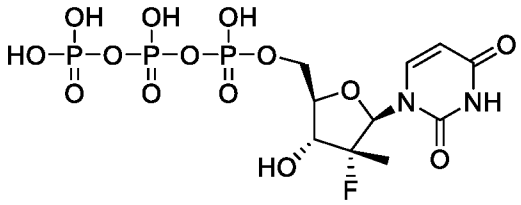
Measurement of Virus Replication- HCV replication from the rep I icon assay system was measured by luciferase activity following 72 hours incubation using the britelite plus luminescence reporter gene kit according to the manufacturer's instructions (Perkin Elmer, Shelton, CT). Briefly, one vial of britelite plus lyophilized substrate was solubilized in 10 ml of britelite reconstitution buffer and mixed gently by inversion. After a 5 minutes incubation at room temperature, the britelite plus reagent was added to the 96 well plates at 100 µl per well. The plates were sealed with adhesive film and incubated at room temperature for approximately 10 minutes to lyse the cells. The well contents were transferred to a white 96-well plate and luminescence was measured within 15 minutes using the Wallac 1450 Microbeta Trilux liquid scintillation counter.

Anti-HCV Evaluation - The system used for analysis of anti-HCV activity consisted of a human liver carcinoma cell line (Huh-7) stably transfected with an autonomously replicating bicistronic HCV subgenomic RNA molecule termed a replicon. Translation of the 5' cistron is driven by the HCV IRES contained in the viral 5' non-coding region (5'-NGR) leading to the synthesis of a firefly luciferase gene-ubiquitin-neomycin phosphotransferase fusion protein.

Synthesis of the fusion protein allows for the monitoring of RNA replication through analysis of reporter firefly luciferase activity and the positive selection of transfected cells with G418 antibiotic. The second cistron consists of HCV non-structural genes (NS3 to NS5B) and the HGV 3'-NCR proceeded by the encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES). EMGV IRES-mediated translation of these downstream genes results in the synthesis of the viral enzymatic proteins necessary for viral RNA replication and polyprotein processing. The use of the replicon system in the analysis of anti-viral activity permits the evaluation of a compound's ability to inhibit several molecular processes in viral replication, including viral RNA synthesis by the NS5B viral RNA-dependent RNA polymerase, IRES-mediated translation of the viral polyprotein, and polyprotein processing by the virally encoded NS3 proteinase. The test articles were evaluated using a high-test concentration of 1 μM and serial logarithmic dilutions.

Sofosbuvir control compound yielded an EC_{50} value of 0.26 μM. Sofosbuvir triphosphate solution RS1 (10 mM) and Sofosbuvir monophosphate solution RS2 (10 mM) were not active against HCV up to 1 μM.

Antiviral	**HCV Replicon EC_{50} (μM)**	**HCV Replicon TC_{50} (μM)**
Formulation 3	**<1**	**>1**
Formulation 4	**<1**	**>1**
Formulation 5	**<1**	**>1**
Sofosbuvir	0.26	**>1**

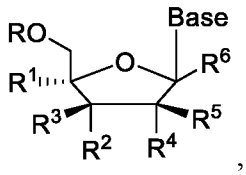
Antiviral	HCV Replicon EC_{50} (μM)	HCV Replicon TC_{50} (μM)
Sofosbuvir monophosphate 	>1	>1
Sofosbuvir triphosphate 	>1	>1

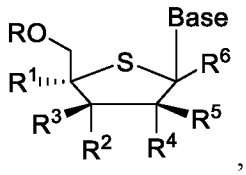
This specification has been described with reference to embodiments of the invention. Given the teaching herein, one of ordinary skill in the art will be able to modify the invention for a desired purpose and such variations are considered within the scope of the invention.

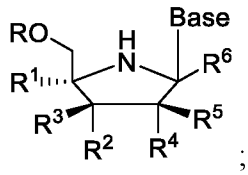
CLAIMS

I claim:

1. A 5'-phosphorylated antiviral monomeric nucleotide in a cationic lipid nano or microparticle composition.
2. The composition of claim 1, wherein the cationic lipid is permanently cationic.
3. The composition of claim 1, wherein the cationic lipid ionizable.
4. The composition of any one of claims 1-3, wherein the 5'-phosphorylated antiviral monomeric nucleotide is a 5'-monophosphate.
5. The composition of any one of claims 1-3, wherein the 5'-phosphorylated antiviral monomeric nucleotide is a 5'-diphosphate.
6. The composition of any one of claims 1-3, wherein the 5'-phosphorylated antiviral monomeric nucleotide is a 5'-triphosphate.
7. The composition of any one of claims 1-6, that also comprises a non-ionizable lipid.
8. The composition of any one of claims 1-7, that also comprises a sterol.
9. The composition of any one of claims 1-8, that also comprises a PEG lipid
10. The composition of any one of claims 1-9, that also comprises a structural lipid.
11. The composition of any one of claims 1-10, that also comprises a phospholipid.
12. A composition comprising a cationic lipid and an active compound of Formula



