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(54) Title: COMPOUNDS, COMPOSITIONS, AND METHODS FOR THE TREATMENT OF DISEASE

(57) Abstract: Disclosed are compounds and compositions for the activation or induction of expression of a pattern recognition receptor (e.g., STING, RIG-I, MDA5), and methods of use thereof.



WO 2020/014127 A1

**COMPOUNDS, COMPOSITIONS, AND METHODS FOR THE
TREATMENT OF DISEASE**

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. provisional patent application No. 62/695,997, filed July 10, 2018, the contents of which is hereby incorporated by reference.

FIELD OF THE INVENTION

Disclosed are compounds and compositions that activate in a host the innate immune defense system and induce expression of pattern recognition receptors, as well as methods of using them for the treatment of a microbial infection or a proliferative disease (e.g., cancer).

BACKGROUND OF THE INVENTION

A key feature of the innate immune system is the recognition and elimination of foreign substances. Identification of these pathogenic invaders occurs through host recognition of evolutionarily conserved microbial structures known as pathogen-associated molecular patterns (PAMPs) (Jensen, S. and Thomsen, A.R. *J Virol* (2012) 86:2900-2910). These PAMPs include a wide array of molecular structures, such as nucleic acids, lipopolysaccharides, and glycoproteins that may be broadly shared by multiple microbial species and are critical to their survival and/or pathogenicity. Host recognition may occur by multiple pathways, such as activation of pattern recognition receptors (PRRs), which ultimately lead to downstream signaling events and culminate in the mounting of an immune response.

To date, several PRRs have been identified that serve as sensors of pathogenic infection. For example, the retinoic acid-inducible gene-I (RIG-I) protein is a RNA helicase that also functions as a sensor of microbial-derived RNA. RIG-I is important factor in host recognition of RNA viruses from a variety of different viral families, including *Flaviviridae* (e.g., West Nile virus, Hepatitis C virus, Japanese encephalitis virus, Dengue virus), *Paramyxoviridae* (e.g., Sendai virus, Newcastle disease virus, Respiratory syncytial virus, Measles virus), *Rhabdoviridae* (e.g., Rabies virus), *Orthomyxoviridae* (e.g., influenza A virus, influenza B virus), and *Arenaviridae* (e.g., Lassa virus). Stimulator of interferon genes (STING) is a cytoplasmic adaptor protein that activates the TBK1-IRF3 signaling complex, resulting in induction of type I interferons (IFN- β

and IFN- α) and other immune pathway proteins. Other PRRs also play a role in sensing microbial-derived nucleic acids, including NOD2, LGP2, MDA5, and a number of Toll-like receptors (TLRs) that are expressed on the cell surface and within endosomal compartments.

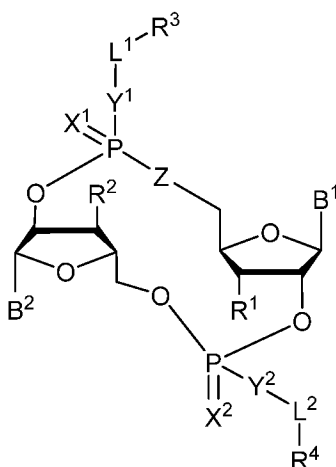
A shortcoming of many current antiviral therapies relates to the emergence of drug resistant variants that occurs upon extended use. In addition, many available treatments require persistent and long-term therapy, which often results in unwanted side effects and the risk of relapse upon conclusion of treatment. Further, many viruses can be subdivided into different genotypes, and certain drugs developed against one genotype may not be active against other genotypes. In contrast, the use of small molecule mimics of viral-derived RNA capable of PRR induction provides an alternate approach to the treatment of viral infection, as these compounds may be agnostic to genotype, may possess both direct antiviral activity as well as the ability to activate the host immune response, and potentially limit the development of drug resistance and toxicity. As such, there exists a need for a new generation of therapies that induce expression of PRRs for use in the treatment of disease and as diagnostic tools.

In addition, RIG-I serves as a biomarker for the prediction of prognosis for certain types of cancer, such as hepatocellular carcinoma (Hou, J. et al, *Cancer Cell* (2014) 25:49-63). Recent publications have highlighted the importance of RIG-I and STING as mediators of innate and adaptive immunity, and RIG-I and STING agonists have been recognized as immuno-oncology agents in cancer therapy (Li, X.Y. et al, *Mol Cell Oncol* (2014) 1:e968016; Woo, S. R. *Trends in Immunol* (2015) 36:250-256). In particular, RIG-I is involved in the regulation of basic cellular processes such as hematopoietic proliferation and differentiation, maintenance of leukemic stemness, and tumorigenesis of hepatocellular carcinoma, indicating that RIG-I performs an essential function as a tumor suppressor. Importantly, the STING pathway of cytosolic DNA sensing has been shown to play an important mechanistic role in innate immune sensing, driving type I IFN production in cancer and in the context of immuno-oncology applications, including therapeutics and diagnostics.

SUMMARY OF THE INVENTION

Cyclic dinucleotide compounds, compositions comprising cyclic dinucleotide compounds, and related methods of use are described herein.

In one aspect, the disclosure features a compound of Formula (I):

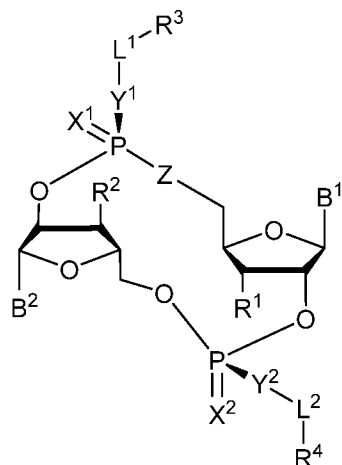


Formula (I)

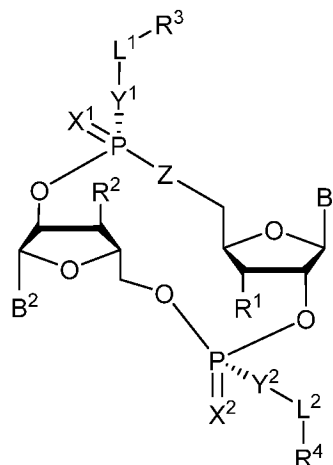
or a pharmaceutically acceptable salt or stereoisomer thereof, wherein: Z is either S or O; each of B¹ and B² is independently a purinyl nucleobase or pyrimidinyl nucleobase; each of X¹ and X² is independently O or S; each of Y¹ and Y² is independently O, S, or NR⁵; each of L¹ and L² is independently absent, C₁-C₆ alkyl or C₁-C₆ heteroalkyl, wherein each alkyl and heteroalkyl is optionally substituted with R⁶; each of R¹ and R² is independently hydrogen, halo, -CN, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), or OR⁷; each of R³ and R⁴ is independently hydrogen, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), C₁-C₂₀ heteroalkyl (e.g., C₁-C₆ heteroalkyl), OC(O)OC₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), cycloalkyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, and heteroaryl is optionally substituted with one or more R⁸; R⁵ is hydrogen or C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl); R⁶ is halo, -CN, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), OR⁷, oxo, cycloalkyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, cycloalkyl, heterocyclyl, aryl, and heteroaryl is optionally substituted with one or more R⁹; R⁷ is hydrogen, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), cycloalkyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, cycloalkyl, heterocyclyl, aryl, and heteroaryl is optionally substituted with one or more R⁹; each R⁸ is independently C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), C₁-C₂₀ heteroalkyl, C(O)-C₁-C₂₀ alkyl, OC(O)-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), C(O)O-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), OC(O)O-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), C(O)N(R⁵)-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), N(R⁵)C(O)-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), OC(O)N(R⁵)-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), O-aryl, O-heteroaryl, C(O)-aryl, C(O)-heteroaryl, OC(O)-aryl, C(O)O-aryl, OC(O)-heteroaryl, C(O)O-heteroaryl, C(O)O-aryl, C(O)O-heteroaryl, C(O)N(R⁵)-aryl, C(O)N(R⁵)-heteroaryl, N(R⁵)C(O)-aryl, N(R⁵)₂C(O)-aryl, or N(R⁵)C(O)-heteroaryl, S(O)₂N(R⁵)-aryl, wherein each alkyl, heteroalkyl, aryl, and heteroaryl is optionally

substituted by one or more R^9 ; and each R^9 is independently C_1 - C_{20} alkyl, O - C_1 - C_{20} alkyl, C_1 - C_{20} heteroalkyl, halo, $-CN$, OH , oxo, aryl, heteroaryl, O -aryl, or O -heteroaryl.

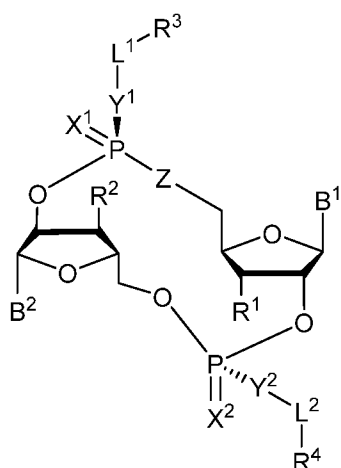
In some embodiments, the compound is a compound of Formula (I-a), (I-b), (I-c), or (I-d):



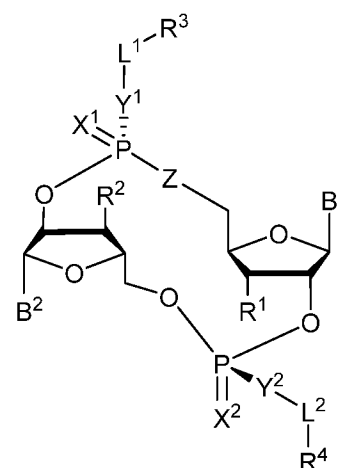
Formula (I-a)



Formula (I-b)



Formula (I-c)



Formula (I-d)

or a pharmaceutically acceptable salt thereof, wherein each of Z , B^1 , B^2 , X^1 , X^2 , Y^1 , Y^2 , L^1 , L^2 , R^1 , R^2 , R^3 , R^4 , and subvariables thereof, are as previously described.

In one aspect, the present disclosure describes a method of inducing the expression of a pattern recognition receptor in a subject suffering from a microbial infection, comprising administering to the subject an effective amount of a compound of Formula (I).

In another aspect, the disclosure features a method of treating cancer in a subject, the method comprising administering to the subject a therapeutically effective amount of a compound of Formula (I).

In another aspect, the disclosure features a composition, comprising a vaccine, and a vaccine adjuvant comprising a compound of Formula (I).

In another aspect, the disclosure features a method of inducing the expression of a pattern recognition receptors (PRR) for immune-modulation in a subject, the method comprising administering to the subject an effective amount of a compound of Formula (I).

In another aspect, the disclosure features a method of inducing the expression of a pattern recognition receptor (PRR) for immunomodulation and inducing a therapeutic response in a subject having cancer, the method comprising administering to the subject an effective amount of a compound of Formula (I).

In another aspect, the present disclosure features a method of inducing an immune response in a subject, the method comprising administering to the subject a therapeutically effective amount of a compound of Formula (I).

In some embodiments, the immune response comprises antitumoral immunity. In some embodiments, the immune response comprises induction of a PRR (e.g., STING, RIG-I, MDA5).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A depicts that compound 1 activates natural allelic variant HAQ to induce IRF. THP1 cells naturally expressing HAQ isoform human STING (hSTING), stably transfected with an ISRE-inducible Lucia and NF- κ B inducible SEAP were treated with various concentrations of Cmd 1 or DMSO control. After 20h incubation, levels of Lucia and SEAP were measured using QUANTI-luc and QUANTI-Blue to measure and ISRE and NF- κ B reporter activity respectively. Percent induction was calculated from fold change in activity compared to DMSO-treated sample.

Figure 1B depicts that compound 1 activates natural allelic variant HAQ to induce IRF. THP1 cells naturally expressing HAQ isoform human STING (hSTING), stably transfected with an ISRE-inducible Lucia and NF- κ B inducible SEAP were treated with various concentrations of Cmd 1 or DMSO control. After 20h incubation, levels of Lucia and SEAP were measured using QUANTI-luc and QUANTI-Blue to measure and ISRE and NF- κ B reporter activity respectively.

Percent induction was calculated from fold change in activity compared to DMSO-treated sample. EC₅₀ values were generated by curve fit in Xlfit.

Figure 2 depicts that compound 1 binds to STING with high affinity. Thermal shift assay was conducted with 0.1 mg/mL of STING CTD with various dilutions of compound 1 from 2 mM to 0.05 μ M in 10 mM HEPES (pH 7.5), 140 mM NaCl and a 5 fold dilution of SYPRO Orange dye (Invitrogen). The fluorescence signal as a function of temperature was recorded using a Real Time PCR machine (*Thermo Fisher*). The temperature gradient was performed in the range of 25–80 °C with a ramp of 0.2 °C over the course of 60 min. Data were analyzed with the Thermal Shift Software (*Thermo Fisher*), DSF analysis, and the Derivative model was used to fit the fluorescence data to obtain the midpoint temperature for the thermal protein unfolding transition (T_m) using the curve-fitting software Prism.

Figure 3A depicts that compound 2 activates natural allelic variant HAQ to induce IRF and NF- κ B. THP1 cells naturally expressing HAQ isoform human STING (hSTING), stably transfected with an ISRE-inducible Lucia and NF- κ B inducible SEAP were treated with various concentrations of Cmd 2 or DMSO control. After 20 h incubation, levels of Lucia and SEAP were measured using QUANTI-luc and QUANTI-Blue to measure and ISRE and NF- κ B reporter activity, respectively, in Xlfit. Percent induction was calculated from fold change in activity compared to DMSO-treated sample.

Figure 3B depicts that compound 2 activates natural allelic variant HAQ to induce IRF and NF- κ B. THP1 cells naturally expressing HAQ isoform human STING (hSTING), stably transfected with an ISRE-inducible Lucia and NF- κ B inducible SEAP were treated with various concentrations of Cmd 2 or DMSO control. After 20 h incubation, levels of Lucia and SEAP were measured using QUANTI-luc and QUANTI-Blue to measure and ISRE and NF- κ B reporter activity, respectively, in Xlfit. Percent induction was calculated from fold change in activity compared to DMSO-treated sample. EC₅₀ values were generated by curve fit.

Figure 3C depicts that compound 2 activates natural allelic variant HAQ to induce IRF and NF- κ B. THP1 cells naturally expressing HAQ isoform human STING (hSTING), stably transfected with an ISRE-inducible Lucia and NF- κ B inducible SEAP were treated with various concentrations of Cmd 2 or DMSO control. After 20 h incubation, levels of Lucia and SEAP were measured using QUANTI-luc and QUANTI-Blue to measure and ISRE and NF- κ B reporter

activity, respectively, in Xlfit. Percent induction was calculated from fold change in activity compared to DMSO-treated sample.

Figure 3D depicts that compound 2 activates natural allelic variant HAQ to induce IRF and NF- κ B. THP1 cells naturally expressing HAQ isoform human STING (hSTING), stably transfected with an ISRE-inducible Lucia and NF- κ B inducible SEAP were treated with various concentrations of Cmd 2 or DMSO control. After 20 h incubation, levels of Lucia and SEAP were measured using QUANTI-luc and QUANTI-Blue to measure and ISRE and NF- κ B reporter activity, respectively, in Xlfit. Percent induction was calculated from fold change in activity compared to DMSO-treated sample. EC₅₀ values were generated by curve fit.

Figure 4A depicts that compound 1 activates mouse STING to induce IRF in RAW macrophages. Mouse derived RAW macrophages stably transfected with an ISRE-inducible Lucia were treated with various concentrations of compound 1 or DMSO control. After 20 h incubation, levels of Lucia were measured using QUANTI-luc to measure and ISRE reporter activity. Percent induction was calculated from fold change in activity compared to DMSO-treated sample.

Figure 4B depicts that compound 1 activates mouse STING to induce IRF in RAW macrophages. Mouse derived RAW macrophages stably transfected with an ISRE-inducible Lucia were treated with various concentrations of compound 1 or DMSO control. After 20 h incubation, levels of Lucia were measured using QUANTI-luc to measure and ISRE reporter activity. Percent induction was calculated from fold change in activity compared to DMSO-treated sample. EC₅₀ values were generated by curve fit in Xlfit.

Figure 5A depicts that compound 2 activates mouse STING to induce IRF in RAW macrophages. Mouse derived RAW macrophages stably transfected with an ISRE-inducible Lucia were treated with various concentrations of compound 2 or DMSO control. After 20h incubation, levels of Lucia were measured using QUANTI-luc to measure and ISRE reporter activity. Percent induction was calculated from fold change in activity compared to DMSO-treated sample.

Figure 5B depicts that compound 2 activates mouse STING to induce IRF in RAW macrophages. Mouse derived RAW macrophages stably transfected with an ISRE-inducible Lucia were treated with various concentrations of compound 2 or DMSO control. After 20h incubation, levels of Lucia were measured using QUANTI-luc to measure and ISRE reporter activity. Percent induction was calculated from fold change in activity compared to DMSO-treated sample. EC₅₀ values were generated by curve fit in Xlfit.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure relates to methods of activating and/or inducing the expression of PRRs (e.g., STING) in a subject, in particular for the treatment of a microbial infection or a proliferative disease (e.g., cancer). In some embodiments, the method comprises administration of a compound of Formula (I) or pharmaceutically acceptable salt thereof. It is to be noted that induction of any PRR with these compounds can stimulate interferon and/or NF-KB production which can induce the expression of a variety of PRRs which are inducible genes by feedback mechanism.

Definitions

As used herein, the articles "a" and "an" refer to one or to more than one (e.g., to at least one) of the grammatical object of the article.

"About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values.

As used herein, the term "acquire" or "acquiring" as the terms are used herein, refer to obtaining possession of a physical entity (e.g., a sample, e.g., blood sample or liver biopsy specimen), or a value, e.g., a numerical value, by "directly acquiring" or "indirectly acquiring" the physical entity or value. "Directly acquiring" means performing a process (e.g., an analytical method) to obtain the physical entity or value. "Indirectly acquiring" refers to receiving the physical entity or value from another party or source (e.g., a third party laboratory that directly acquired the physical entity or value). Directly acquiring a value includes performing a process that includes a physical change in a sample or another substance, e.g., performing an analytical process which includes a physical change in a substance, e.g., a sample, performing an analytical method, e.g., a method as described herein, e.g., by sample analysis of bodily fluid, such as blood by, e.g., mass spectroscopy, e.g., LC-MS.

As used herein, the terms "induce" or "induction of" refer to the increase or enhancement of a function, e.g., the increase or enhancement of the expression of a pattern recognition receptor (e.g., STING). In some embodiments, "induction of PRR expression" refers to induction of transcription of PRR RNA, e.g., STING RNA (e.g., mRNA, e.g., an increase or enhancement of), or the translation of a PRR protein, e.g., the STING protein (e.g., an increase or enhancement of).

In some embodiments, induction of PRR expression (e.g., STING expression) refers to the increase or enhancement of the concentration of a PRR RNA, e.g., or STING RNA (e.g., mRNA) or the STING protein, e.g., in a cell. In some embodiments, induction of PRR expression (e.g., STING expression) refers to the increase of the number of copies of PRR RNA, e.g., STING RNA (e.g., mRNA) or PRR protein, e.g., the STING protein, e.g., in a cell. In some embodiments, to induce expression of a PRR (e.g., STING) may refer to the initiation of PRR RNA (e.g., STING RNA (e.g., mRNA)) or transcription or PRR protein (e.g., STING protein) translation. In some embodiments, to induce expression of a PRR (e.g., STING) may refer to an increase in the rate of PRR RNA (e.g., STING RNA (e.g., mRNA)) transcription or an increase in the rate of PRR protein (e.g., STING protein) expression.

As used herein, the terms “activate” or “activation” refer to the stimulation or triggering of a function, e.g., of a downstream pathway, e.g., a downstream signaling pathway. In some embodiments, activation of a pattern recognition receptor (PRR) (e.g., STING) refers to the stimulation of a specific protein or pathway, e.g., through interaction with a downstream signaling partner (e.g., IFN- β promoter stimulator 1 (IPS-1), IRF3, IRF7, NF- κ B, interferons (e.g., IFN- α or IFN- β) and/or cytokines). In some embodiments, activation is distinct from the induction of expression of a PRR. In some embodiments, a PRR may be activated without resulting in an induction of PRR expression (e.g., expression of STING). In some embodiments, activation may include induction of expression of a PRR (e.g., STING). In some embodiments, activation of a PRR may trigger the induction of expression of a PRR (e.g., STING) by about 0.1%, about 0.5%, about 1%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, or more compared to a reference standard (e.g., basal expression levels of a PRR (e.g., STING)).

As used herein, an amount of a compound, conjugate, or substance effective to treat a disorder (e.g., a disorder described herein), “therapeutically effective amount,” “effective amount” or “effective course” refers to an amount of the compound, substance, or composition which is effective, upon single or multiple dose administration(s) to a subject, in treating a subject, or in curing, alleviating, relieving or improving a subject with a disorder (e.g., a microbial infection) beyond that expected in the absence of such treatment.

As used herein, the terms “prevent” or “preventing” as used in the context of a disorder or disease, refer to administration of an agent to a subject, e.g., the administration of a compound of

the present disclosure (e.g., compound of Formula (I)) to a subject, such that the onset of at least one symptom of the disorder or disease is delayed as compared to what would be seen in the absence of administration of said agent.

As used herein, the terms “reference treatment” or “reference standard” refer to a standardized level or standardized treatment that is used as basis for comparison. In some embodiments, the reference standard or reference treatment is an accepted, well known, or well characterized standard or treatment in the art. In some embodiments, the reference standard describes an outcome of a method described herein. In some embodiments, the reference standard describes a level of a marker (e.g., a level of induction of a PRR, e.g., STING) in a subject or a sample, e.g., prior to initiation of treatment, e.g., with a compound or composition described herein. In some embodiments, the reference standard describes a measure of the presence of, progression of, or severity of a disease or the symptoms thereof, e.g., prior to initiation of treatment, e.g., with a compound or composition described herein.