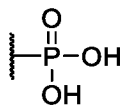
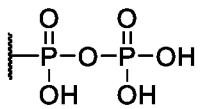
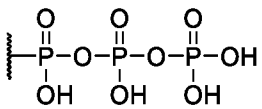
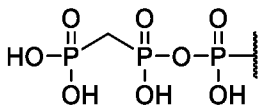
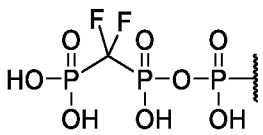
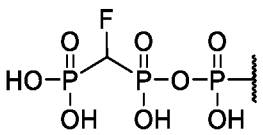
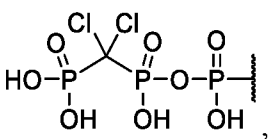
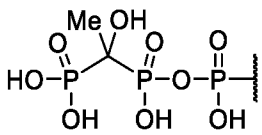
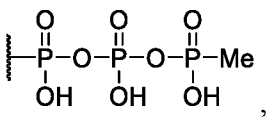
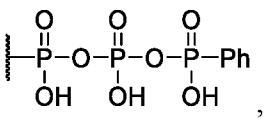
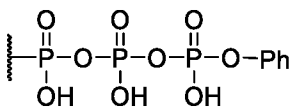
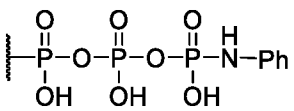
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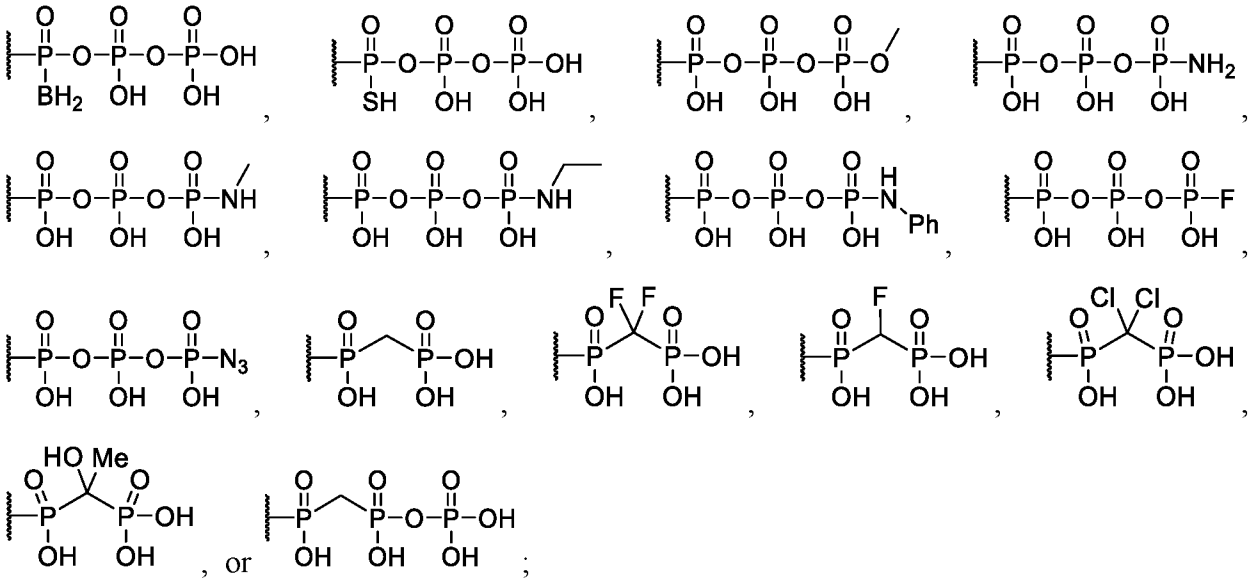
, or


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or a pharmaceutically acceptable salt thereof;

wherein:

R is

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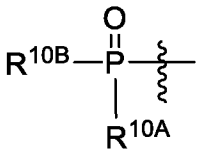


R^1, R^2, R^3, R^4, R^5 and R^6 are independently selected from the group consisting of H, NR^7R^8, $-C(O)NR^7R^8$, $-OC(O)NR^7R^8$, $-C(O)OR^7$, $-OC(O)OR^7$, $-S(O)_nR^9$, $-S(O)_2NR^7R^8$, N_3, $-OR^{10}$, CN, halogen, C_1-C_8alkyl, C_1-C_8haloalkyl, $C_{1\text{-}8}$alkoxy, $-(C_0$-C_2alkyl)(cycloalkyl), $-C_2$-C_8alkenyl, $-C_2$-C_8alkynyl, and aryl(C_1-C_8alkyl)-;

or any two of R^1, R^2, R^3, R^4, R^5 and R^6 on adjacent carbons when taken together are $-OC(O)O-$ or $-O(CR^7R^8)O-$ or when taken together with the ring carbon atoms to which are attached form a double bond;

R^7 and R^8 are independently selected from the group consisting of H, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, $-(C_0$-C_2alkyl)(cycloalkyl), aryl(C_1-C_8alkyl)-, $-(C_0$-C_2alkyl)(heterocyclo), aryl, heteroaryl, $-C(O)R^{10C}$, and $-S(O)_n(C_1$-$C_8)$alkyl or R^7 and R^8 taken together with a nitrogen to which they are both attached form a 3 to 7 membered heterocyclic ring;

R^9 is selected from the group consisting of C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, $-(C_0$-C_2alkyl)(cycloalkyl), aryl(C_1-C_8alkyl)-, $-(C_0$-C_2alkyl)(heterocyclo), aryl, and heteroaryl;

R^{10} is selected from the group consisting of H, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, $-(C_0$-C_2alkyl)(cycloalkyl), aryl(C_1-C_8alkyl)-, $-(C_0$-C_2alkyl)(heterocyclo), aryl, heteroaryl, $-C(O)R^{10C}$, $-S(O)_n(C_1$-$C_8)$alkyl, , diphosphate, triphosphate, an optionally substituted carbonyl linked amino acid, or $-C(O)R^{10C}$;

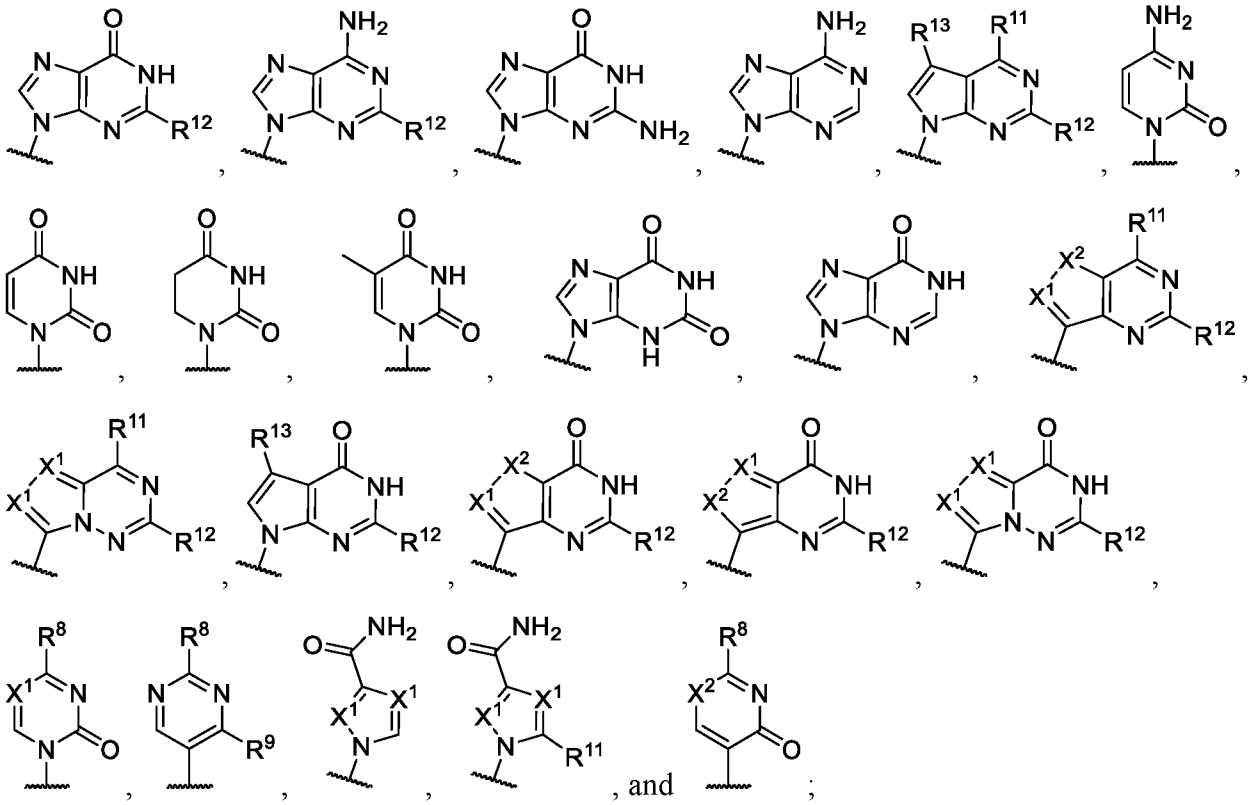
R^{10A} is selected from the group consisting of OH, an –O-optionally substituted aryl, an –O-optionally substituted heteroaryl, and an optionally substituted heterocyclyl;

R^{10B} is selected from the group consisting of OH, an optionally substituted N-linked amino acid, and an optionally substituted N-linked amino acid ester; and

R^{10C} is alkyl, alkenyl, alkynyl, -(C_0-C_2alkyl)(cycloalkyl), -(C_0-C_2alkyl)(heterocyclo), -(C_0-C_2alkyl)(aryl), -(C_0-C_2alkyl)(heteroaryl), -O-alkyl, -O-alkenyl, -O-alkynyl, -O-(C_0-C_2alkyl)(cycloalkyl), -O-(C_0-C_2alkyl)(heterocyclo), -O-(C_0-C_2alkyl)(aryl), or -O-(C_0-C_2alkyl)(heteroaryl);

n is independently selected at each instance from 0, 1, 2, and 3;