As used herein, the term “subject” is intended to include human and non-human animals. Exemplary human subjects include a human patient having a disorder, e.g., a disorder described herein, or a normal subject. The term “non-human animals” includes all vertebrates, e.g., non-mammals (such as chickens, amphibians, reptiles) and mammals, such as non-human primates, domesticated and/or agriculturally useful animals, e.g., sheep, dogs, cats, cows, pigs. In exemplary embodiments of the disclosure, the subject is a woodchuck (e.g., an Eastern woodchuck (*Marmota monax*)).

As used herein, the terms “treat” or “treating” a subject having a disorder or disease refer to subjecting the subject to a regimen, e.g., the administration of a compound of Formula (I) or pharmaceutically acceptable salt thereof, or a composition comprising Formula (I) or pharmaceutically acceptable salt thereof, such that at least one symptom of the disorder or disease is cured, healed, alleviated, relieved, altered, remedied, ameliorated, or improved. Treating includes administering an amount effective to alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder or disease, or the symptoms of the disorder or disease. The treatment may inhibit deterioration or worsening of a symptom of a disorder or disease.

As used herein, the term “Cmd” refers to the word “compound” or “Compound”, and all of the terms are used interchangeably.

Numerous ranges, *e.g.*, ranges for the amount of a drug administered per day, are provided herein. In some embodiments, the range includes both endpoints. In other embodiments, the range excludes one or both endpoints. By way of example, the range can exclude the lower endpoint. Thus, in such an embodiment, a range of 250 to 400 mg/day, excluding the lower endpoint, would cover an amount greater than 250 that is less than or equal to 400 mg/day.

The term “alkyl,” as used herein, refers to a monovalent saturated, straight- or branched-chain hydrocarbon such as a straight or branched group of 1-12, 1-10, or 1-6 carbon atoms, referred to herein as C₁-C₁₂ alkyl, C₁-C₁₀ alkyl, and C₁-C₆ alkyl, respectively. Examples of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, sec-pentyl, iso-pentyl, tert-butyl, n-pentyl, neopentyl, n-hexyl, sec-hexyl, and the like.

The terms “alkenyl” and “alkynyl” are art-recognized and refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond, respectively. Exemplary alkenyl groups include, but are not limited to, -CH=CH₂ and -CH₂CH=CH₂.

The term “alkylene” refers to the diradical of an alkyl group.

The terms “alkenylene” and “alkynylene” refer to the diradicals of an alkenyl and an alkynyl group, respectively.

The term “methylene unit” refers to a divalent -CH₂- group present in an alkyl, alkenyl, alkynyl, alkylene, alkenylene, or alkynylene moiety.

The term “carbocyclic ring system”, as used herein, means a monocyclic, or fused, spiro-fused, and/or bridged bicyclic or polycyclic hydrocarbon ring system, wherein each ring is either completely saturated or contains one or more units of unsaturation, but where no ring is aromatic.

The term “carbocyclyl” refers to a radical of a carbocyclic ring system. Representative carbocyclyl groups include cycloalkyl groups (*e.g.*, cyclopentyl, cyclobutyl, cyclopentyl, cyclohexyl and the like), and cycloalkenyl groups (*e.g.*, cyclopentenyl, cyclohexenyl, cyclopentadienyl, and the like).

The term “aromatic ring system” is art-recognized and refers to a monocyclic, bicyclic or polycyclic hydrocarbon ring system, wherein at least one ring is aromatic.

The term “aryl” refers to a radical of an aromatic ring system. Representative aryl groups include fully aromatic ring systems, such as phenyl, naphthyl, and anthracenyl, and ring systems where an aromatic carbon ring is fused to one or more non-aromatic carbon rings, such as indanyl,

phthalimidyl, naphthimidyl, or tetrahydronaphthyl, and the like. Each instance of an aryl group may be independently optionally substituted, *i.e.*, unsubstituted (an "unsubstituted aryl") or substituted (a "substituted aryl") with one or more substituents; *e.g.*, for instance from 1 to 5 substituents, 1 to 4 substituents, 1 to 3 substituents, 1 to 2 substituents or just 1 substituent. The aromatic ring may be substituted at one or more ring positions with one or more substituents, such as halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, fluoroalkyl (such as trifluoromethyl), cyano, or the like. For example, in certain embodiments, the aryl group can be an unsubstituted C₅-C₁₂ aryl and in certain embodiments, the aryl group can be a substituted C₅-C₁₀ aryl.

The term "heteroalkyl" refers to an "alkyl" moiety wherein at least one of the carbone molecules has been replaced with a heteroatom such as O, S, or N.

The term "heteroaromatic ring system" is art-recognized and refers to monocyclic, bicyclic or polycyclic ring system wherein at least one ring is both aromatic and comprises a heteroatom; and wherein no other rings are heterocyclyl (as defined below). In certain instances, a ring which is aromatic and comprises a heteroatom contains 1, 2, 3, or 4 independently selected ring heteroatoms in such ring.

The term "heteroaryl" refers to a radical of a heteroaromatic ring system. Representative heteroaryl groups include ring systems where (i) each ring comprises a heteroatom and is aromatic, *e.g.*, imidazolyl, oxazolyl, thiazolyl, triazolyl, pyrrolyl, furanyl, thiophenyl pyrazolyl, pyridinyl, pyrazinyl, pyridazinyl, pyrimidinyl, indoliziny, purinyl, naphthyridinyl, and pteridinyl; (ii) each ring is aromatic or carbocyclyl, at least one aromatic ring comprises a heteroatom and at least one other ring is a hydrocarbon ring or *e.g.*, indolyl, isoindolyl, benzothieryl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolyl, phthalazinyl, quinazolinyl, quinoxalinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, pyrido[2,3-*b*]-1,4-oxazin-3(4H)-one, 5,6,7,8-tetrahydroquinolyl and 5,6,7,8-tetrahydroisoquinolyl; and (iii) each ring is aromatic or carbocyclyl, and at least one aromatic ring shares a bridgehead heteroatom with another aromatic ring, *e.g.*, 4H-quinoliziny. In certain embodiments, the heteroaryl is a monocyclic or bicyclic ring, wherein each of said rings contains 5 or 6 ring atoms where 1, 2, 3, or 4 of said

ring atoms are a heteroatom independently selected from N, O, and S. The aromatic heterocycle may be substituted at one or more ring positions with one or more substituents, such as halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxy, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, fluoroalkyl (such as trifluoromethyl), cyano, or the like.

The term “heterocyclic ring system” refers to monocyclic, or fused, spiro-fused, and/or bridged bicyclic and polycyclic ring systems where at least one ring is saturated or partially unsaturated (but not aromatic) and comprises a heteroatom. A heterocyclic ring system can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted.

The term “heterocyclyl” refers to a radical of a heterocyclic ring system. Representative heterocyclyls include ring systems in which (i) every ring is non-aromatic and at least one ring comprises a heteroatom, e.g., tetrahydrofuranyl, tetrahydrothienyl, pyrrolidinyl, pyrrolidonyl, piperidinyl, pyrrolinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and quinuclidinyl; (ii) at least one ring is non-aromatic and comprises a heteroatom and at least one other ring is an aromatic carbon ring, e.g., 1,2,3,4-tetrahydroquinolinyl, 1,2,3,4-tetrahydroisoquinolinyl; and (iii) at least one ring is non-aromatic and comprises a heteroatom and at least one other ring is aromatic and comprises a heteroatom, e.g., 3,4-dihydro-1H-pyrano[4,3-c]pyridine, and 1,2,3,4-tetrahydro-2,6-naphthyridine. In certain embodiments, the heterocyclyl is a monocyclic or bicyclic ring, wherein each of said rings contains 3-7 ring atoms where 1, 2, 3, or 4 of said ring atoms are a heteroatom independently selected from N, O, and S.

The term “saturated heterocyclyl” refers to a radical of heterocyclic ring system wherein every ring is saturated, e.g., tetrahydrofuran, tetrahydro-2H-pyran, pyrrolidine, piperidine and piperazine.

“Partially unsaturated” refers to a group that includes at least one double or triple bond. A “partially unsaturated” ring system is further intended to encompass rings having multiple sites of unsaturation, but is not intended to include aromatic groups (e.g., aryl or heteroaryl groups) as herein defined. Likewise, “saturated” refers to a group that does not contain a double or triple bond, *i.e.*, contains all single bonds.

The term “nucleobase” as used herein, is a nitrogen-containing biological compound found linked to a sugar within a nucleoside—the basic building blocks of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The primary, or naturally occurring, nucleobases are cytosine (DNA and RNA), guanine (DNA and RNA), adenine (DNA and RNA), thymine (DNA) and uracil (RNA), abbreviated as C, G, A, T, and U, respectively. Because A, G, C, and T appear in the DNA, these molecules are called DNA-bases; A, G, C, and U are called RNA-bases. Adenine and guanine belong to the double-ringed class of molecules called purines (abbreviated as R). Cytosine, thymine, and uracil are all pyrimidines. Other nucleobases that do not function as normal parts of the genetic code are termed non-naturally occurring.

As described herein, compounds of the disclosure may contain “optionally substituted” moieties. In general, the term “substituted”, whether preceded by the term “optionally” or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an “optionally substituted” group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at each position. Combinations of substituents envisioned under this disclosure are preferably those that result in the formation of stable or chemically feasible compounds. The term “stable”, as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

As used herein, the definition of each expression, *e.g.*, alkyl, m, n, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

As described herein, compounds of the disclosure may contain “optionally substituted” moieties. In general, the term “substituted”, whether preceded by the term “optionally” or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Possible “substituents” include, for example one or more: (i) D, F, Cl, Br or I atoms; or (ii) methyl, ethyl, propyl, trichloromethyl, trifluoromethyl, carbonyl (*i.e.* C=O), nitrile (*i.e.* -C≡N), hydroxyl (*i.e.* -OH), alkoxy (*i.e.* -OR”), nitro (*i.e.* -NO₂) or amino groups, each independently chosen for each possible position for substitution of a hydrogen atom. Other substituents are contemplated,

such as halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxy, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, fluoroalkyl (such as trifluoromethyl), cyano, or the like. Unless otherwise indicated, an “optionally substituted” group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at each position. Combinations of substituents envisioned under this disclosure are preferably those that result in the formation of stable or chemically feasible compounds. The term “stable”, as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

Pattern Recognition Receptors

The disclosure presented herein features methods for the activation and induction of PRR expression (e.g., STING expression) in a subject, e.g., a subject with a microbial infection (e.g., a viral infection, bacterial infection, fungal infection, or parasitic infection) or a proliferative disease (e.g., cancer).

Pattern recognition receptors (PRRs) are a broad class of proteins which recognize pathogen-associated molecular patterns (PAMPs) conserved within pathogenic invaders. PAMPs are typically products of biosynthetic pathways that are essential to the survival and/or infectivity of the pathogen, e.g., lipopolysaccharides, glycoproteins, and nucleic acids. Recognition of PAMPs by their cognate PRRs activates signaling pathways that result in the production of immune defense factors such as pro-inflammatory and anti-inflammatory cytokines, type I interferons (IFN- α , IFN- β), and/or interferon stimulated genes (ISGs). It is well known that induction of innate immune signaling also results in the activation of T cell responses as well as the induction of adaptive immunity. These downstream immune effects are essential for clearance of the virus through apoptosis and killing of infected cells through cytotoxic T lymphocytes and other defense mechanisms. It is also well known that interferons act on ISRE (interferon response elements) that can trigger the production of ISGs, which play an important role in antiviral cellular defense.

The stimulator of interferon genes (STING) is a cytosolic microbial-derived DNA sensor that has been shown to be particularly sensitive to double-stranded DNA and cyclic dinucleotides (e.g., cyclic di-GMP) (Burdette, D. L. and Vance, R. E. (2013) *Nat Immunol* 14:19-26). Two molecules of STING form a homodimer mediated by an α -helix present in the C-terminal dimerization domain, and molecular binding studies have revealed that each STING dimer binds one molecule of microbial nucleic acids, e.g., DNA or a cyclic dinucleotide. Upon ligand binding, STING activates the innate immune response through interaction with RIG-I and IPS-1, resulting in interferon production (e.g., IFN- α and IFN- β) and other downstream signaling events. Since its discovery, STING has been shown to function as a critical sensor of viruses (e.g., adenovirus, herpes simplex virus, hepatitis B virus, vesicular stomatitis virus, hepatitis C virus), bacteria (e.g., *Listeria monocytogenes*, *Legionella pneumopholia*, *Mycobacterium tuberculosis*) and protozoa (*Plasmodium falciparum*, *Plasmodium berghei*). In addition, STING has been shown to play a major role in the innate immune response against tumor antigens, driving dendritic cell activation and subsequent T cell priming in several cancers (Woo, S.R. et al. *Trends in Immunol* (2015) 36:250-256).

Another class of PRRs includes RIG-I, which is the founding member of a family of PRRs termed RIG-I-like receptors (RLRs) that primarily detect RNA derived from foreign sources. It is a critical sensor of microbial infection (e.g., viral infection) in most cells and is constitutively expressed at low levels in the cytosol. After ligand binding, the expression of RIG-I is rapidly enhanced, leading to increased RIG-I concentrations in the cell (Jensen, S. and Thomsen, A.R. *J Virol* (2012) 86:2900-2910; Yoneyama M. et al. *Nat Immunol* (2004) 5:730-737). RIG-I is an ATP-dependent helicase containing a central DExD/H box ATPase domain and tandem N-terminal caspase-recruiting domains (CARDs) that mediate downstream signaling. The C-terminus of RIG-I comprises an ssRNA/dsRNA-binding domain that when unbound acts to silence CARD function at the N-terminus. Without wishing to be bound by theory, it is believed that upon recognition of target RNA structures, two N-terminal CARDs are exposed, allowing for interaction with the CARD of a downstream binding partner, IFN- β promoter stimulator 1 (IPS-1), also known as mitochondrial antiviral signaling molecule (MAVS) and CARDIF. This interaction in turn triggers further downstream signaling, such as induction of IRF3, IRF7, NF- κ B, IFNs, and cytokine production that results in the initiation of the host immune response.

Other RLRs are homologous to RIG-I and function in a similar manner, including MDA5, LGP2, and RNase L. MDA5 is highly homologous to RIG-I, and has been shown to be crucial for triggering a cytokine response upon infection with picornaviruses (e.g., encephalomyocarditis virus (EMCV), Theiler's virus, and Mengo virus), Sendai virus, rabies virus, West Nile virus, rabies virus, rotavirus, murine hepatitis virus, and murine norovirus. LGP2 lacks a CARD domain found in RIG-I and MDA5, which is responsible for direct interaction with IPS-1 to initiate downstream signaling. As such, LGP2 is believed to behave as a modulator of the innate immune response in conjunction with other CARD-bearing RLRs such as RIG-I and MDA5.

Another class of PRRs encompasses the nucleotide-binding and oligomerization domain (NOD)-like receptors, or NLR family (Caruso, R. et al, *Immunity* (2014) 41:898-908), which includes the microbial sensor NOD2. NOD2 is composed of an N-terminal CARD, a centrally-located nucleotide-binding oligomerization domain, and a C-terminal leucine rich repeat domain that is responsible for binding microbial PAMPs, such as bacterial peptidoglycan fragments and microbial nucleic acids. Ligand binding activates NOD2 and is believed to drive interaction with the CARD-containing kinase RIPK2, which in turn activates a number of downstream proteins including NF- κ B, MAPK, IRF7, and IRF3, the latter of which results in the induction of type 1 interferons. NOD2 is expressed in a diverse set of cell types, including macrophages, dendritic cells, paneth cells, epithelial cells (e.g., lung epithelial cells, intestinal epithelia), and osteoblasts. NOD2 has been established as a sensor of infection by variety of pathogenic invaders, such as protozoa (e.g., *Toxoplasma gondii* and *Plasmodium berghei*), bacteria (e.g., *Bacillus anthracis*, *Borrelia burgdorferi*, *Burkholderia pseudomallei*, *Helicobacter hepaticus*, *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Propionibacterium acne*, *Porphyromonas gingivalis*, *Salmonella enterica*, and *Streptococcus pneumonia*), and viruses (e.g., respiratory syncytial virus and murine norovirus-1) (Moreira, L. O. and Zamboni, D. S. *Front Immunol* (2012) 3:1-12). Recent work has shown that mutation of NOD2 may contribute to inflammatory diseases such as Crohn's disease, resulting in an aberrant inflammatory response upon stimulation.

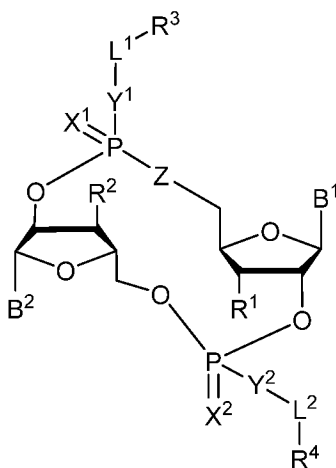
Representative Compounds

The present disclosure features compounds and methods for the induction of PRR expression (e.g., STING expression) in a subject (e.g., a subject with a microbial infection (e.g., a viral infection, bacterial infection, fungal infection, or parasitic infection) or a proliferative disease

(e.g., cancer)), comprising administration of an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt or stereoisomer thereof.

In some embodiments, the present disclosure features a compound of Formula (I) in which the 2'-OH end of a first nucleoside is joined to the 5'-OH of a second nucleoside through a linkage; and the 2'-OH end of the second nucleoside is joined to the 5'-OH of the first nucleoside through a linkage.

In some embodiments, the compound is a compound of Formula (I):



Formula (I)

or a pharmaceutically acceptable salt or stereoisomer thereof, wherein: Z is either S or O; each of B¹ and B² is independently a purinyl nucleobase or pyrimidinyl nucleobase; each of X¹ and X² is independently O or S; each of Y¹ and Y² is independently O, S, or NR⁵; each of L¹ and L² is independently absent, C₁-C₆ alkyl or C₁-C₆ heteroalkyl, wherein each alkyl and heteroalkyl is optionally substituted with R⁶; each of R¹ and R² is independently hydrogen, halo, -CN, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), or OR⁷; each of R³ and R⁴ is independently hydrogen, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), C₁-C₂₀ heteroalkyl (e.g., C₁-C₆ heteroalkyl), OC(O)OC₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), cycloalkyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, and heteroaryl is optionally substituted with one or more R⁸; R⁵ is hydrogen or C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl); R⁶ is halo, -CN, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), OR⁷, oxo, cycloalkyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, cycloalkyl, heterocyclyl, aryl, and heteroaryl is optionally substituted with one or more R⁹; R⁷ is hydrogen, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), cycloalkyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, cycloalkyl, heterocyclyl, aryl, and heteroaryl is optionally substituted with one or more R⁹; each R⁸ is independently C₁-C₂₀

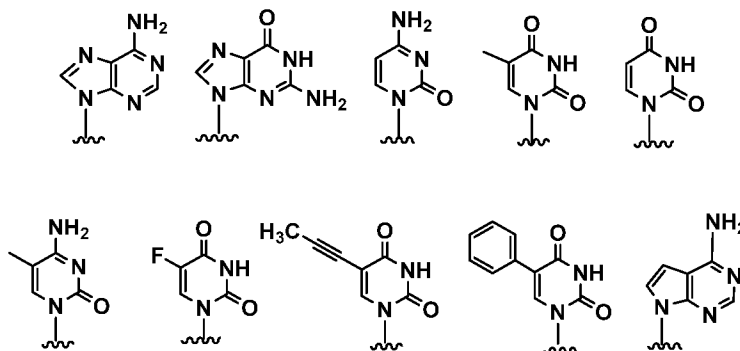
alkyl (e.g., C₁-C₆ alkyl), C₁-C₂₀ heteroalkyl, C(O)-C₁-C₂₀ alkyl, OC(O)-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), C(O)O-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), OC(O)O-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), C(O)N(R⁵)-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), N(R⁵)C(O)-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), OC(O)N(R⁵)-C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), O-aryl, O-heteroaryl, C(O)-aryl, C(O)-heteroaryl, OC(O)-aryl, C(O)O-aryl, OC(O)-heteroaryl, C(O)O-heteroaryl, C(O)O-aryl, C(O)O-heteroaryl, C(O)N(R⁵)-aryl, C(O)N(R⁵)-heteroaryl, N(R⁵)C(O)-aryl, N(R⁵)₂C(O)-aryl, or N(R⁵)C(O)-heteroaryl, S(O)₂N(R⁵)-aryl, wherein each alkyl, heteroalkyl, aryl, and heteroaryl is optionally substituted by one or more R⁹; and each R⁹ is independently C₁-C₂₀ alkyl, O-C₁-C₂₀ alkyl, C₁-C₂₀ heteroalkyl, halo, -CN, OH, oxo, aryl, heteroaryl, O-aryl, or O-heteroaryl.

In some embodiments Z is S. In some embodiments Z is O. In some embodiments, at least one of B¹ or B² is a purinyl nucleobase. In some embodiments, each of B¹ or B² is independently a purinyl nucleobase. In some embodiments, B¹ is a purinyl nucleobase. In some embodiments, B² is a pyrimidinyl nucleobase. In some embodiments, B¹ is a purinyl nucleobase and B² is a pyrimidinyl nucleobase.

In some embodiments Z is S. In some embodiments Z is O.

In some embodiments, at least one of B¹ or B² is a purinyl nucleobase. In some embodiments, each of B¹ or B² is independently a purinyl nucleobase. In some embodiments, B¹ is a purinyl nucleobase. In some embodiments, B² is a pyrimidinyl nucleobase. In some embodiments, B¹ is a purinyl nucleobase and B² is a pyrimidinyl nucleobase. In some embodiments, B¹ is a pyrimidinyl nucleobase. In some embodiments, B² is a purinyl nucleobase. In some embodiments, B¹ is a pyrimidinyl nucleobase and B² is a purinyl nucleobase.

In some embodiments, each of B¹ or B² is selected from a naturally occurring nucleobase or a modified nucleobase. In some embodiments, each of B¹ or B² is selected from adenosinyl, guanosinyl, cytosinyl, thyminyl, uracilyl, 5'-methylcytosinyl, 5'-fluorouracilyl, 5'-propynyluracilyl, and 7-deazaadenosinyl. In some embodiments, each of B¹ or B² is selected from:



wherein “” indicates the linkage of the nucleobase to the ribose ring.