Base is selected from:



each X^1 is independently N or CR^{13};

X^2 is independently NR^7, O, or $S(O)_n$;

R^{11} is independently halogen, -NR^7R^8, N_3, NO, NO_2, $C(O)R^{10C}$, CN, —CH(=NR^7), —CH=NNHR^7, —CH=N(OR^7), —CH(OR^7)$_2$, —C(=O)NR^7R^8, -C(=S)NR^7R^8, -C(=O)OR^7, C_1-C_8alkyl, C_2-C_8alkenyl, C_2-C_8alkynyl, -(C_0-C_2alkyl)(cycloalkyl),

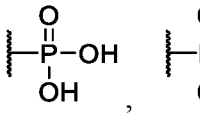
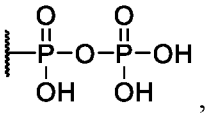
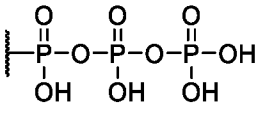
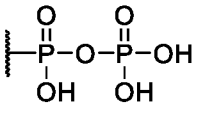
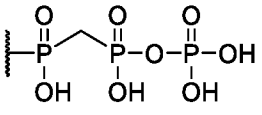
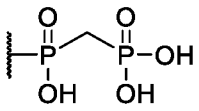
aryl, -(C_0-C_2alkyl)(heterocyclo), heteroaryl, —C(O)R^{10C}, —S(O)$_n$(C_1-C_8)alkyl, aryl(C_1-C_8alkyl)-, or $OR^{10;}$ and

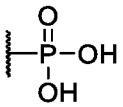
R^{12} and R^{13} are independently selected at each instance from the group consisting of H, halogen, -NR^7R^8, N_3, NO, NO_2, C(O)R^{10C}, CN, —CH(=NR^7), —CH=NHNR^7, —CH=N(OR^7), —CH(OR^7)$_2$, —C(=O)NR^7R^8, -C(=S)NR^7R^8, —C(=O)OR^7, R^7, OR^{10}, and SR^8.

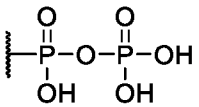
13. The composition of claim 12, wherein the composition comprises nanoparticles.

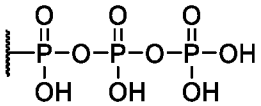
14. The composition of claim 12, wherein the composition comprises microparticles.

15. The composition of any one of claims 12-14, wherein R is


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16. The composition of any one of claims 12-14, wherein R is 
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17. The composition of any one of claims 12-14, wherein R is 
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18. The composition of any one of claims 12-14, wherein R is 
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19. The composition of any one of claims 12-18, wherein R^1 is hydrogen.

20. The composition of any one of claims 12-18, wherein R^1 is methyl.

21. The composition of any one of claims 12-18, wherein R^1 is F.

22. The composition of any one of claims 12-21, wherein R^2 is hydrogen.

23. The composition of any one of claims 12-21, wherein R^2 is methyl.

24. The composition of any one of claims 12-21, wherein R^2 is F.

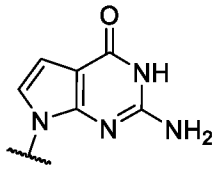
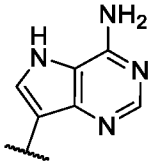
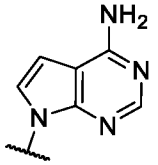
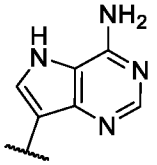
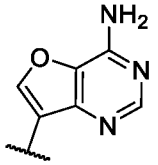
25. The composition of any one of claims 12-24, wherein R^3 is hydrogen.

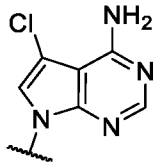
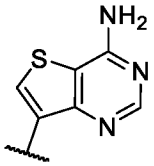
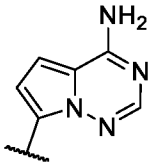
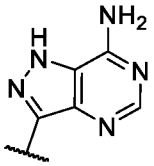
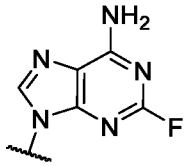
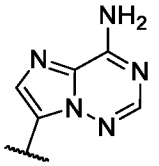
26. The composition of any one of claims 12-24, wherein R^3 is methyl.

27. The composition of any one of claims 12-24, wherein R^3 is F.

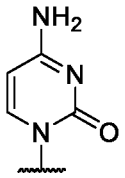
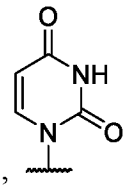
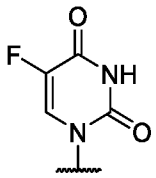
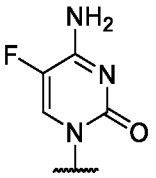
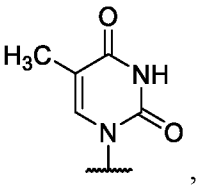
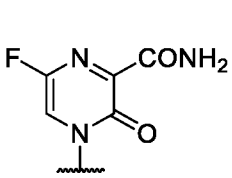
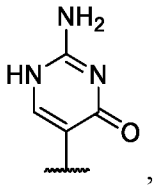
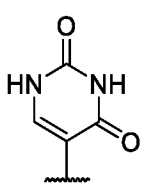
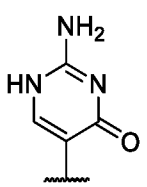
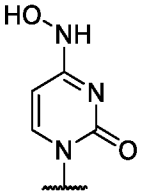
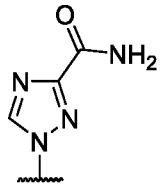
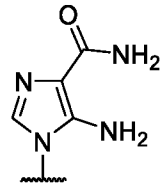
28. The composition of any one of claims 12-27, wherein R^4 is hydrogen.

29. The composition of any one of claims 12-27, wherein R^4 is methyl.
30. The composition of any one of claims 12-27, wherein R^4 is F.
31. The composition of any one of claims 12-30, wherein R^5 is hydrogen.
32. The composition of any one of claims 12-30, wherein R^5 is methyl.
33. The composition of any one of claims 12-30, wherein R^5 is F.
34. The composition of any one of claims 12-33, wherein R^6 is hydrogen.
35. The composition of any one of claims 12-34, wherein Base is selected from the group consisting of:

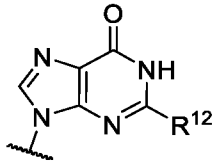
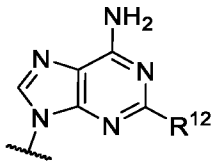
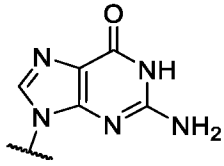
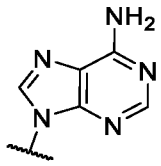
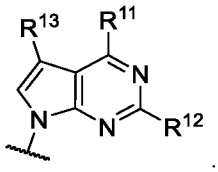

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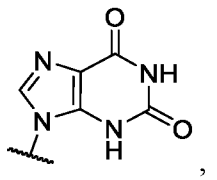
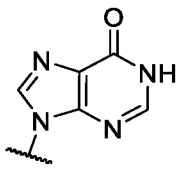
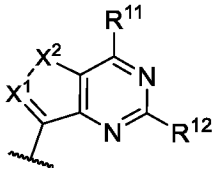
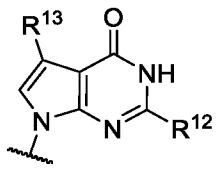
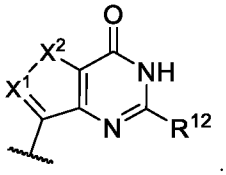
36. The composition of any one of claims 12-34, wherein Base is selected from the group consisting of:


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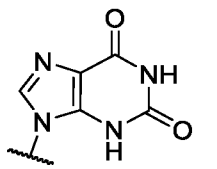
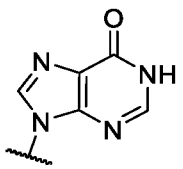
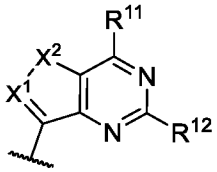
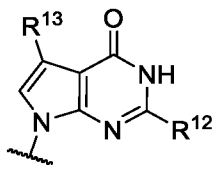
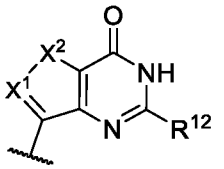
37. The composition of any one of claims 12-34, wherein Base is selected from the group consisting of:

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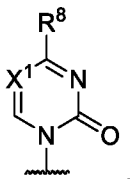
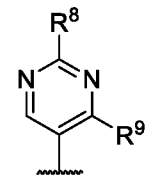
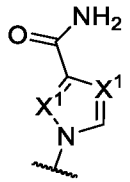
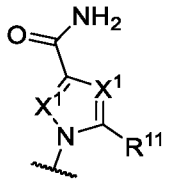
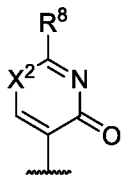
38. The composition of any one of claims 12-34, wherein Base is selected from the group consisting of:

, , , , and .

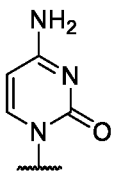
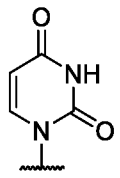
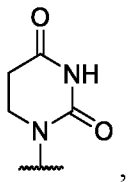
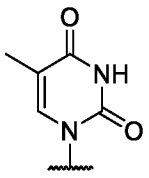
39. The composition of any one of claims 12-34, wherein Base is selected from the group consisting of:

, , , , and .

40. The composition of any one of claims 12-34, wherein Base is selected from the group consisting of:

, , , , and .

41. The composition of any one of claims 12-34, wherein Base is selected from the group consisting of:

, , , and .