In some embodiments, one of B¹ or B² is selected from a naturally occurring nucleobase and the other of B¹ or B² is a modified nucleobase. In some embodiments, one of B¹ or B² is adenosinyl, guanosinyl, thyminyl, cytosinyl, or uracilyl, and the other of B¹ or B² is 5'-methylcytosinyl, 5'-fluorouracilyl, 5'-propynyluracilyl, or 7-deazaadenosinyl.

In some embodiments, B¹ is adenosinyl or guanosinyl. In some embodiments, B² is cytosinyl, thyminyl, or uracilyl. In some embodiments, B¹ is adenosinyl or guanosinyl and B² is cytosinyl, thyminyl, or uracilyl. In some embodiments, B² is adenosinyl or guanosinyl. In some embodiments, B¹ is cytosinyl, thyminyl, or uracilyl. In some embodiments, B² is adenosinyl or guanosinyl and B¹ is cytosinyl, thyminyl, or uracilyl.

In some embodiments, each of B¹ and B² is independently uracilyl. In some embodiments, each of B¹ and B² is independently adenosinyl.

In some embodiments, each of R¹ and R² is independently hydrogen, halo, or OR⁷. In some embodiments, each of R¹ and R² is independently halo (e.g., fluoro). In some embodiments, each of R¹ and R² is not hydrogen or OR⁷.

In some embodiments, X¹ is O. In some embodiments, X² is O. In some embodiments, each of X¹ and X² is independently O.

In some embodiments, Y¹ is O or S. In some embodiments, Y² is O or S. In some embodiments, each of Y¹ and Y² is independently O or S. In some embodiments, one of Y¹ or Y² is O and the other of Y¹ or Y² is S. In some embodiments, each of Y¹ or Y² is independently S. In some embodiments, each of Y¹ or Y² is independently O.

In some embodiments, L¹ is C₁-C₆ alkyl (e.g., CH₂). In some embodiments, L² is C₁-C₆ alkyl (e.g., CH₂). In some embodiments, each of L¹ and L² is independently C₁-C₆ alkyl (e.g., CH₂).

In some embodiments, R^3 is hydrogen, aryl, or heteroaryl, wherein aryl and heteroaryl is optionally substituted with 1-5 R^8 . In some embodiments, R^3 is aryl or heteroaryl, each of which is optionally substituted with 1-5 R^8 . In some embodiments, R^3 is phenyl substituted with 1 R^8 .

In some embodiments, R^4 is independently hydrogen, aryl, or heteroaryl, wherein aryl and heteroaryl is optionally substituted with 1-5 R^8 . In some embodiments, R^4 is aryl or heteroaryl, each of which is optionally substituted with 1-5 R^8 . In some embodiments, R^4 is phenyl substituted with 1 R^8 .

In some embodiments, each of R^3 and R^4 is independently hydrogen, aryl, or heteroaryl, wherein aryl and heteroaryl is optionally substituted with 1-5 R^8 . In some embodiments, R^3 is aryl or heteroaryl, each of which is optionally substituted with 1-5 R^8 , and R^4 is hydrogen. In some embodiments, R^3 is phenyl substituted with 1 R^8 and R^4 is hydrogen. In some embodiments, each of R^3 and R^4 is independently phenyl substituted with 1 R^8 .

In some embodiments, each of Y^1 and Y^2 is O and each of R^3 and R^4 is independently hydrogen. In some embodiments, Y^2 is O and R^4 is hydrogen. In some embodiments, each of Y^1 and Y^2 is independently S and each of R^3 and R^4 is independently substituted with 1 R^8 . In some embodiments, Y^1 is S and R^3 is substituted with 1 R^8 .

In some embodiments, each R^8 is independently C_1 - C_{20} alkyl (e.g., C_1 - C_6 alkyl), C_1 - C_{20} heteroalkyl, $C(O)$ - C_1 - C_{20} alkyl, $OC(O)$ - C_1 - C_{20} alkyl, $OC(O)O$ - C_1 - C_{20} alkyl, $OC(O)N(R^5)$ - C_1 - C_{20} alkyl, O-aryl, $C(O)$ -aryl, $OC(O)$ -aryl, or $C(O)N(R^5)$ -aryl, wherein each alkyl, heteroalkyl, aryl, and heteroaryl is optionally substituted by one or more R^9 .

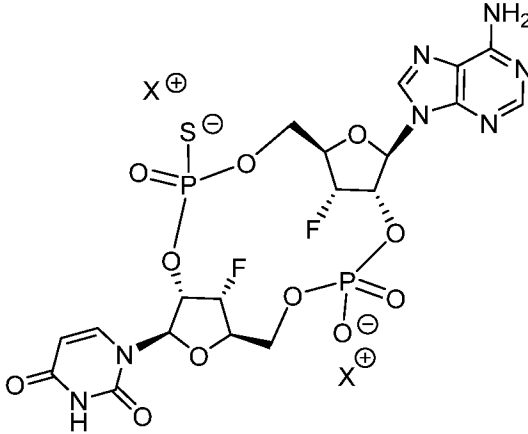
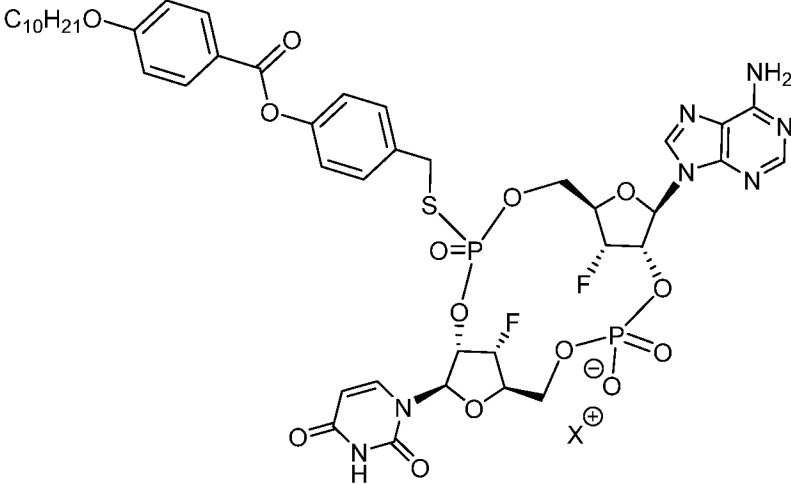
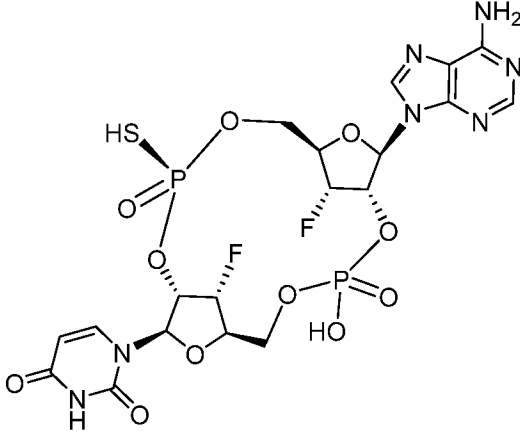
In some embodiments, R^8 is $OC(O)$ -aryl optionally substituted by 1-5 R^9 (e.g., 1 R^9). In some embodiments, R^8 is $C(O)$ -aryl optionally substituted by 1-5 R^9 (e.g., 1 R^9).

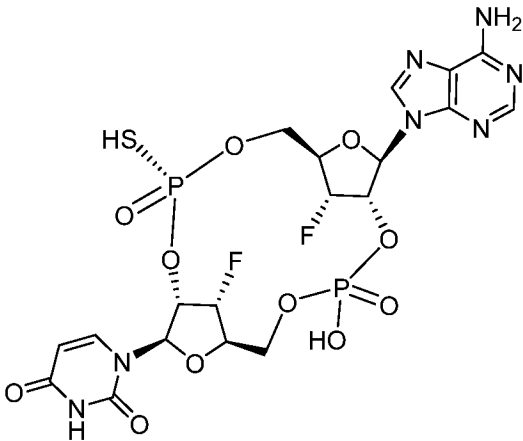
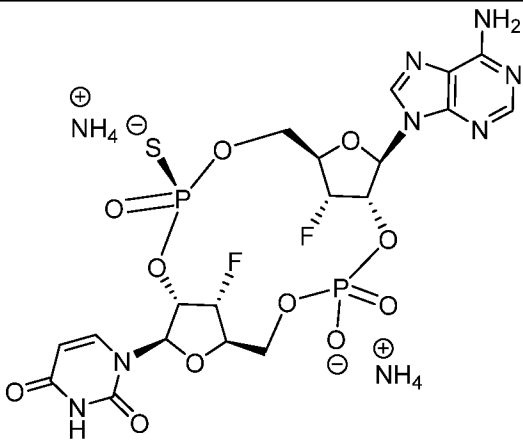
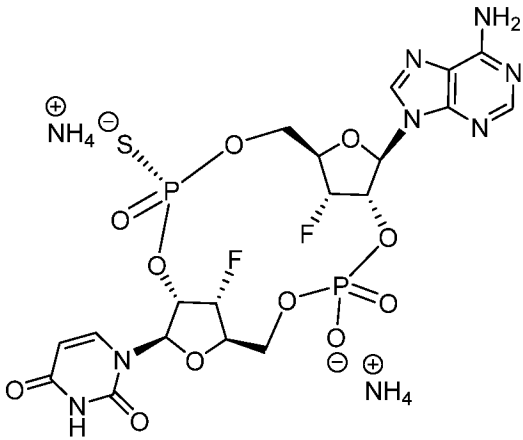
In some embodiments, R^9 is O- C_1 - C_{12} alkyl (e.g., O- $CH_2(CH_2)_8CH_3$). In some embodiments, R^9 is O- C_1 - C_{10} alkyl (e.g., O- $CH_2(CH_2)_8CH_3$). In some embodiments, R^9 is O- C_1 - C_8 alkyl (e.g., O- $CH_2(CH_2)_6CH_3$). In some embodiments, R^9 is O- C_1 - C_6 alkyl (e.g., O- $CH_2(CH_2)_4CH_3$).

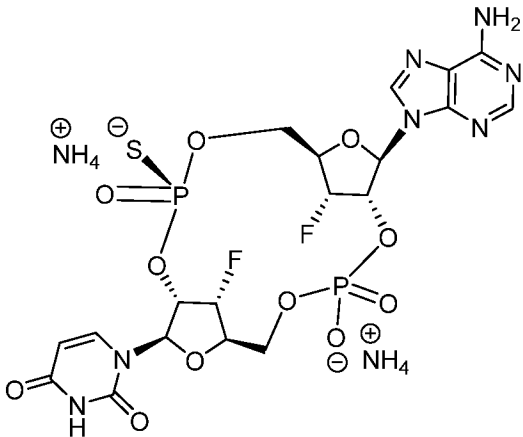
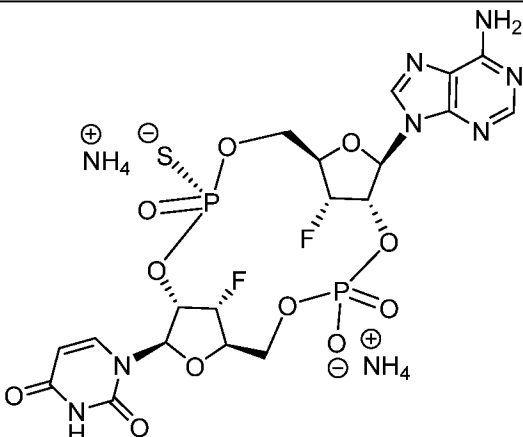
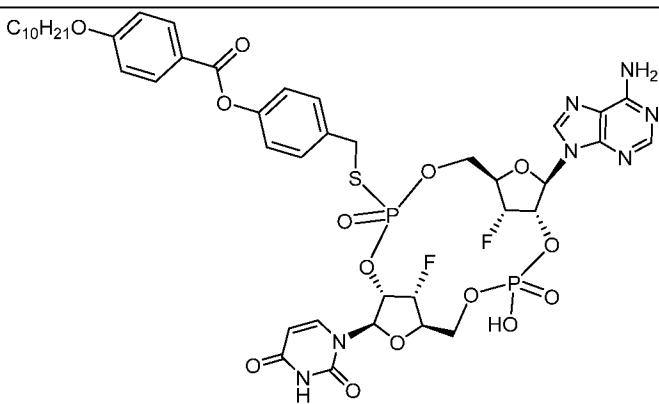
substituted by 1-5 R^9 ; and each R^9 is independently O- C_1 - C_{20} alkyl.

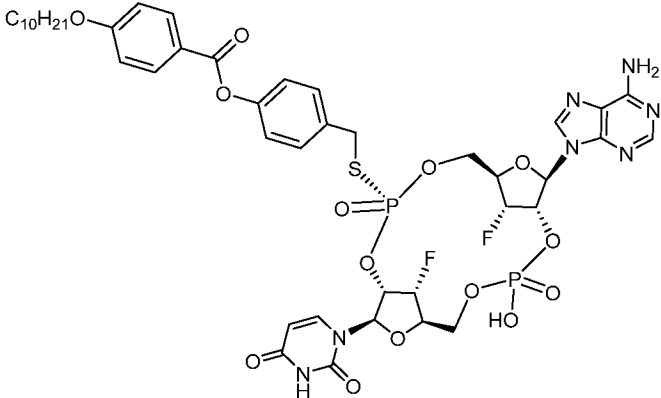
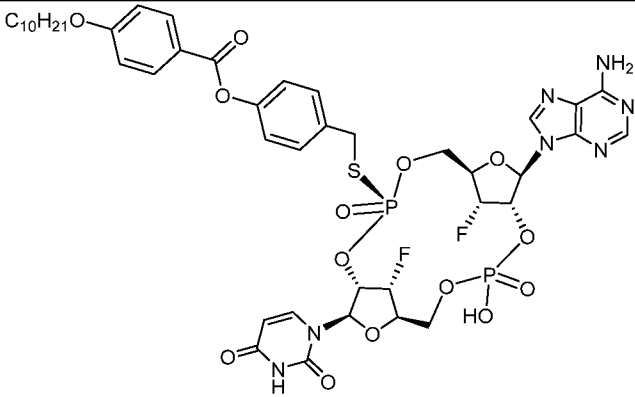
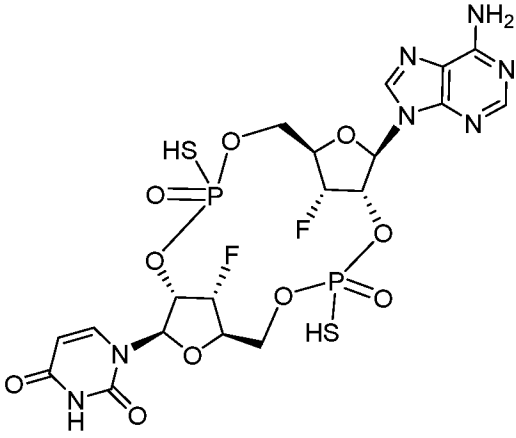
In some embodiments, the compound of Formula (I) is selected from those depicted in Table 1.

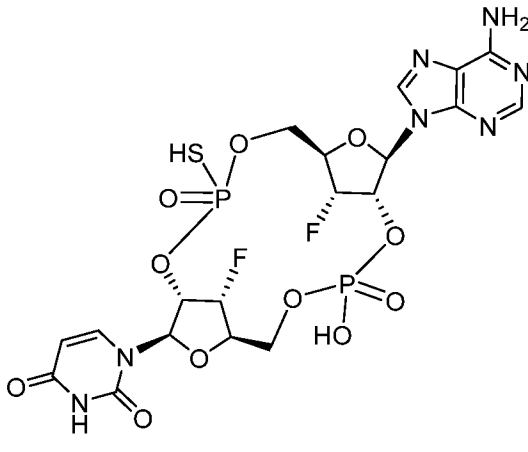
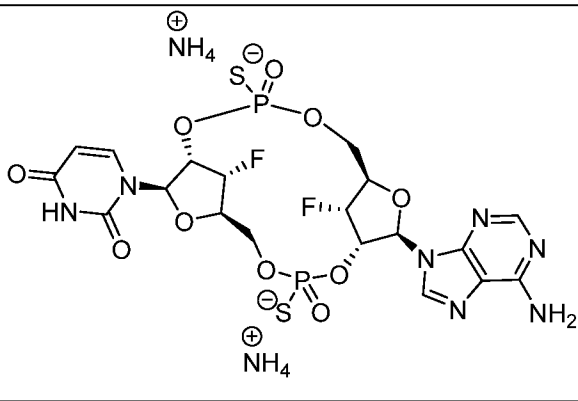
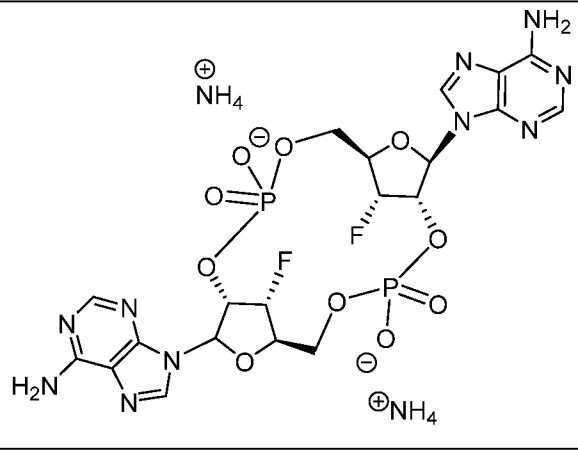
Table 1 (Unless otherwise indicated in the table, a formula $C_nH_{(2n+1)}$ refers to an n-alkyl group. For example, $C_{10}H_{21}$ refers to n-decyl unless otherwise indicated.).