42. The composition of any one of claims 12-41, wherein X^1 is N.

43. The composition of any one of claims 12-41, wherein X^1 is CR^{13}.

44. The composition of any one of claims 12-43, wherein X^2 is NR^7.

45. The composition of any one of claims 12-43, wherein X^2 is O.

46. The composition of any one of claims 12-45, wherein R^{11} is halogen.

47. The composition of any one of claims 12-45, wherein R^{11} is $-NR^7R^8$.

48. The composition of any one of claims 12-45, wherein R^{11} is halogen.

49. The composition of any one of claims 12-45, wherein R^{11} is F.

50. The composition of any one of claims 12-45, wherein R^{11} is Cl.

51. The composition of any one of claims 12-45, wherein R^{11} is cyano.

52. The composition of any one of claims 12-51, wherein R^{12} is halogen.

53. The composition of any one of claims 12-51, wherein R^{12} is $-NR^7R^8$.

54. The composition of any one of claims 12-51, wherein R^{12} is halogen.

55. The composition of any one of claims 12-51, wherein R^{12} is F.

56. The composition of any one of claims 12-51, wherein R^{12} is Cl.

57. The composition of any one of claims 12-51, wherein R^{12} is cyano.

58. The composition of any one of claims 12-57, wherein R^{13} is halogen.

59. The composition of any one of claims 12-57, wherein R^{13} is $-NR^7R^8$.

60. The composition of any one of claims 12-57, wherein R^{13} is halogen.

61. The composition of any one of claims 12-57, wherein R^{13} is F.

62. The composition of any one of claims 12-57, wherein R^{13} is Cl.

63. The composition of any one of claims 12-57, wherein R^{13} is cyano.

64. The composition of any one of claims 12-63, wherein R^7 is hydrogen.

65. The composition of any one of claims 12-63, wherein R^7 is methyl.

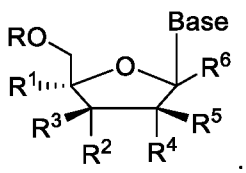
66. The composition of any one of claims 12-63, wherein R^7 is ethyl.

67. The composition of any one of claims 12-66, wherein R^8 is hydrogen.

68. The composition of any one of claims 12-66, wherein R^8 is methyl.

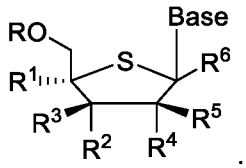
69. The composition of any one of claims 12-66, wherein R^8 is ethyl.

70. The composition of any one of claims 12-69, wherein the compound is of Formula



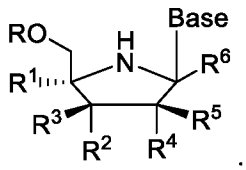
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71. The composition of any one of claims 12-69, wherein the compound is of Formula



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72. The composition of any one of claims 12-69, wherein the compound is of Formula



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73. The composition of any one of claims 1-72, wherein the cationic lipid is selected from the group consisting of PEG-DMG, PEG-DSG, DODMA, DODAP, DOTMA, DOTAP, KC2 and MC3.
74. The composition of any one of claims 1-72, wherein the cationic lipid is DODAP.
75. The composition of any one of claims 1-72, wherein the cationic lipid is DODMA.
76. The composition of any one of claims 1-72, wherein the cationic lipid is DOTAP.
77. The composition of any one of claims 1-72, wherein the cationic lipid is KC2.
78. The composition of any one of claims 1-72, wherein the cationic lipid is MC3.
79. The composition of any one of claims 1-78, comprising a second cationic lipid.
80. The composition of any one of claims 1-79, comprising a phospholipid.
81. The composition of claim 80, wherein the phospholipid is selected from DDPC, DEPA, DEPC, DEPE, DEPG, DLOPC, DLPA, DLPC, DLPE, DLPG, DLPS, DMG, DMPA, DMPC, DMPE, DMPG, DMPS, DOPA, DOPC, DOPE, DOPG, DOPS, DPPA, DPPC, DPPE, DPPG, DPPS, DPyPE, DSPA, DSPC, DSPE, DSPG, DSPS, EPC, HEPC, HSPC, HSPC, STEARIC, Milk Sphingomyelin MPPC, MSPC, PMPC, POPC, POPE, POPG, PSPC, SMPC, SOPC, and SPPC.
82. The composition of claim 80, wherein the phospholipid is selected from DDPC, DEPA, DEPC, DEPE, DEPG, DLOPC, DLPA, DLPC, DLPE, DLPG, DLPS, DMG, and DMPA.
83. The composition of claim 80, wherein the phospholipid is selected from DMPC, DMPE, DMPG, DMPS, DOPA, DOPC, DOPE, DOPG, DOPS, DPPA, DPPC, DPPE, and DPPG.
84. The composition of claim 80, wherein the phospholipid is selected from DPPS, DPyPE, DSPA, DSPC, DSPE, DSPG, DSPS, EPC, HEPC, HSPC, and HSPC.