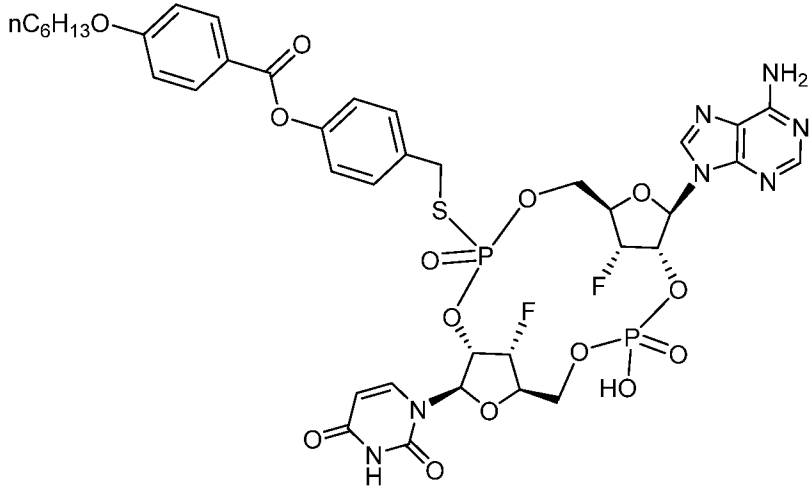
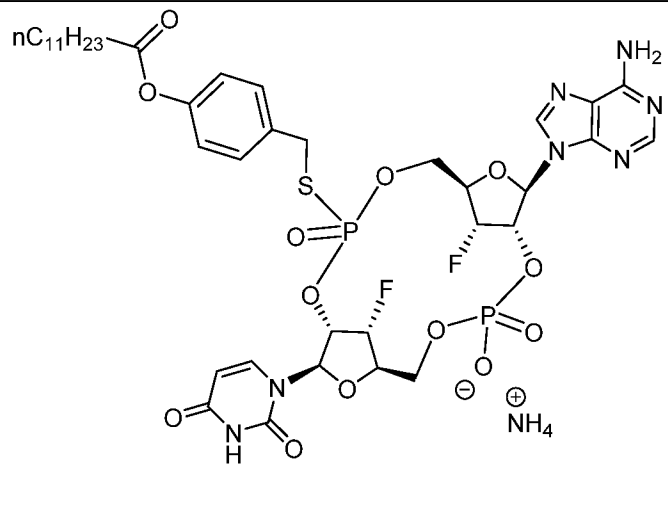
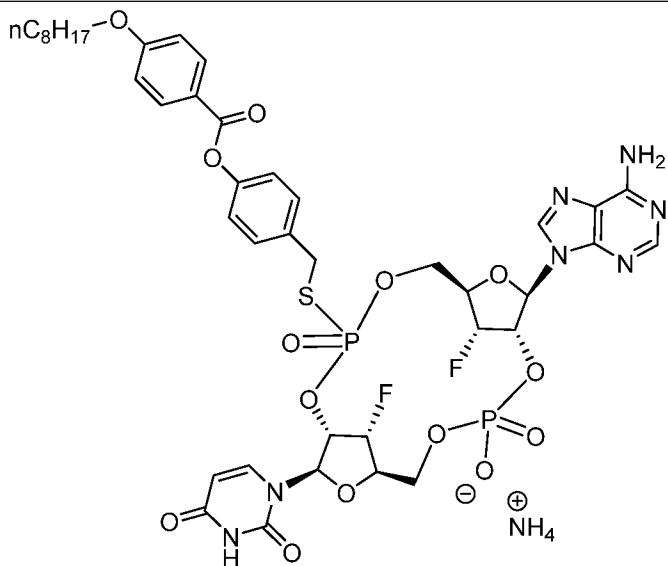
Compound No.	Structure
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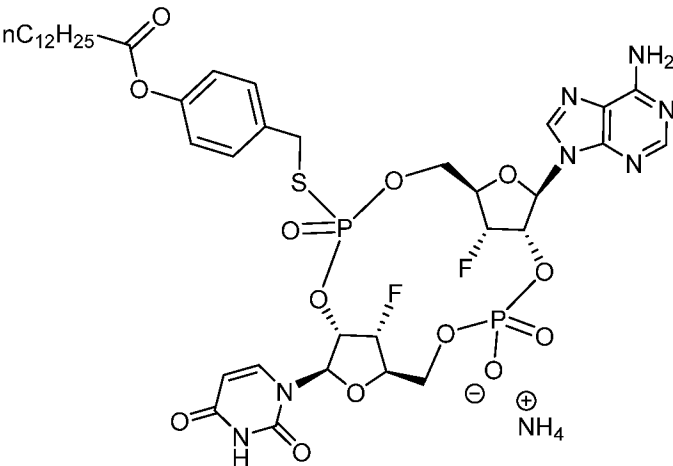
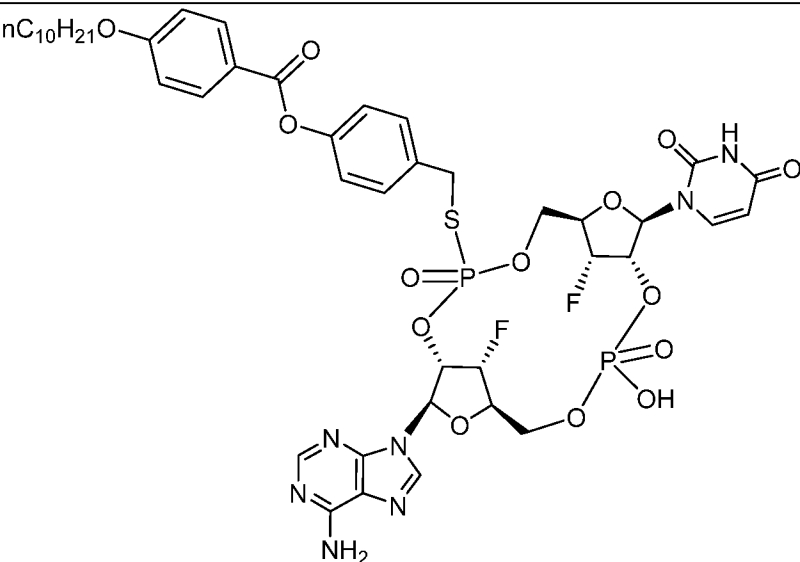
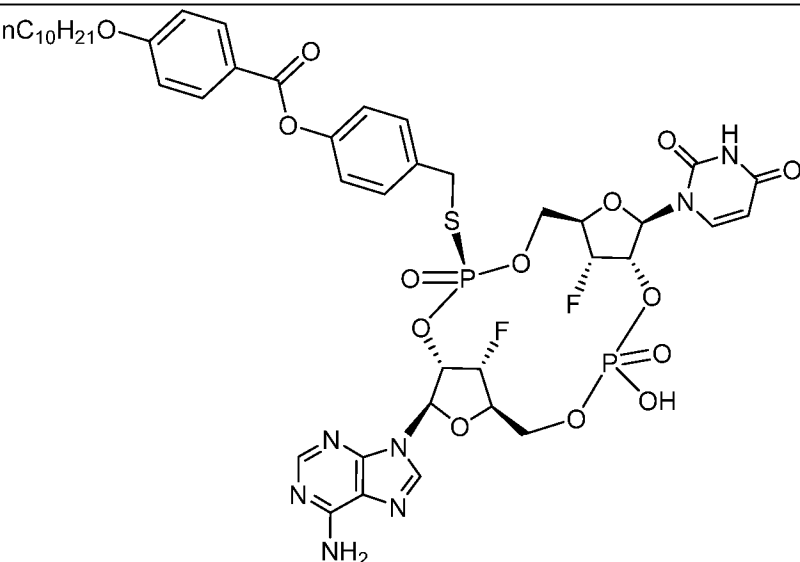
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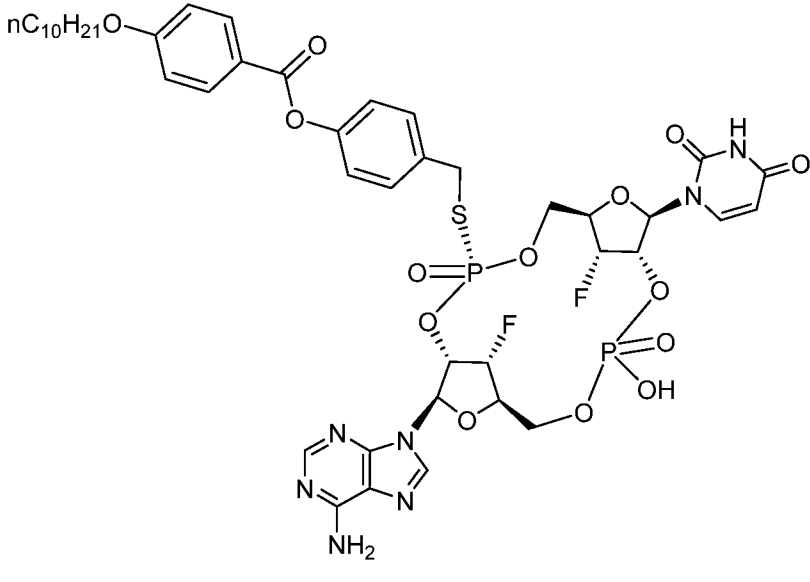
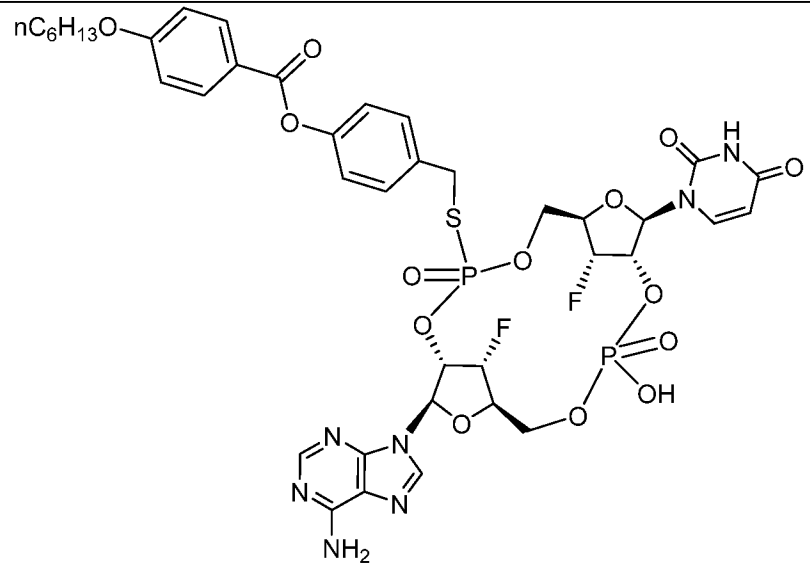
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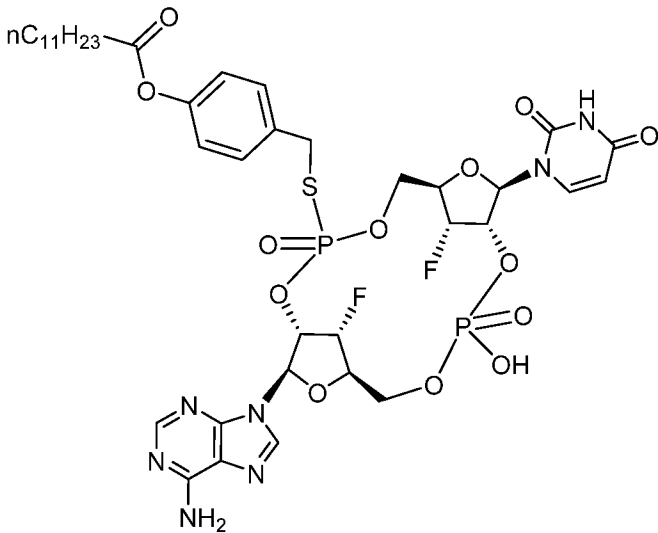
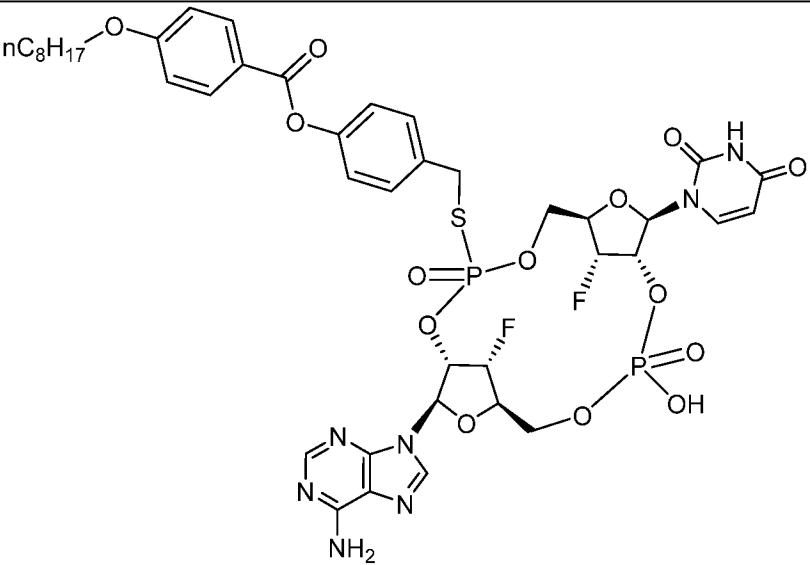
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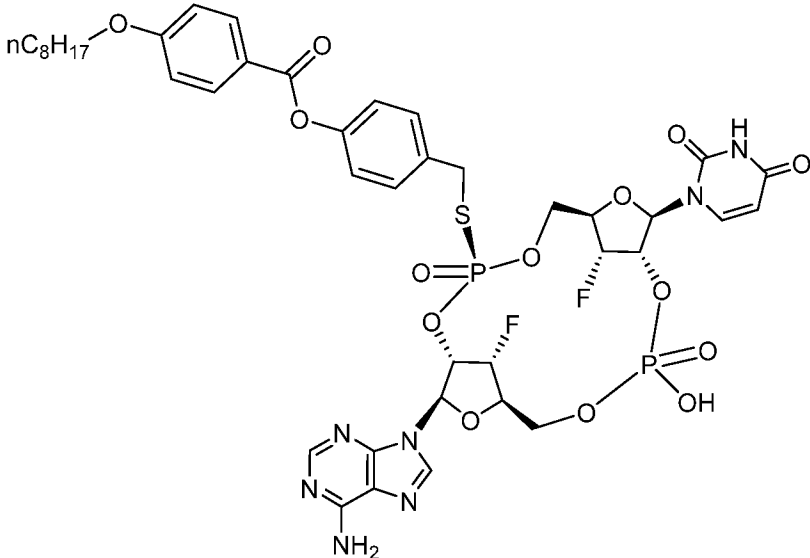
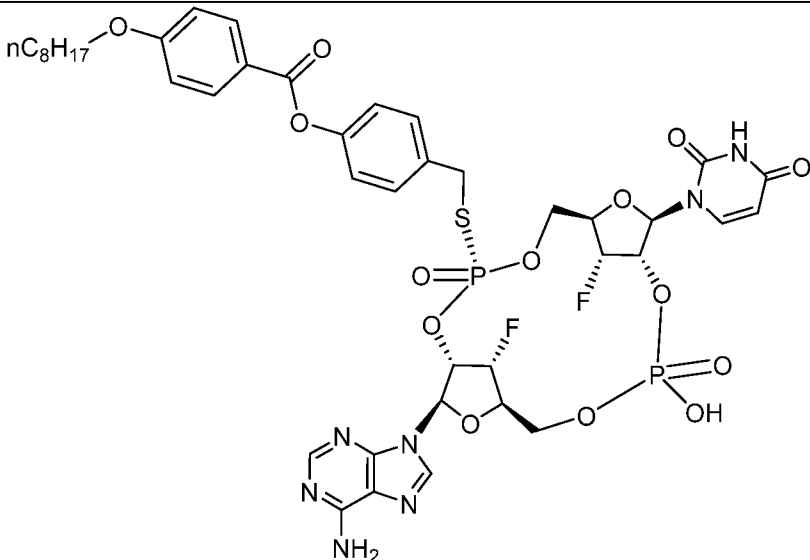
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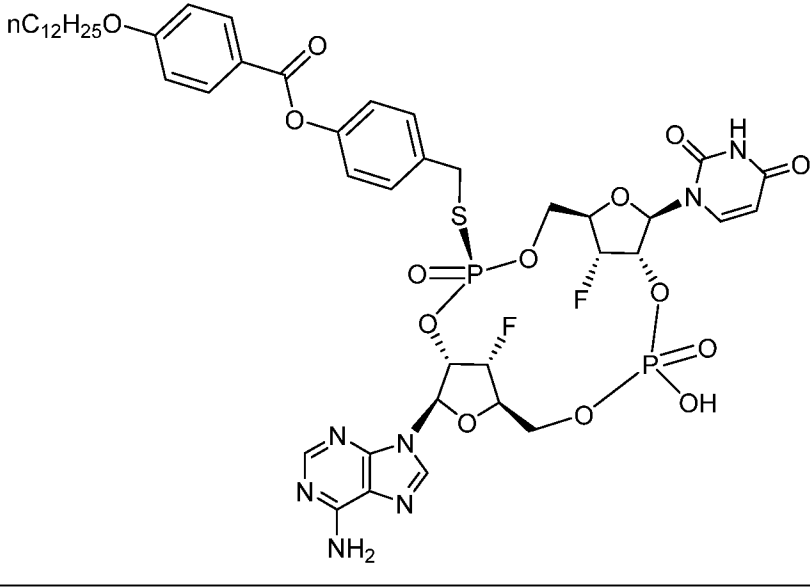
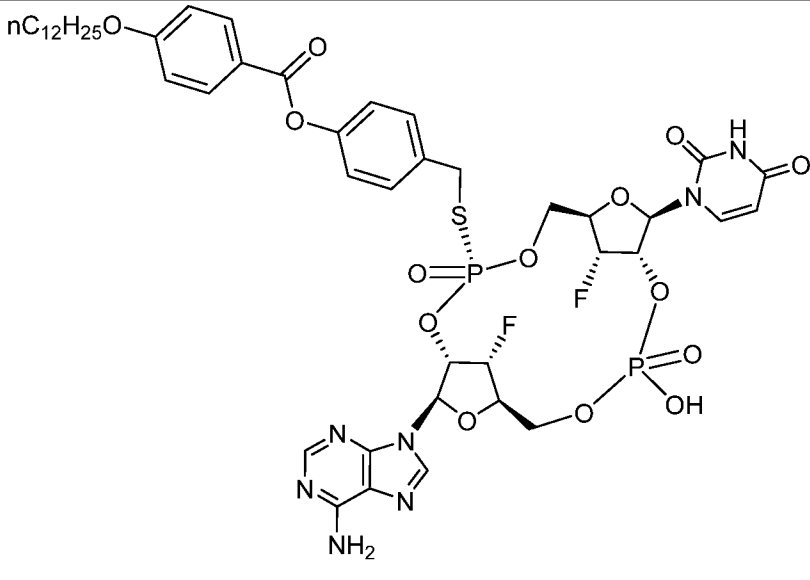
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24	Chemical structure 24: A complex molecule featuring a central phosphorus atom (P) bonded to a sulfur atom (S) and an oxygen atom (O). The sulfur atom is part of a chain that includes a benzene ring with a nC6H13O group and a carbonyl group. The phosphorus atom is also bonded to a fluorine atom (F) and an oxygen atom (O). The oxygen atom is part of a chain that includes a pyrimidine ring and a phosphorus atom (P) bonded to two hydroxyl groups (OH).
25	Chemical structure 25: A complex molecule featuring a central phosphorus atom (P) bonded to a sulfur atom (S) and an oxygen atom (O). The sulfur atom is part of a chain that includes a benzene ring with a nC6H13O group and a carbonyl group. The phosphorus atom is also bonded to a fluorine atom (F) and an oxygen atom (O). The oxygen atom is part of a chain that includes a pyrimidine ring and a phosphorus atom (P) bonded to two hydroxyl groups (OH).

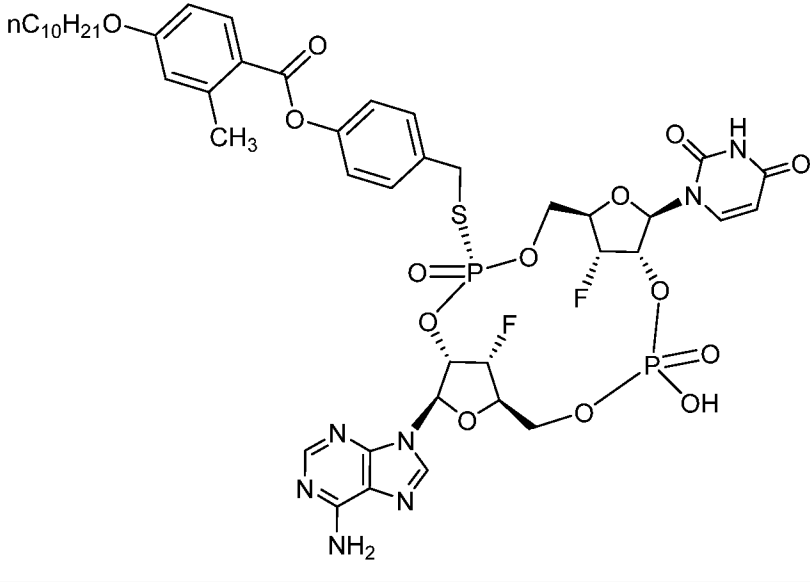
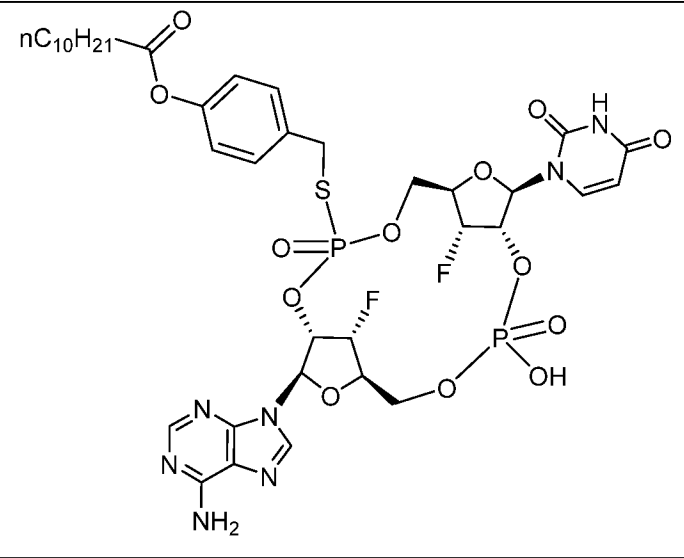
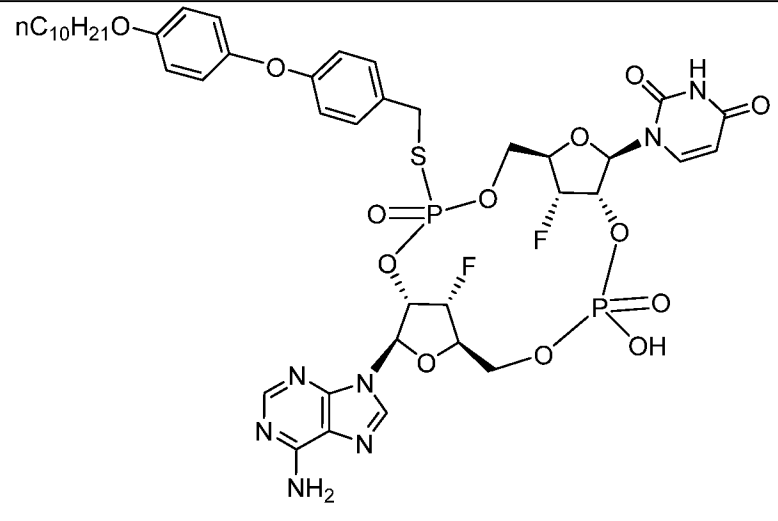
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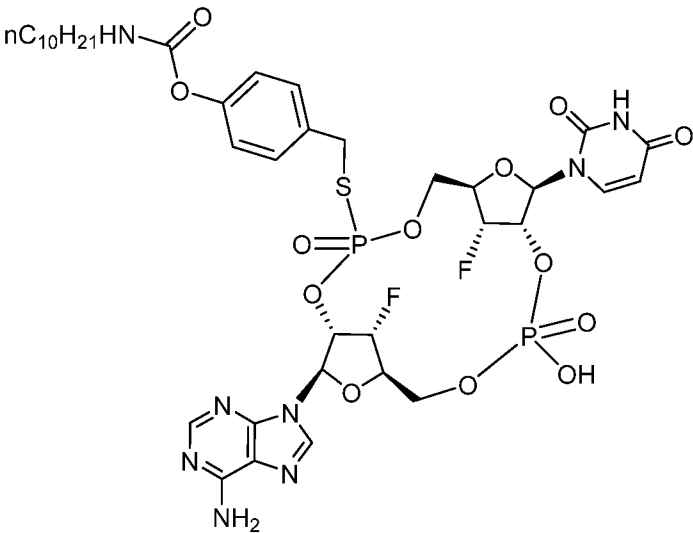
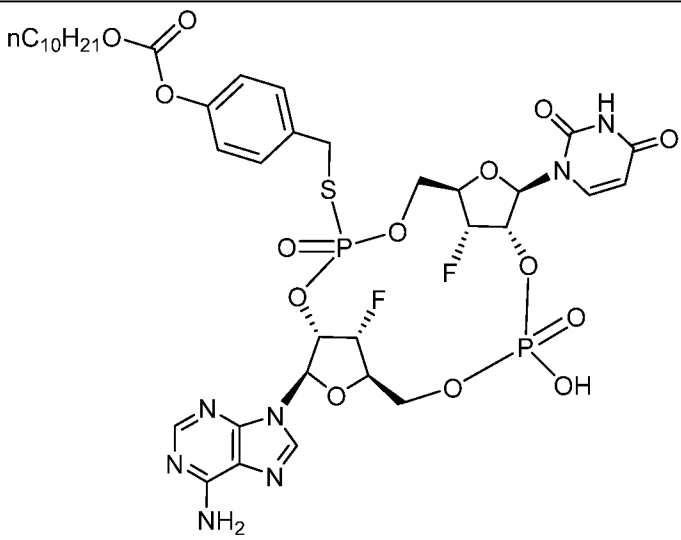
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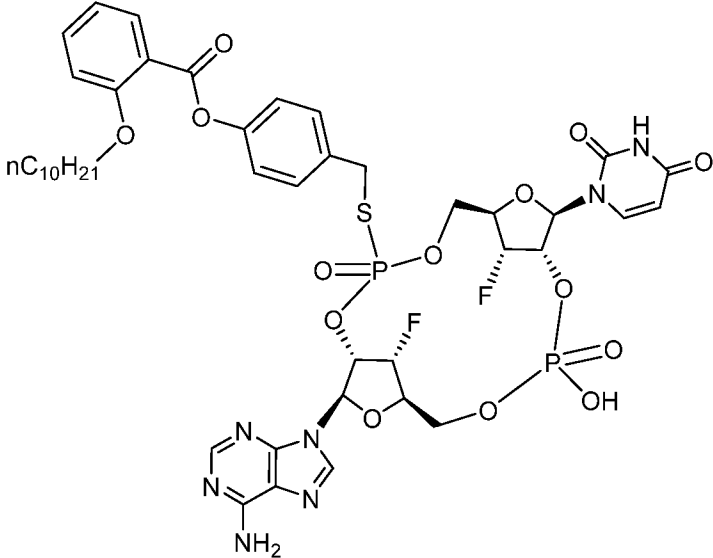
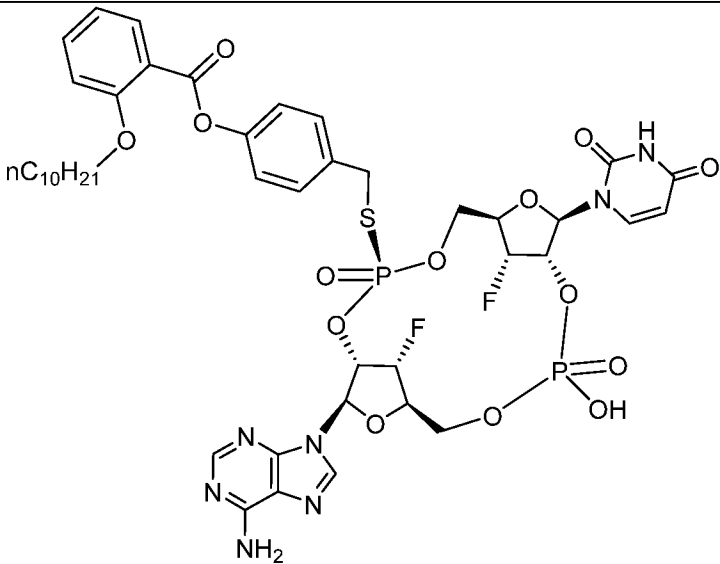
32	 Chemical structure 32 is a complex molecule. It features a 4-(n-dodecyloxy)benzoyl group (nC₁₂H₂₅O-C₆H₄-C(=O)-O-C₆H₄-CH₂-S-) linked via an ester to a 4-(methylthio)phenyl group. This is connected to a phosphorus atom, which is part of a cyclic phosphate system. The system includes a ribose derivative with a uracil base, a fluorine atom, and an adenine derivative. The phosphorus atom is also bonded to a hydroxyl group.
33	 Chemical structure 33 is a complex molecule. It features a 4-(n-dodecyloxy)benzoyl group (nC₁₂H₂₅O-C₆H₄-C(=O)-O-C₆H₄-CH₂-S-) linked via an ester to a 4-(methylthio)phenyl group. This is connected to a phosphorus atom, which is part of a cyclic phosphate system. The system includes a ribose derivative with a uracil base, a fluorine atom, and an adenine derivative. The phosphorus atom is also bonded to a hydroxyl group.

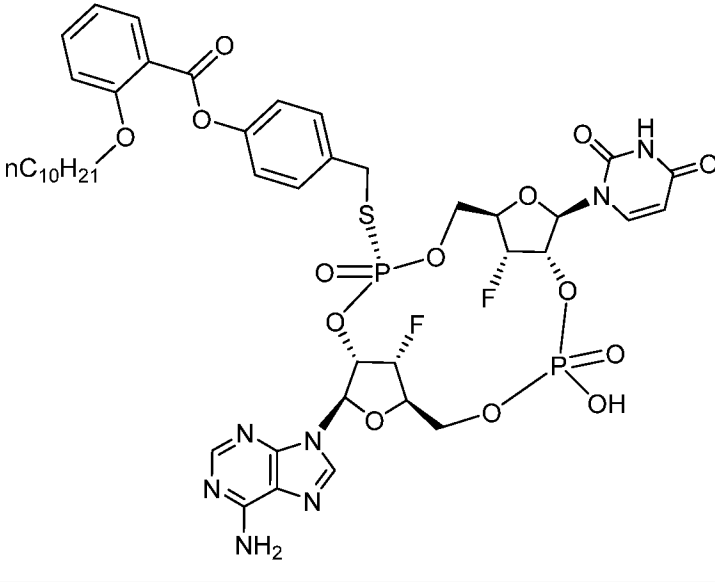
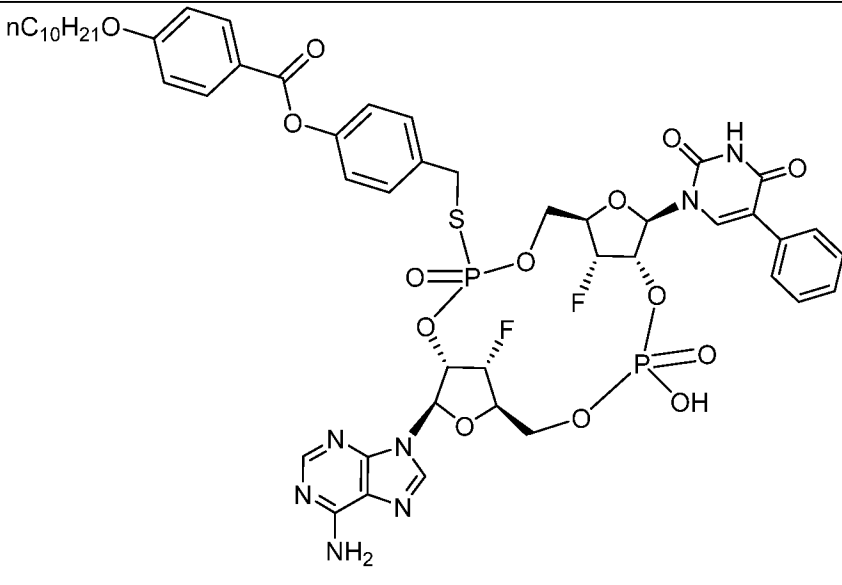
34	Chemical structure 34: A complex molecule featuring a central dinucleotide core. The core consists of two deoxyribose sugars linked by a 3'-5' phosphodiester bond. The 5' sugar is substituted with a 2-amino-6-oxo-1,2,3,4-tetrahydropyrimidin-9-yl group. The 3' sugar is substituted with a 2,4,6-trioxypyrimidin-5-yl group. A phosphate group is attached to the 3' carbon of the 5' sugar, which is further linked to a thioether group (-S-). This thioether group is connected to a benzyl group, which is part of a larger side chain. The side chain includes a 4-methoxyphenyl group, a carbonyl group, and a long alkyl chain (nC₁₀H₂₁O-).
35	Chemical structure 35: A complex molecule featuring a central dinucleotide core. The core consists of two deoxyribose sugars linked by a 3'-5' phosphodiester bond. The 5' sugar is substituted with a 2-amino-6-oxo-1,2,3,4-tetrahydropyrimidin-9-yl group. The 3' sugar is substituted with a 2,4,6-trioxypyrimidin-5-yl group. A phosphate group is attached to the 3' carbon of the 5' sugar, which is further linked to a thioether group (-S-). This thioether group is connected to a benzyl group, which is part of a larger side chain. The side chain includes a 4-methoxyphenyl group, a carbonyl group, and a long alkyl chain (nC₁₀H₂₁O-).

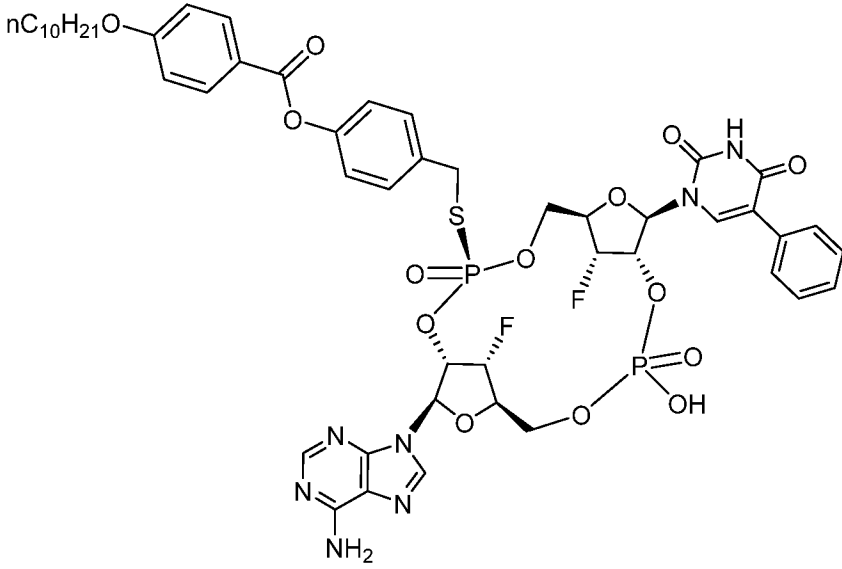
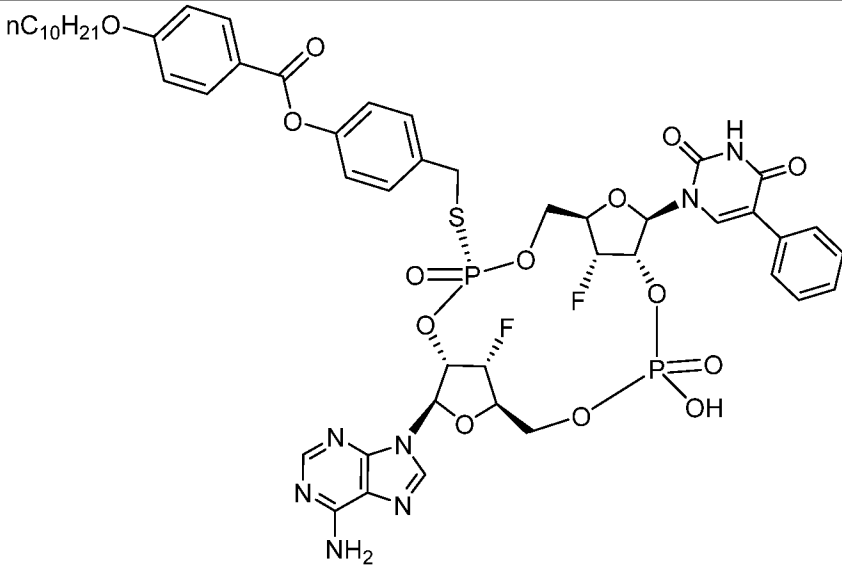
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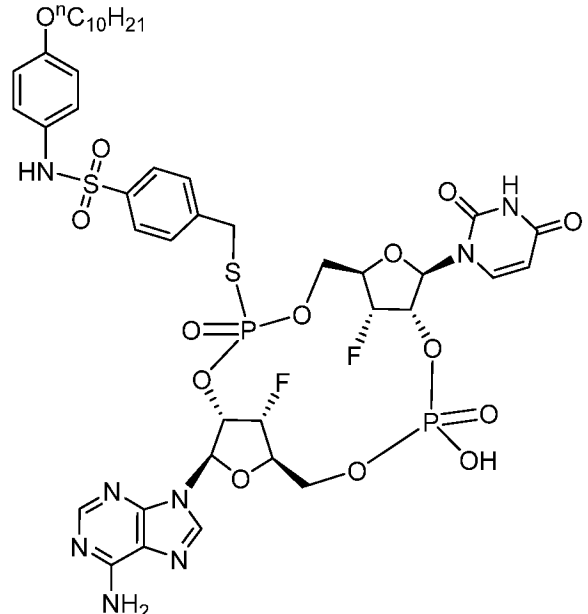
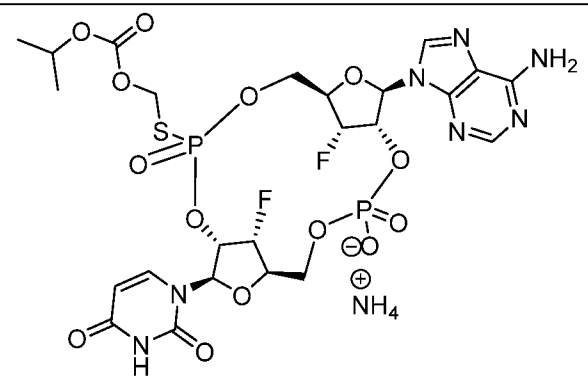
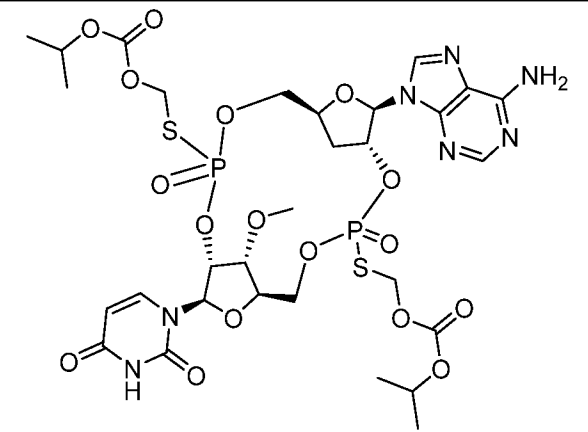
39	Chemical structure 39 is a complex molecule. It features a central bicyclic core consisting of a purine-like base (2-aminopurine) and a pyrimidine-like base (2,4-dioxypyrimidin-5(1H)-one). The core is linked via a phosphorus atom to a long chain containing a thioether linkage and a long alkyl chain (nC₁₀H₂₁O).
40	Chemical structure 40 is a complex molecule. It features a central bicyclic core consisting of a purine-like base (2-aminopurine) and a pyrimidine-like base (2,4-dioxypyrimidin-5(1H)-one). The core is linked via a phosphorus atom to a long chain containing a thioether linkage and a long alkyl chain (nC₁₀H₂₁O).

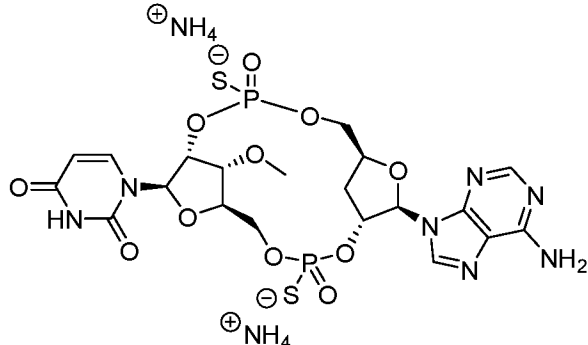
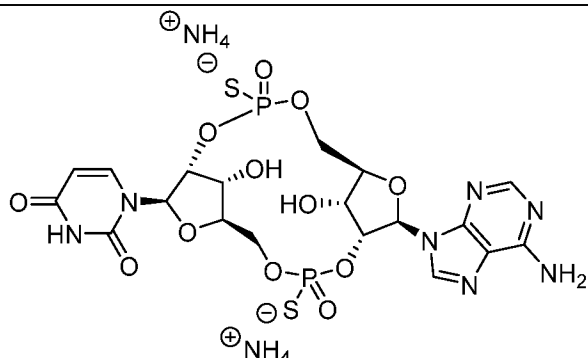
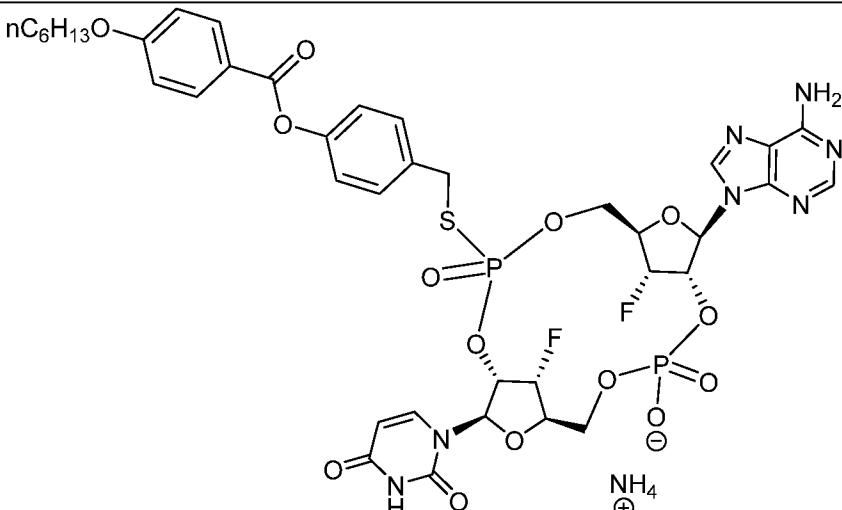
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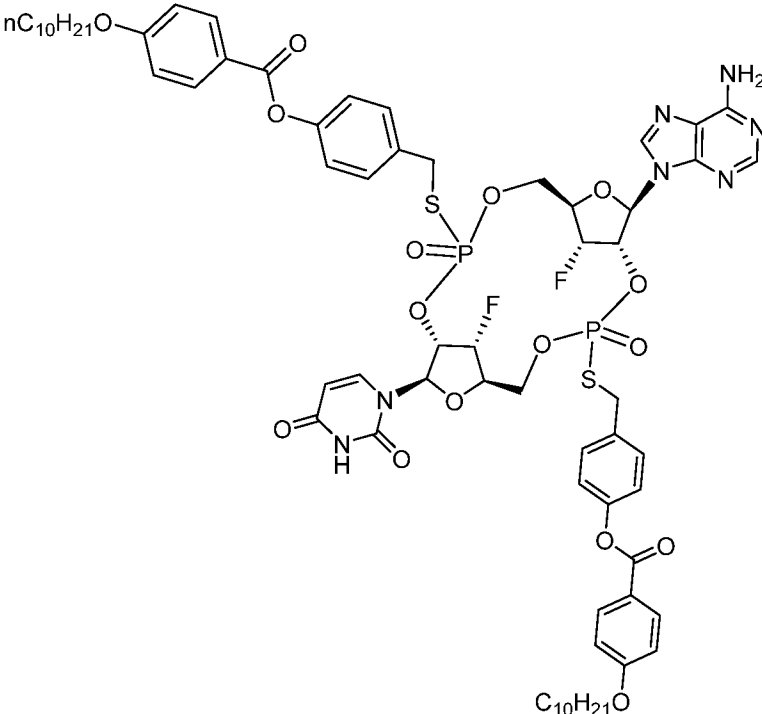
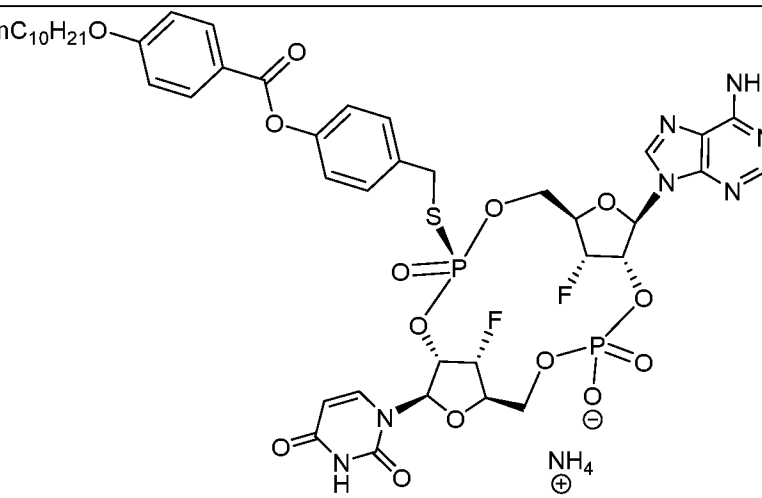
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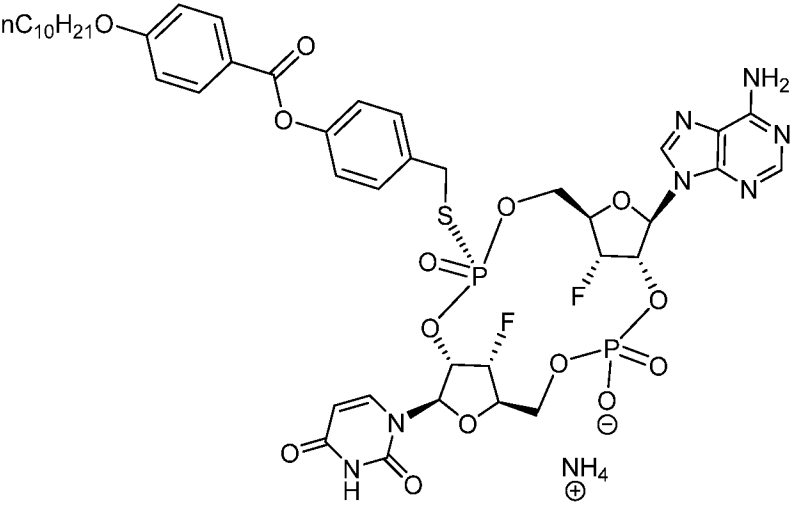
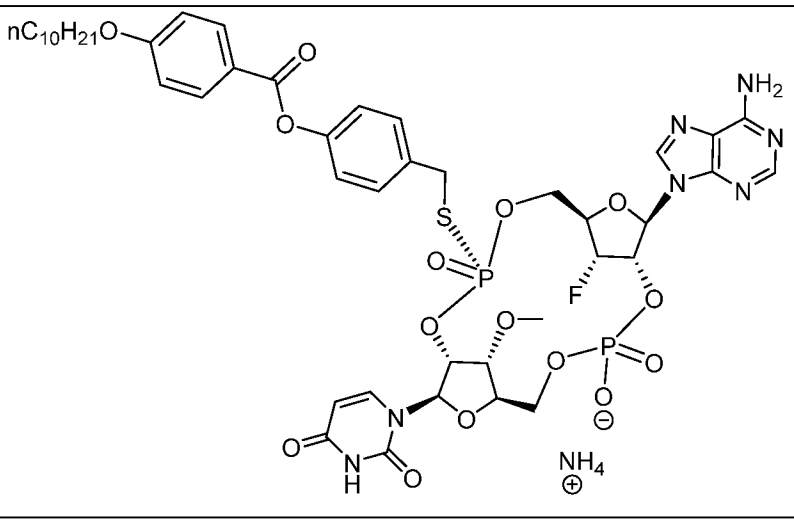
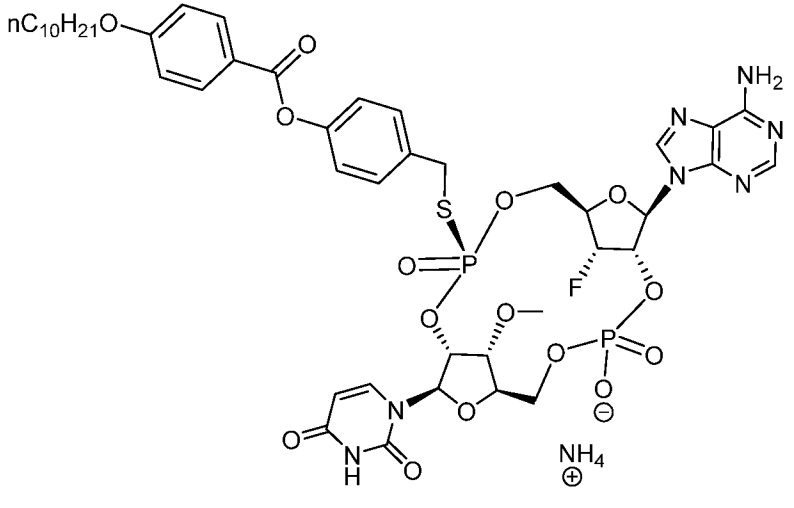
47	 Chemical structure 47: A complex molecule featuring a diphosphate core. One phosphate is linked via a sulfonate group to a 4-(n-decyloxy)benzoyl group. The other phosphate is linked via an ester to a ribose derivative substituted with a fluorine atom and a 2-aminopurine-9-yl group. A third phosphate group is also attached to the ribose derivative via an ester linkage.
48	 Chemical structure 48: A complex molecule featuring a diphosphate core. One phosphate is linked via a sulfonate group to a 4-(n-decyloxy)benzoyl group. The other phosphate is linked via an ester to a ribose derivative substituted with a fluorine atom and a 2-aminopurine-9-yl group. A third phosphate group is also attached to the ribose derivative via an ester linkage.