85. The composition of claim 80, wherein the phospholipid is selected from MPPC, MSPC, PMPC, POPC, POPE, POPG, PSPC, SMPC, SOPC, and SPPC.
86. The composition of claim 80, wherein the phospholipid is DSPC.
87. The composition of any one of claims 1-86 comprising a structural lipid.
88. The composition of claim 87 comprising cholesterol.
89. A pharmaceutical composition comprising a composition of any one of claims 1-88, wherein the composition is suitable for pharmaceutical administration.
90. A method of treating a virus comprising administering an effective amount of a composition of any one of claims 1-89 to a patient in need thereof.
91. The method of claim 90, wherein the patient is a human.
92. The method of claim 90 or 91, wherein the virus is a DNA virus.
93. The method of claim 90 or 91, wherein the virus is a RNA virus.
94. The method of claim 90 or 91, wherein the virus is selected from the group consisting of HIV, HBV, HCV, SARS-CoV-2, EV-68, EV-71, Respiratory Syncytial virus, Dengue Fever virus, Yellow Fever virus, West Nile virus, Chikungunya virus, Tick-borne encephalitis virus, Zika virus, Japanese encephalitis virus, Semliki Forest virus, Venezuelan equine encephalitis virus, Sindbis virus, Hanta virus, Rift valley fever virus, Crimean Congo Hemorrhagic fever virus, ebola virus, Marburg virus, California encephalitis, Eastern equine encephalitis virus, Western equine encephalitis virus, St. Louis encephalitis virus, Nipah virus, Tickborne encephalitis viruses, Omsk hemorrhagic fever virus, Alkhurma virus, Kyasanur Forest virus, John Cunningham (JC) virus, Norovirus and Norwalk virus, HHV-6, HHV-8, Junin virus, Guanarito, Chopare, Lujo, Hepatitis A, Rotavirus, Papilloma Virus, Lacrosse, Metapneumovirus, Parainfluenza virus, Influenza A virus, Influenza B virus, Coxsackie virus, and Poliovirus.
95. The method of claim 90 or 91, wherein the virus is SARS-CoV-2.
96. The method of claim 90 or 91, wherein the virus is HCV.
97. The method of claim 90 or 91, wherein the virus is HIV.
98. Use of a composition of any one of claims 1-89 in the manufacture of a medicament to treat a virus.
99. The composition of any one of claims 1-89 for use in the treatment of a virus.

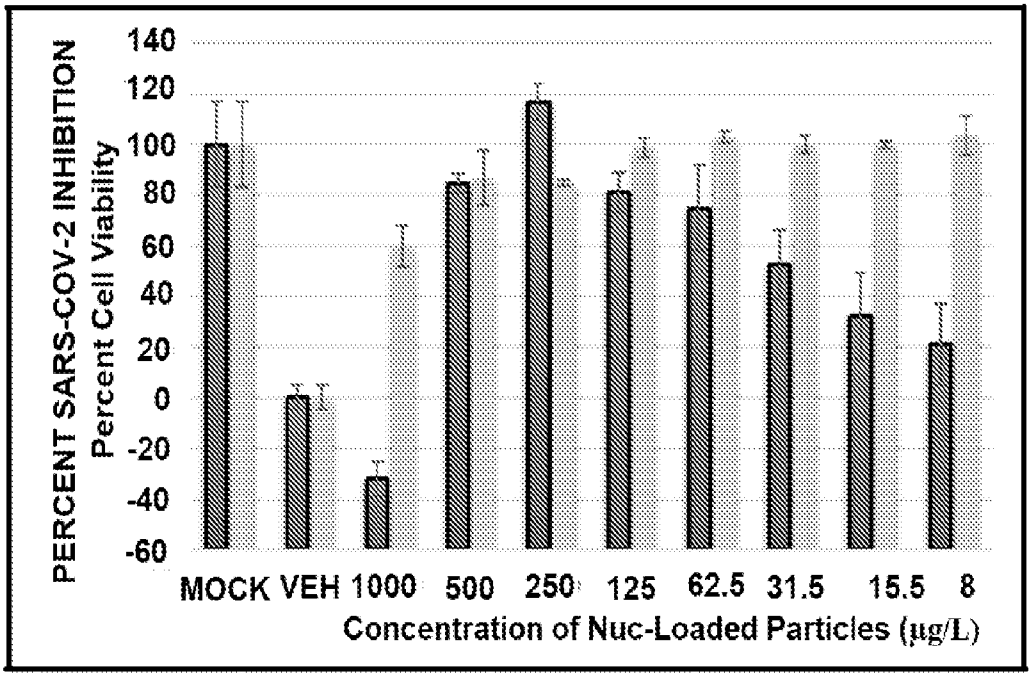

PERCENT SARS-COV-2 INHIBITION
Percent Cell Viability
140
120
100
80
60
40
20
0
-20
-40
-60
MOCK VEH 1000 500 250 125 62.5 31.5 15.5 8
Concentration of Nuc-Loaded Particles (µg/L)