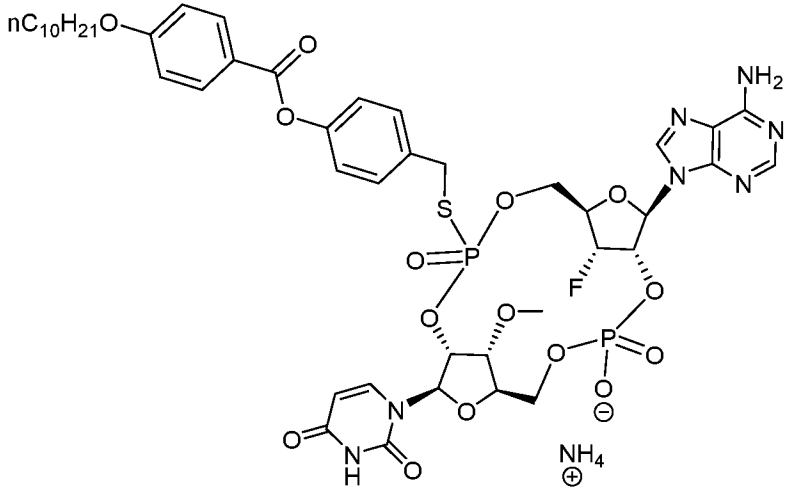
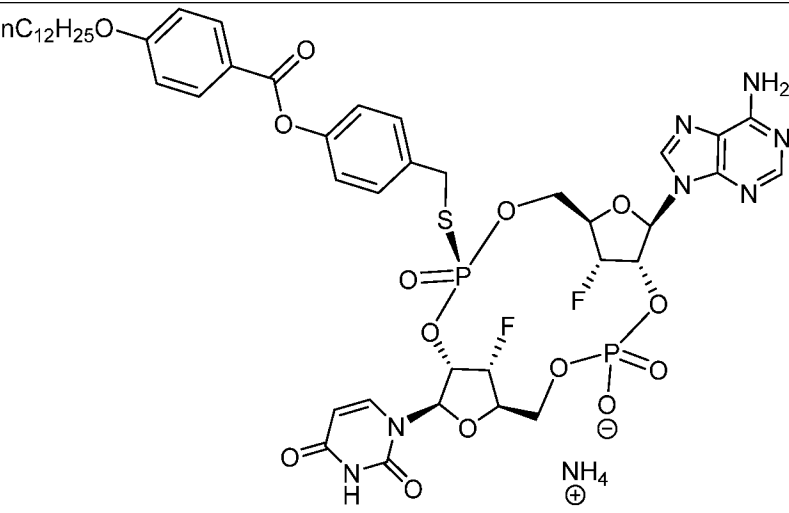
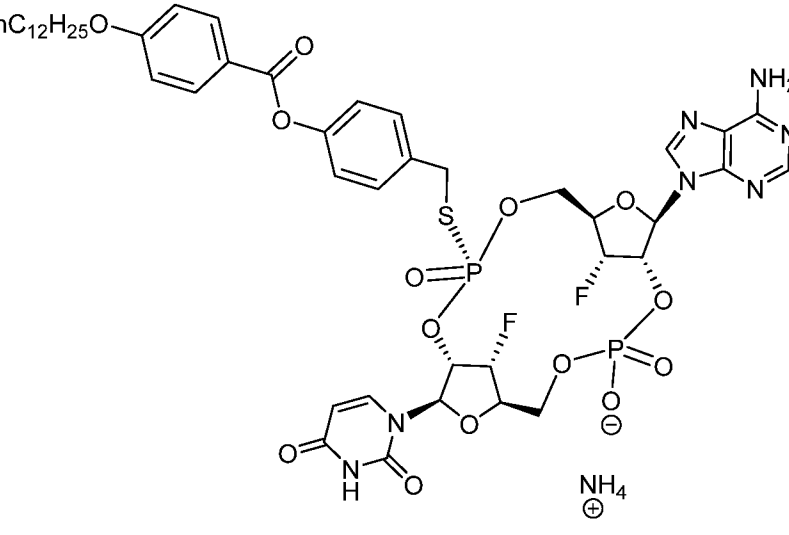
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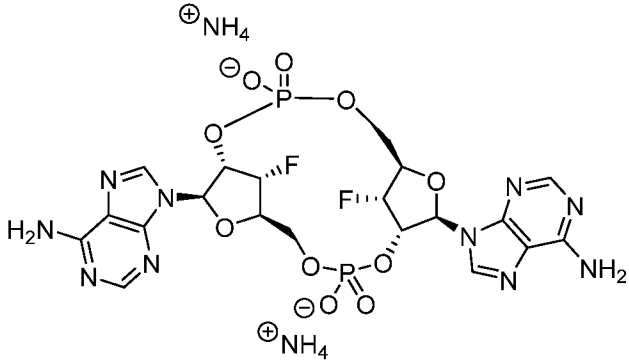
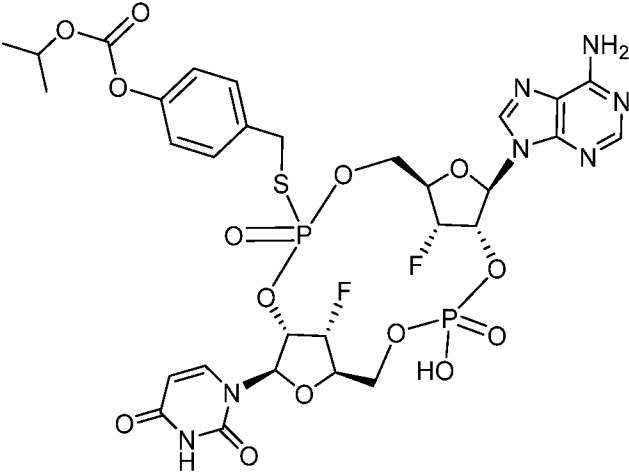
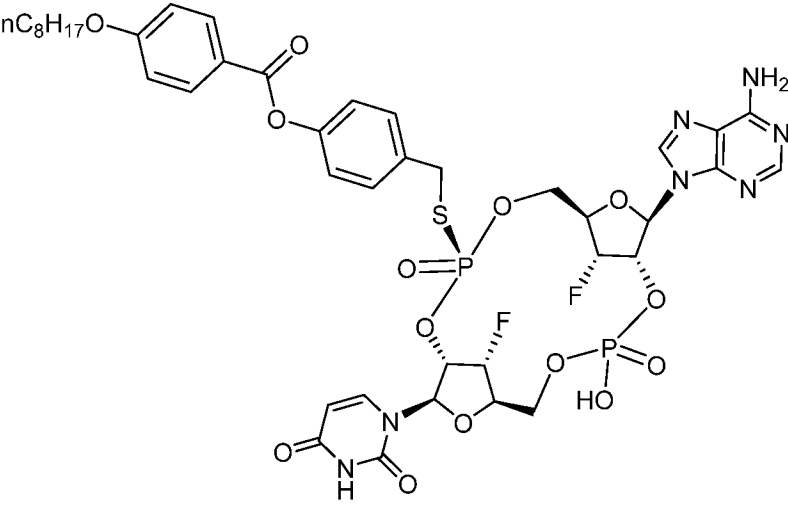
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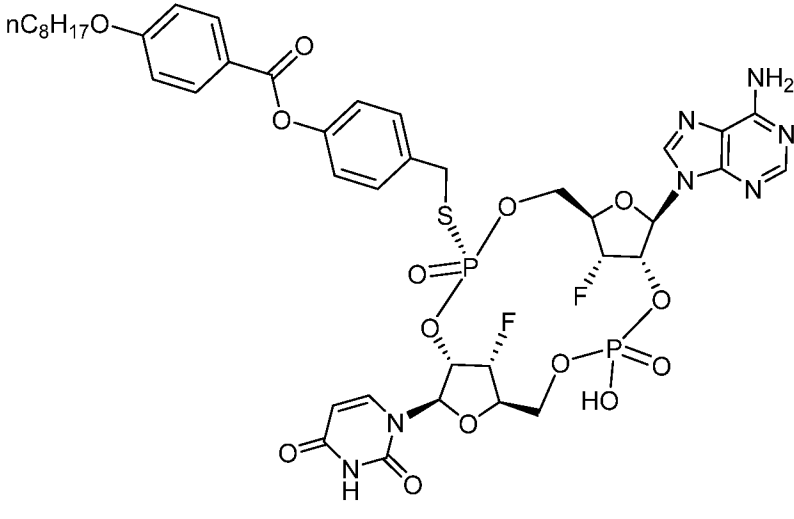
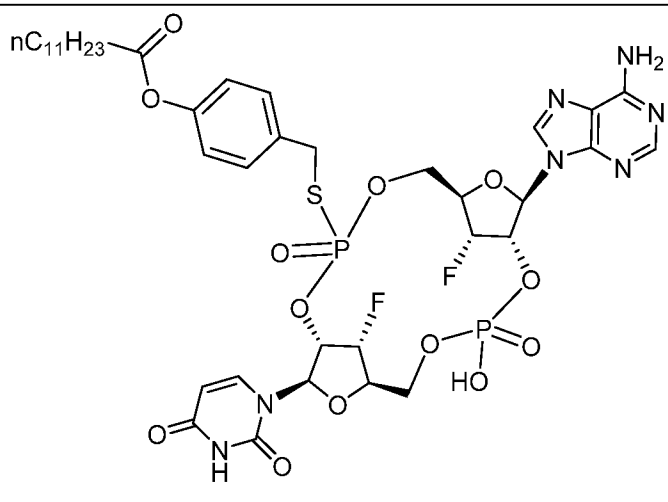
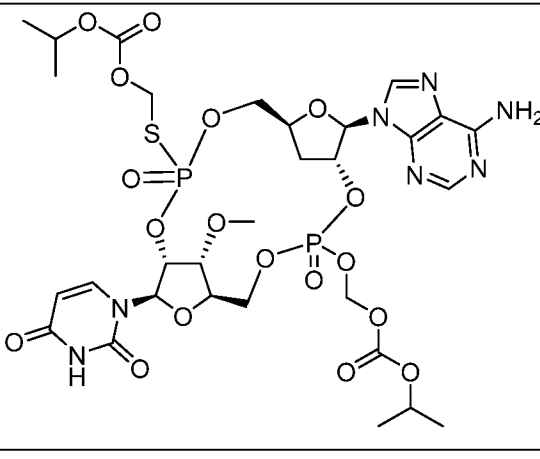
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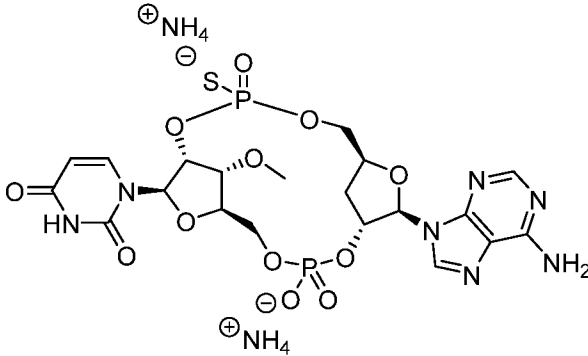
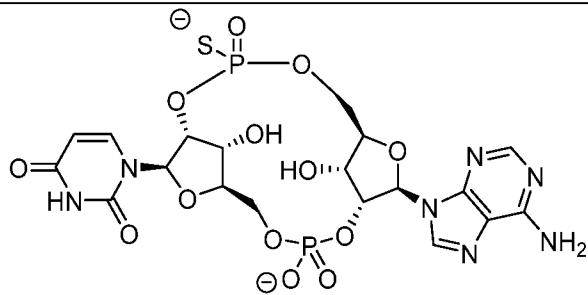
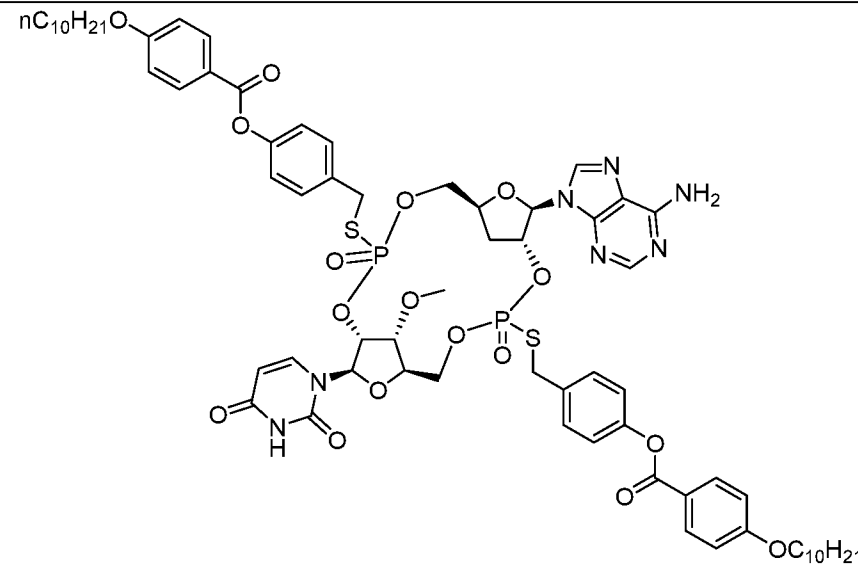
58	 Chemical structure 58 is a complex molecule featuring a central ribose derivative. The ribose is substituted with a thiazine ring at the C1 position, a thymine base at the C2 position, and a 4'-phosphonate group at the C4' position. The phosphonate group is linked via a thioether to a 4'-phenoxybenzoate group, which is further linked via an ester to a 4'-n-decylphenoxy group. The structure is labeled with $nC_{10}H_{21}O$ at the top left and $C_{10}H_{21}O$ at the bottom right.
59	 Chemical structure 59 is a complex molecule similar to 58, but with a different phosphonate group at the C4' position. It features a thiazine ring at C1, a thymine base at C2, and a 4'-phosphonate group at C4'. The phosphonate is linked via a thioether to a 4'-phenoxybenzoate group, which is further linked via an ester to a 4'-n-decylphenoxy group. The structure is labeled with $nC_{10}H_{21}O$ at the top left and NH_4^+ at the bottom right.

60	 Chemical structure 60: A complex molecule featuring a diphosphate core. One phosphate is linked to a thioether group, which is connected to a 4-(n-decyloxybenzoyloxy)benzyl group. The other phosphate is linked to a ribose derivative substituted with a pyrimidin-2(1H)-one and a fluorine atom. The second ribose is substituted with an adenine group and a fluorine atom. A separate ammonium ion (NH_4^+) is shown.
61	 Chemical structure 61: Similar to structure 60, but with a different stereochemistry at the phosphate linkage.
62	 Chemical structure 62: Similar to structure 60, but with a different stereochemistry at the phosphate linkage.

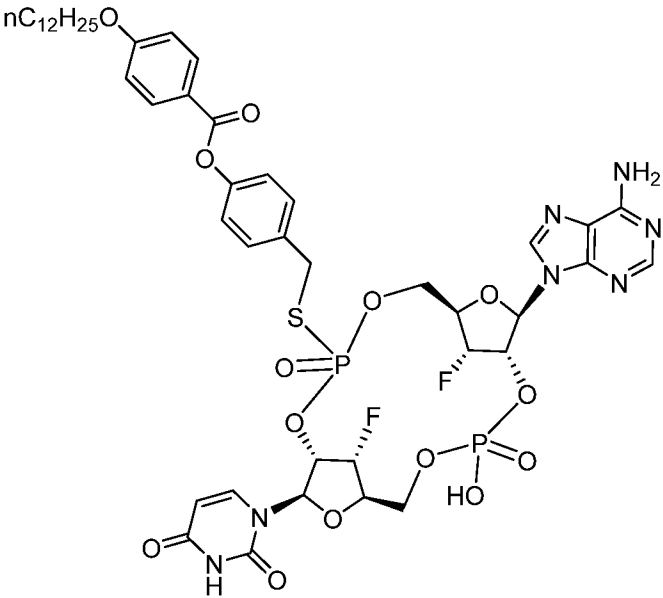
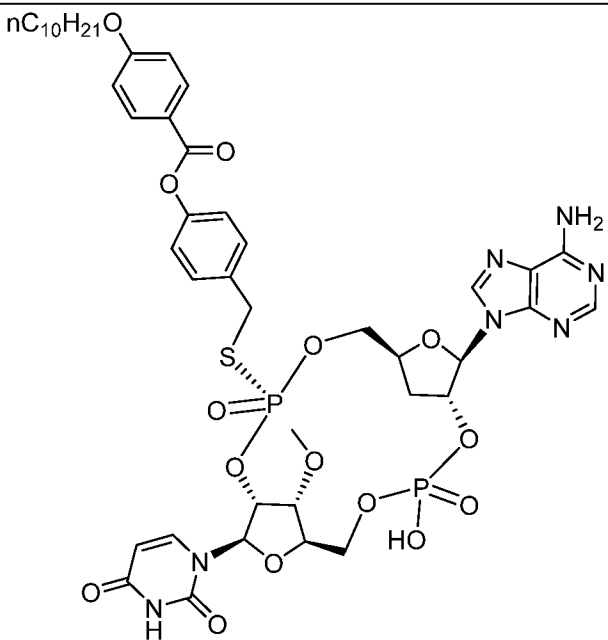
63	 Chemical structure 63: A complex molecule featuring a nucleoside core. The sugar moiety is a ribose derivative with a fluorine atom at the 2' position and a phosphate group at the 3' position. The phosphate group is linked to a thioether bridge, which is connected to a benzyl group. The benzyl group is further linked to a p-tert-butylbenzoate group. The base is an adenine derivative. The counterion is NH₄⁺.
64	 Chemical structure 64: A complex molecule featuring a nucleoside core. The sugar moiety is a ribose derivative with a fluorine atom at the 2' position and a phosphate group at the 3' position. The phosphate group is linked to a thioether bridge, which is connected to a benzyl group. The benzyl group is further linked to a p-tert-butylbenzoate group. The base is an adenine derivative. The counterion is NH₄⁺.
65	 Chemical structure 65: A complex molecule featuring a nucleoside core. The sugar moiety is a ribose derivative with a fluorine atom at the 2' position and a phosphate group at the 3' position. The phosphate group is linked to a thioether bridge, which is connected to a benzyl group. The benzyl group is further linked to a p-tert-butylbenzoate group. The base is an adenine derivative. The counterion is NH₄⁺.

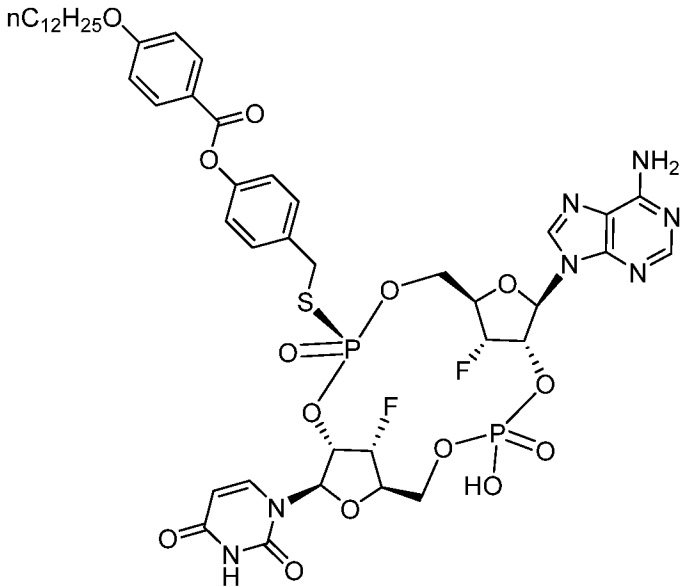
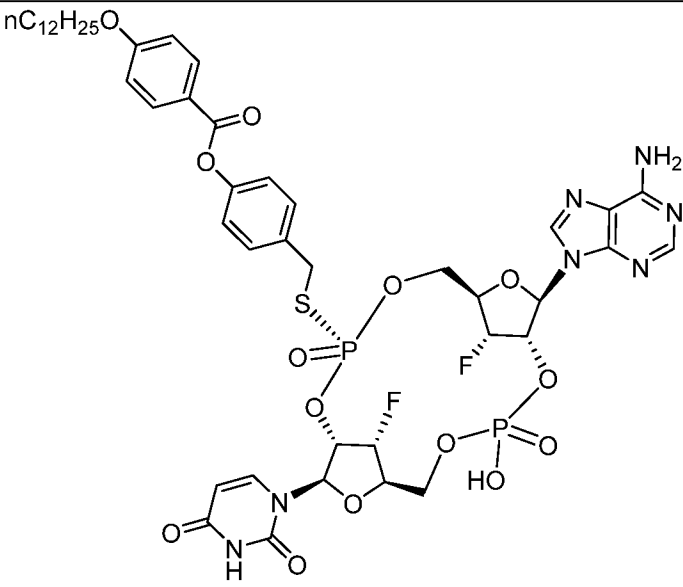
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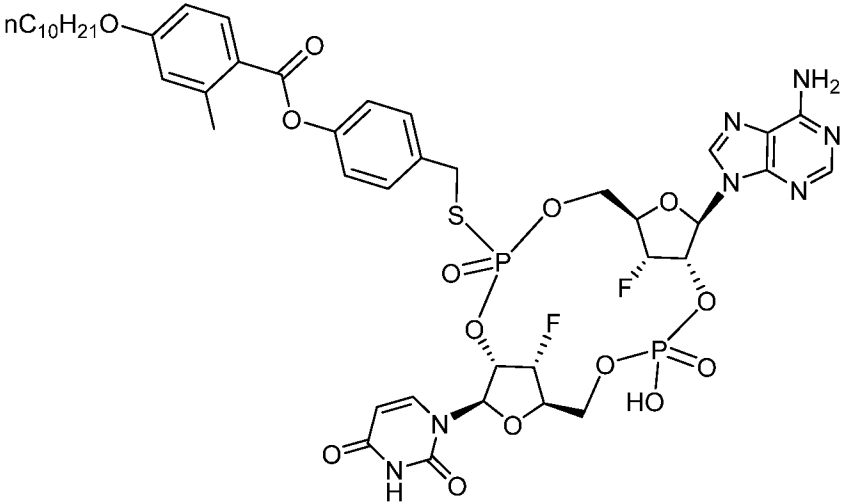
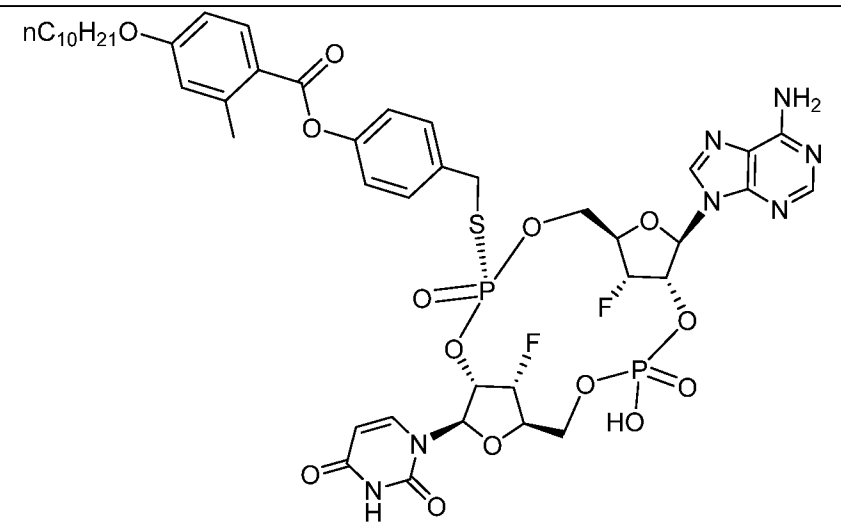
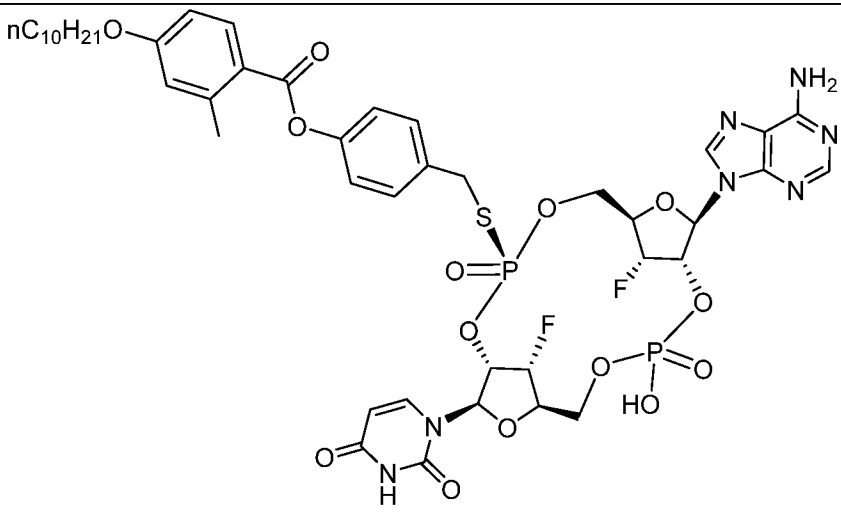
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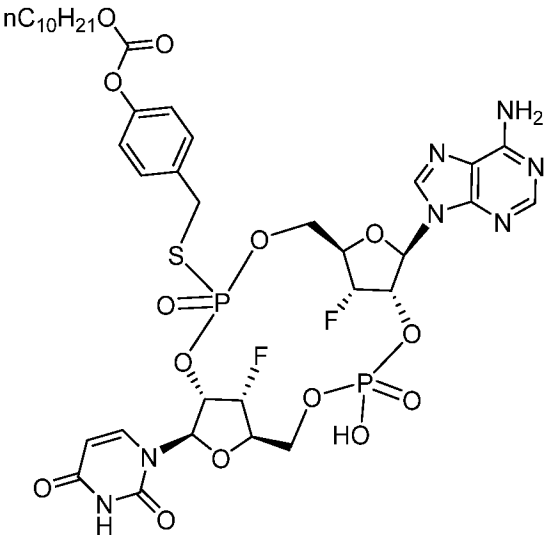
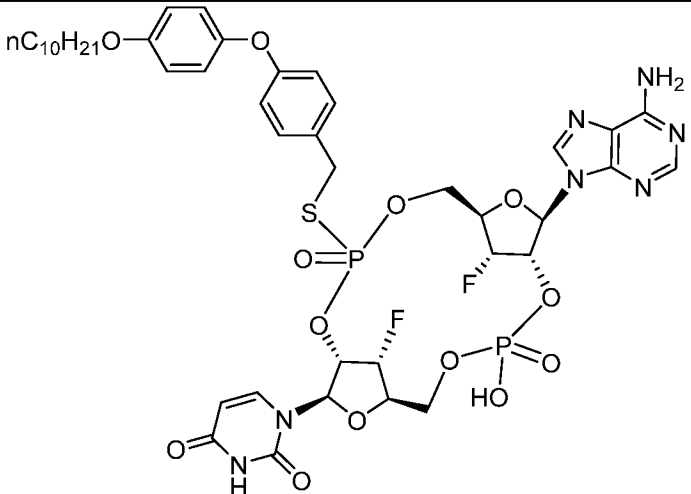
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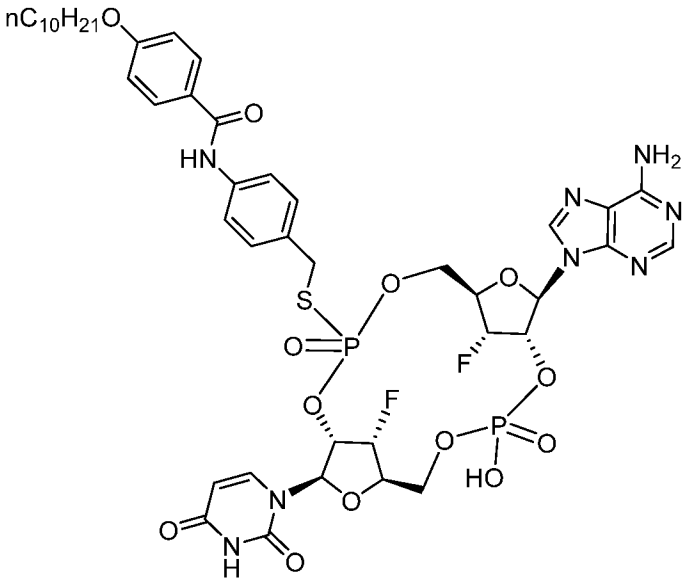
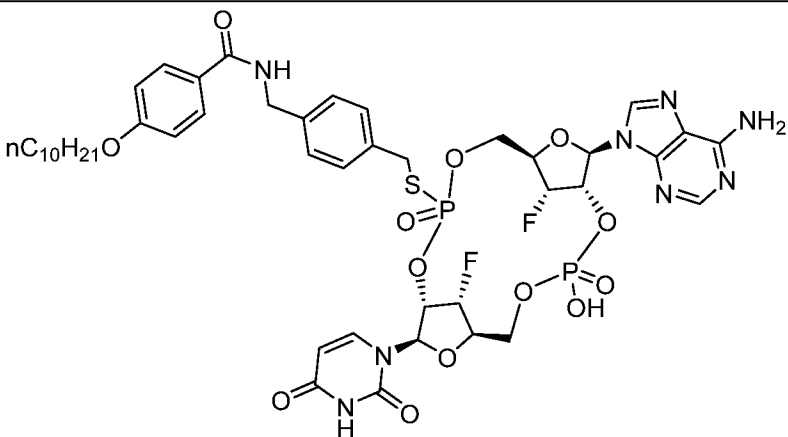
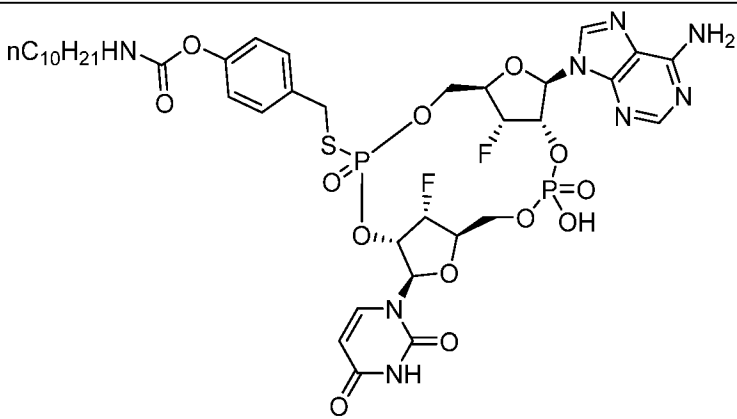
75	Chemical structure 75: A complex molecule featuring a central bicyclic core with a thiazine ring and a pyrimidine ring. The structure includes two phosphate groups, a thiol group, and a long alkyl chain (nC₁₀H₂₁O).
76	Chemical structure 76: A complex molecule featuring a central bicyclic core with a thiazine ring and a pyrimidine ring. The structure includes two phosphate groups, a thiol group, and a long alkyl chain (nC₁₀H₂₁O).
77	Chemical structure 77: A complex molecule featuring a central bicyclic core with a thiazine ring and a pyrimidine ring. The structure includes two phosphate groups, a thiol group, and a long alkyl chain (nC₈H₁₇O).

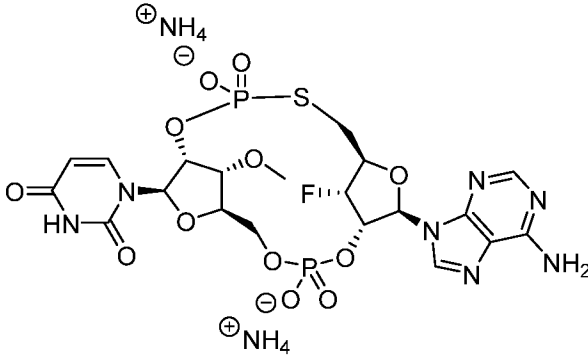
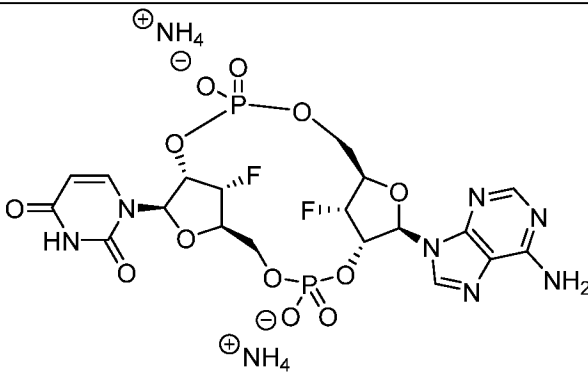
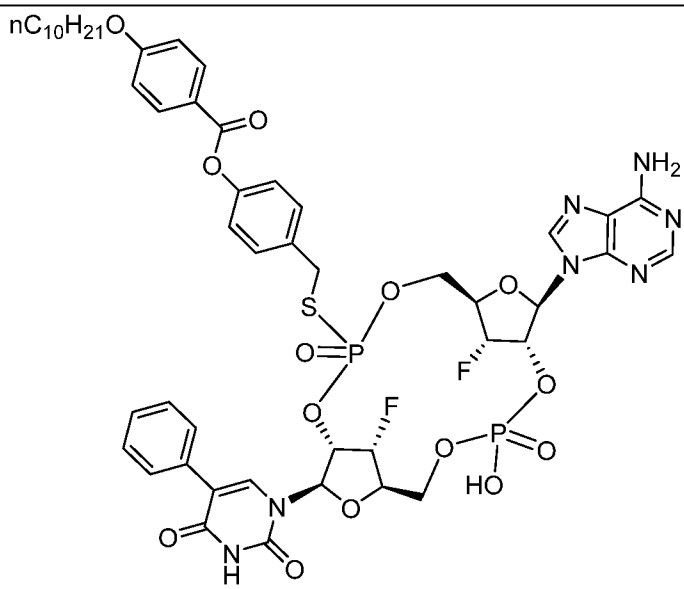
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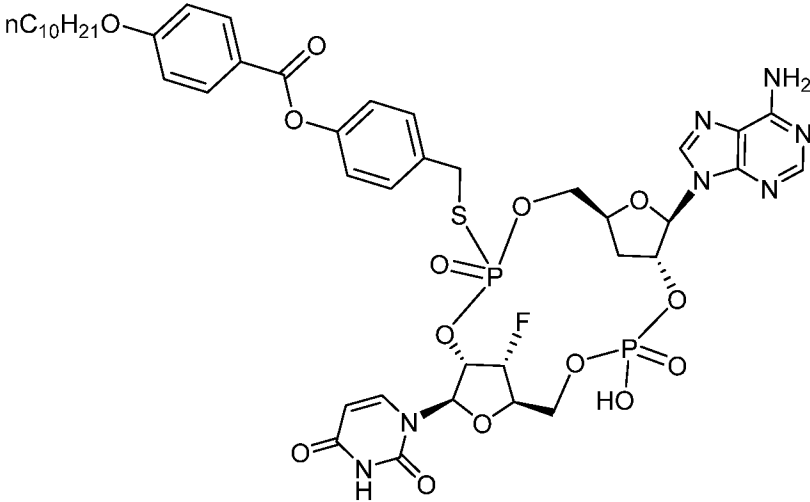
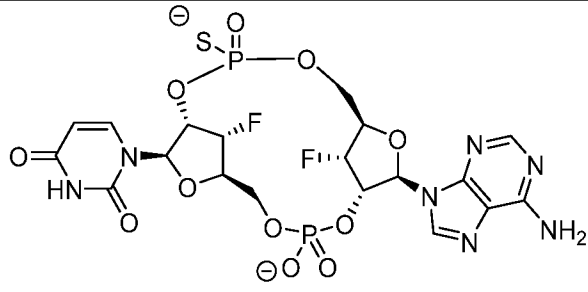
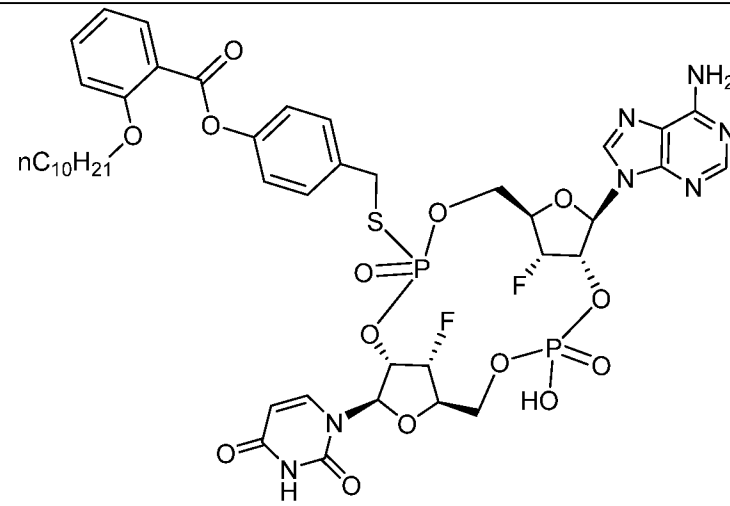
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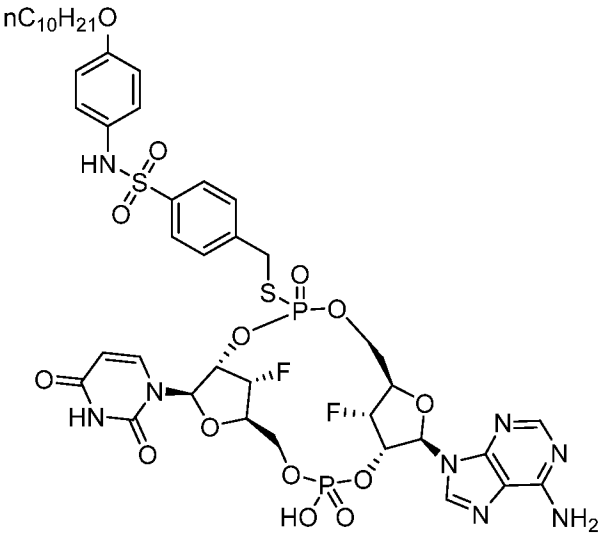
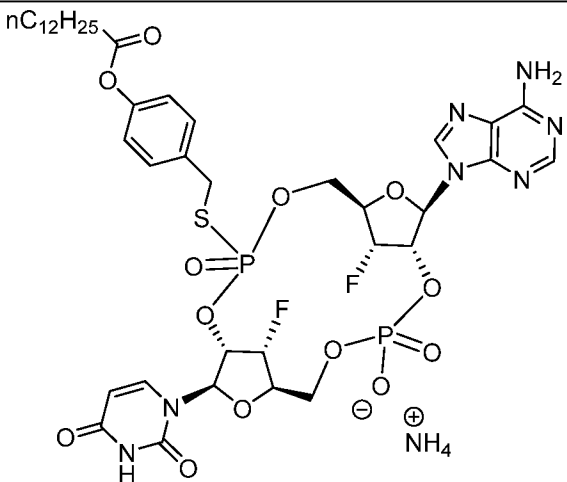
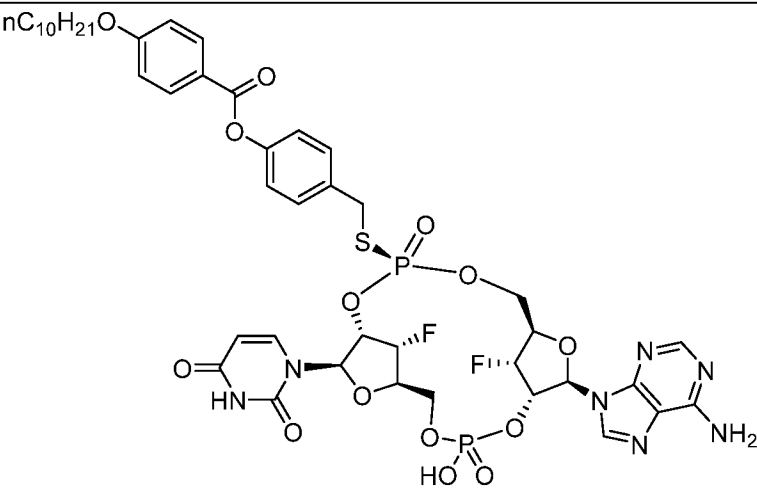
85	 Chemical structure 85: A complex molecule featuring a pyrimidine ring system (adenine derivative) linked via a sugar moiety to a phosphate group. The phosphate is further linked to a thioether group, which is connected to a benzyl group. The benzyl group is linked to a phenyl ring, which is connected to a long alkyl chain (nC₁₀H₂₁O). The molecule also includes a pyridine ring system and a hydroxyl group.
86	 Chemical structure 86: A complex molecule featuring a pyrimidine ring system (adenine derivative) linked via a sugar moiety to a phosphate group. The phosphate is further linked to a thioether group, which is connected to a benzyl group. The benzyl group is linked to a phenyl ring, which is connected to a long alkyl chain (nC₁₀H₂₁O). The molecule also includes a pyridine ring system and a hydroxyl group.