FIG. 1

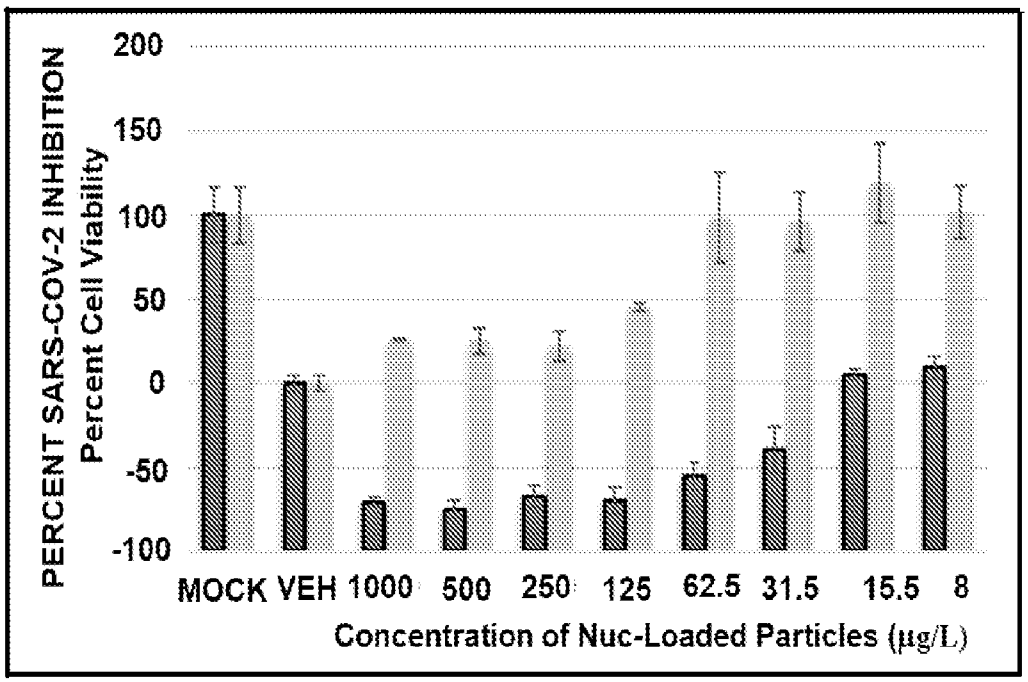

PERCENT SARS-COV-2 INHIBITION
Percent Cell Viability
200
150
100
50
0
-50
-100
MOCK VEH 1000 500 250 125 62.5 31.5 15.5 8
Concentration of Nuc-Loaded Particles (µg/L)

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/14633

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/12; A61K 39/145; A61P 31/16 (2022.01)

CPC - A61K3 9/12; A61K 39/145; A61P 31/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHIEN et al. "Nucleotide Analogues as Inhibitors of SARS-CoV-2 Polymerase, a Key Drug Target for COVID-19", J. Proteome Res. 2020. 19, pp 4690-4697, especially: abstract, formula; pg 4692, col 1, para 2.	1-6
Y	DELSHADI et al. "Development of nanoparticle-delivery systems for antiviral agents: A review", Journal of Controlled Release. January 2021. 331, pp 30-44, especially: abstract; pg 40, col 1, para 6 to col 2, para 1; pg 40, col 2, para 3.	1-6
A	SIEGEL et al. "Discovery and Synthesis of a Phosphoramidate Prodrug of a Pyrrolo[2,1-f][triazin -4-amino] Adenine C-Nucleoside (GS-5734) for the Treatment of Ebola and Emerging Viruses", J. Med. Chem. 2017. 60, pp 1648-1661, entire document.	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 June 2022	JUN 28 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/14633

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-11, 19-99
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
(see extra sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

--BOX III - LACK OF UNITY--

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-6, drawn to a 5'-phosphorylated antiviral monomeric nucleotide in a cationic lipid nano or microparticle composition.

Group II: Claims 12-18, drawn to a composition comprising a cationic lipid and an active compound of Formula indicated in the instant claim, or a pharmaceutically acceptable salt thereof.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I requires a 5'-phosphorylated antiviral monomeric nucleotide, which is not required by Group II.

Group II requires an active compound of Formula indicated in the instant claim 2, or a pharmaceutically acceptable salt thereof, which is not required by Group I.

Shared Common Features

The only feature shared by Groups I and II that would otherwise unify the groups is a cationic lipid nano or microparticle composition. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by the article entitled "Development of nanoparticle-delivery systems for antiviral agents: A review" by Delshadi et al. (hereinafter 'DELSHADI').

Delshadi teaches a cationic lipid nano or microparticle composition (pg 40, col 1, para 6 to col 2, para 1, In many respects, the structure of solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) is similar to that of O/W nanoemulsions, except that the lipid phase inside the nanoparticles is solid rather than liquid; see also pg 40, col 2, para 3, El-Gizawy, El-Maghraby et al. [54] designed a SLN-based system for brain targeting of acyclovir. The SLNs were formulated from a variety of lipids and surfactants, and the nanoparticles were coated with chitosan to make them cationic).

As the technical features were known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups. Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Claims 7-11 and 19-99 are unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).