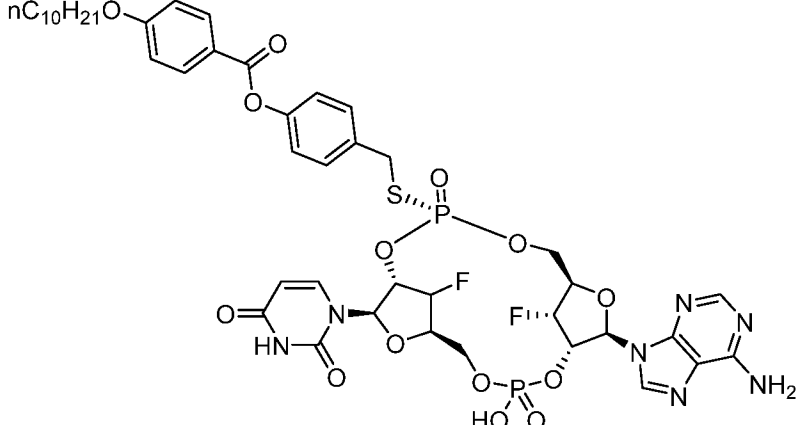
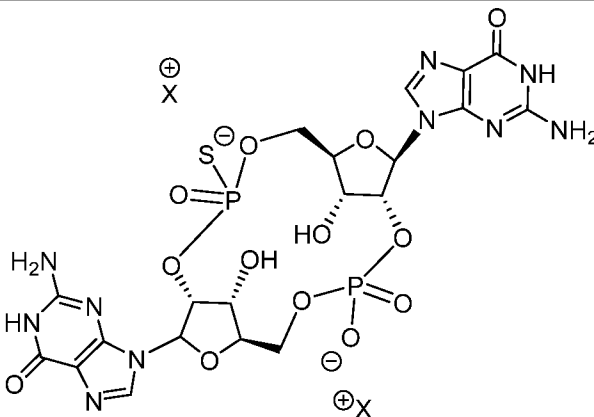
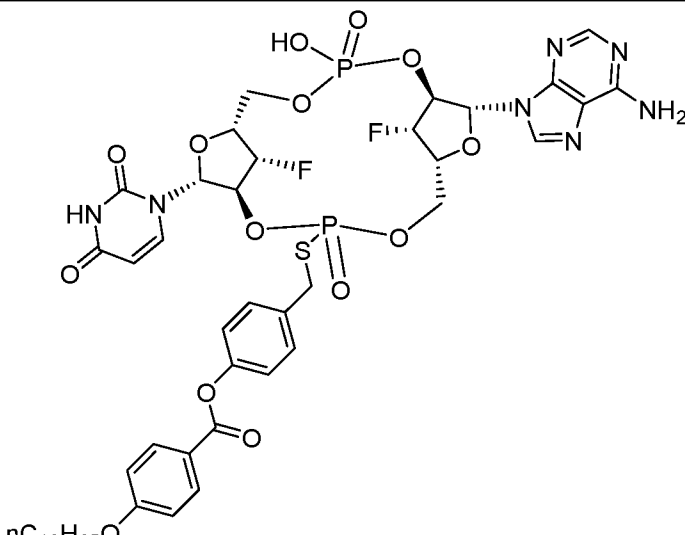
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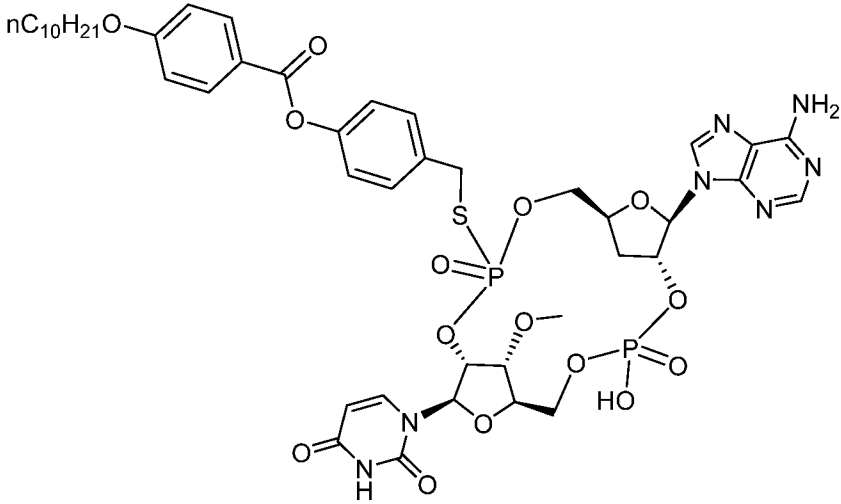
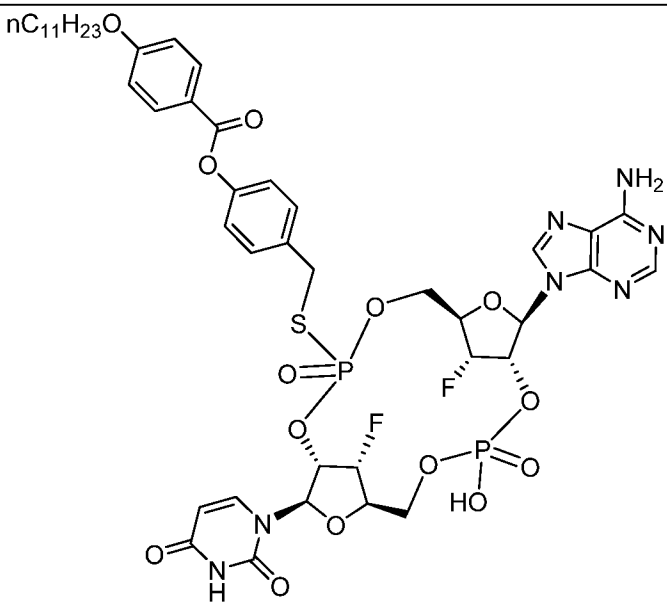
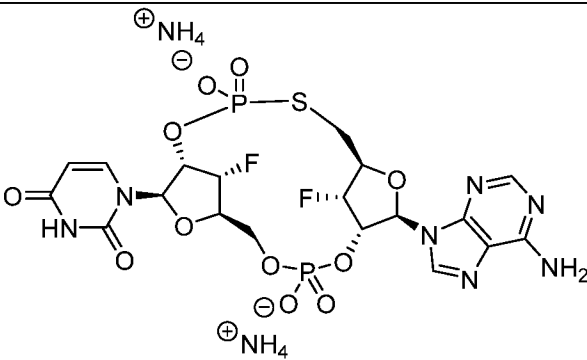
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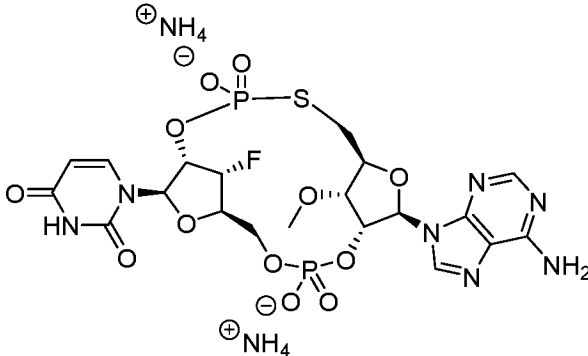
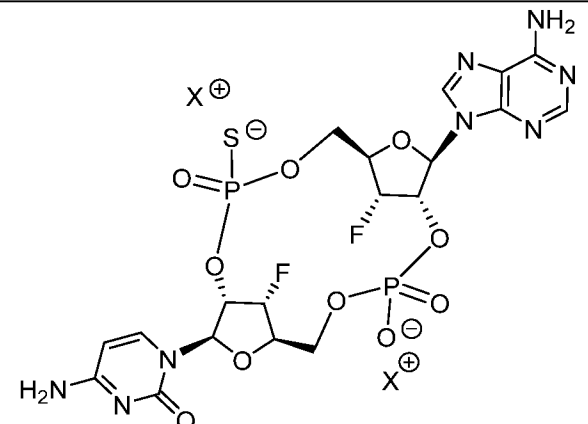
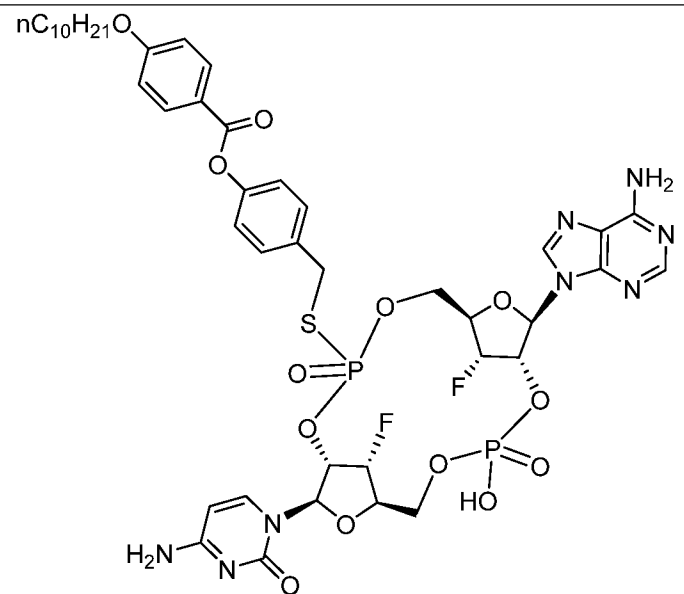
93	 <chem>Nc1ncnc2nc(NC(=O)c3ccccc3C(=O)N2)cnc1[C@@H]3O[C@H](COP(=O)([O-])[O-])[C@@H](F)[C@H](O[C@H]4C[C@H](COP(=O)([O-])[O-])[C@@H](F)[C@H](O4)c5c[nH]c6c(=O)c7ccccc7c(=O)n56)[C@H]3O</chem>
94	 <chem>Nc1ncnc2nc(NC(=O)c3ccccc3C(=O)N2)cnc1[C@@H]3O[C@H](COP(=O)([O-])[O-])[C@@H](F)[C@H](O[C@H]4C[C@H](COP(=O)([O-])[O-])[C@@H](F)[C@H](O4)c5c[nH]c6c(=O)c7ccccc7c(=O)n56)[C@H]3O</chem>
95	 <chem>Nc1ncnc2nc(NC(=O)c3ccccc3C(=O)N2)cnc1[C@@H]3O[C@H](COP(=O)([O-])[O-])[C@@H](F)[C@H](O[C@H]4C[C@H](COP(=O)([O-])[O-])[C@@H](F)[C@H](O4)c5c[nH]c6c(=O)c7ccccc7c(=O)n56)[C@H]3O</chem>

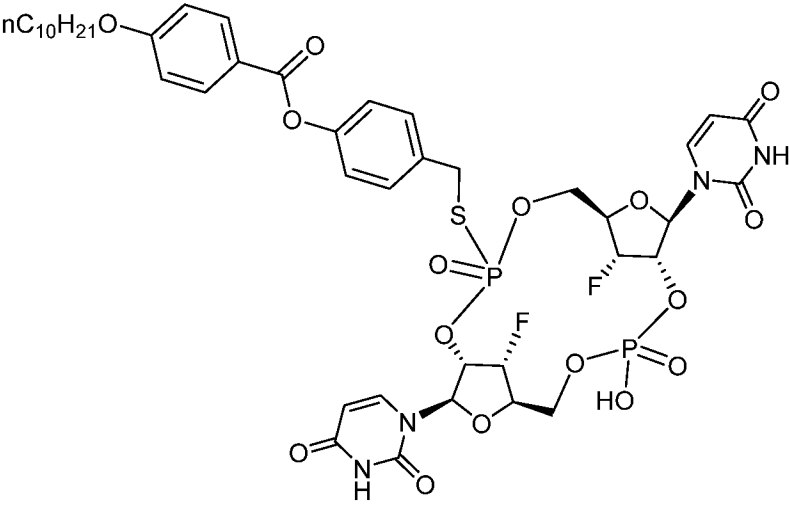
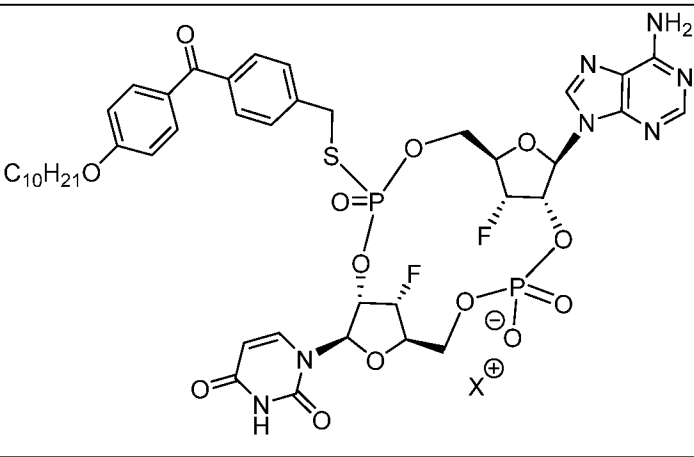
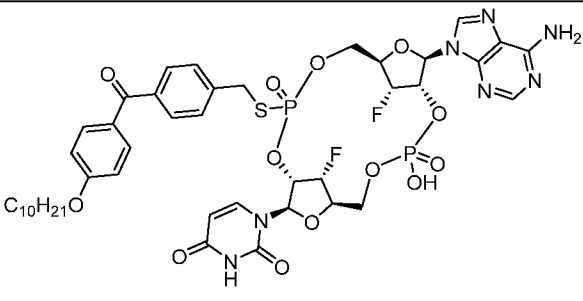
96	 Chemical structure 96: A complex molecule featuring a central sugar moiety (ribose) linked to a thiazine ring system. The sugar is substituted with a phosphate group (HO-P(=O)(O⁻)-O⁻) and a fluorine atom. The thiazine ring is linked to a benzyl group, which is further substituted with a long alkyl chain (nC₁₀H₂₁O) and a carbonyl group.
97	 Chemical structure 97: A complex molecule featuring a central sugar moiety (ribose) linked to a thiazine ring system. The sugar is substituted with a phosphate group (S-P(=O)(O⁻)-O⁻) and a fluorine atom. The thiazine ring is linked to a benzyl group, which is further substituted with a long alkyl chain (nC₁₀H₂₁O) and a carbonyl group.
98	 Chemical structure 98: A complex molecule featuring a central sugar moiety (ribose) linked to a thiazine ring system. The sugar is substituted with a phosphate group (HO-P(=O)(O⁻)-O⁻) and a fluorine atom. The thiazine ring is linked to a benzyl group, which is further substituted with a long alkyl chain (nC₁₀H₂₁O) and a carbonyl group.

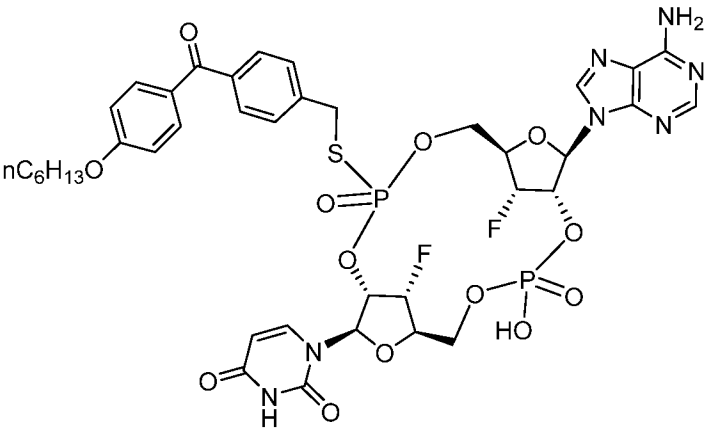
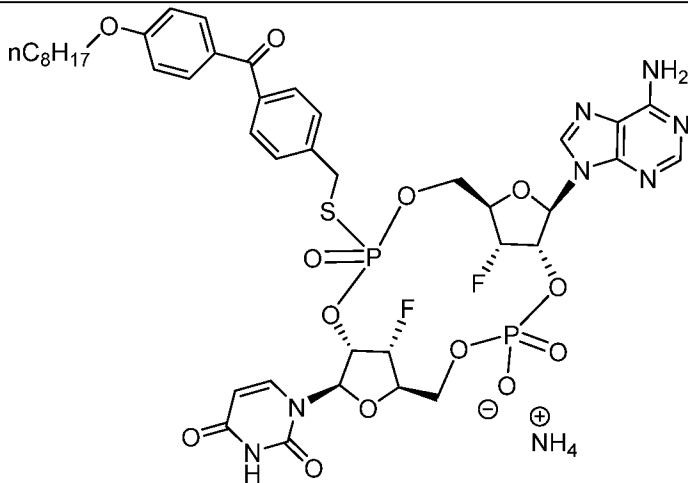
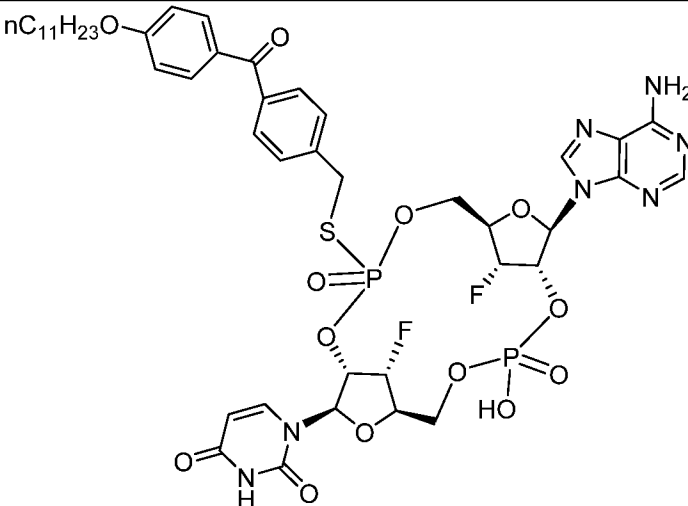
99	 Chemical structure 99: A complex molecule featuring a central sugar moiety (ribose) linked via phosphonate groups to a thymine base and an adenine base. The thymine base is further linked via a sulfonate group to a phenyl ring, which is connected to a long alkyl chain (nC₁₀H₂₁O).
100	 Chemical structure 100: A complex molecule featuring a central sugar moiety (ribose) linked via phosphonate groups to a thymine base and an adenine base. The thymine base is further linked via a sulfonate group to a phenyl ring, which is connected to a long alkyl chain (nC₁₂H₂₅).
101	 Chemical structure 101: A complex molecule featuring a central sugar moiety (ribose) linked via phosphonate groups to a thymine base and an adenine base. The thymine base is further linked via a sulfonate group to a phenyl ring, which is connected to a long alkyl chain (nC₁₀H₂₁O).

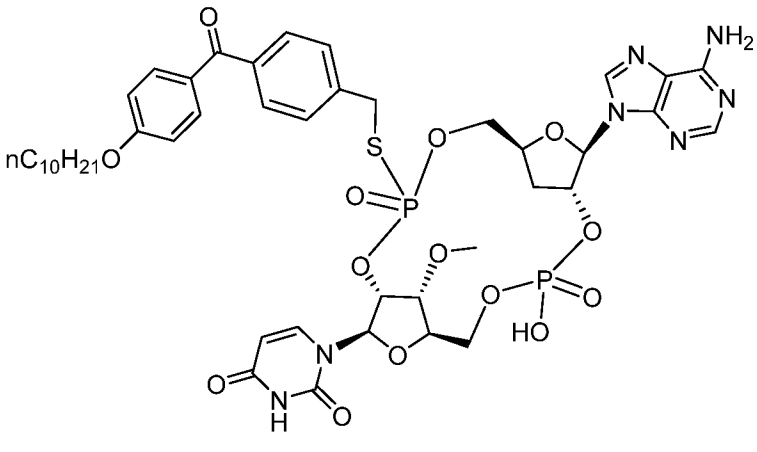
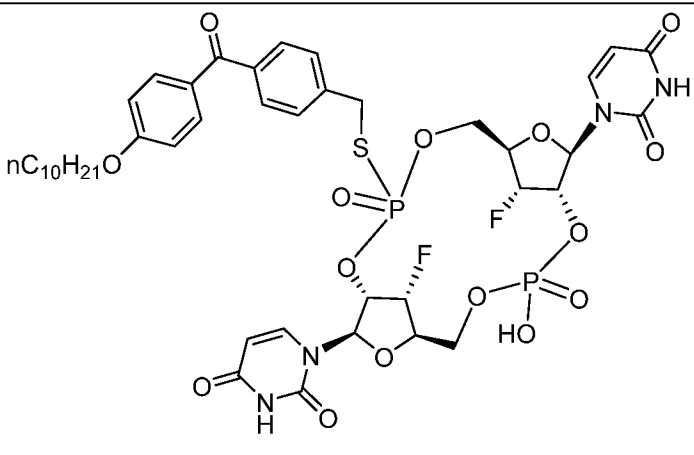
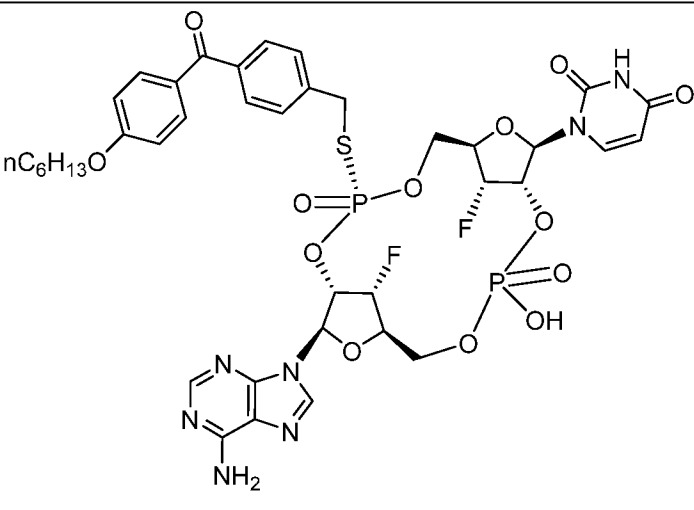
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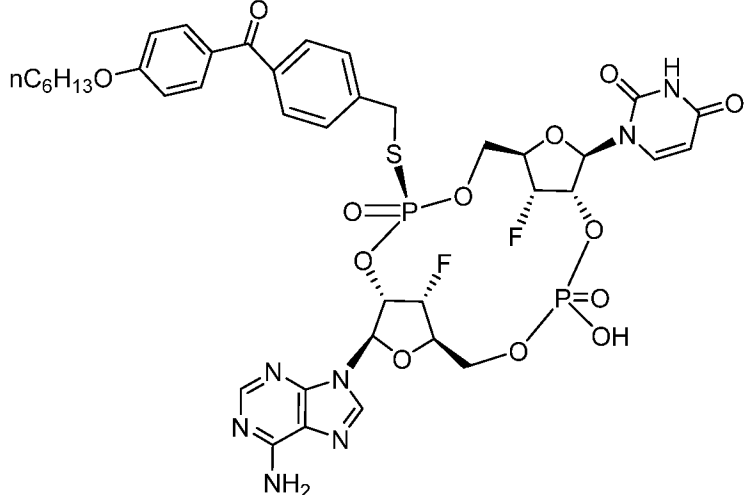
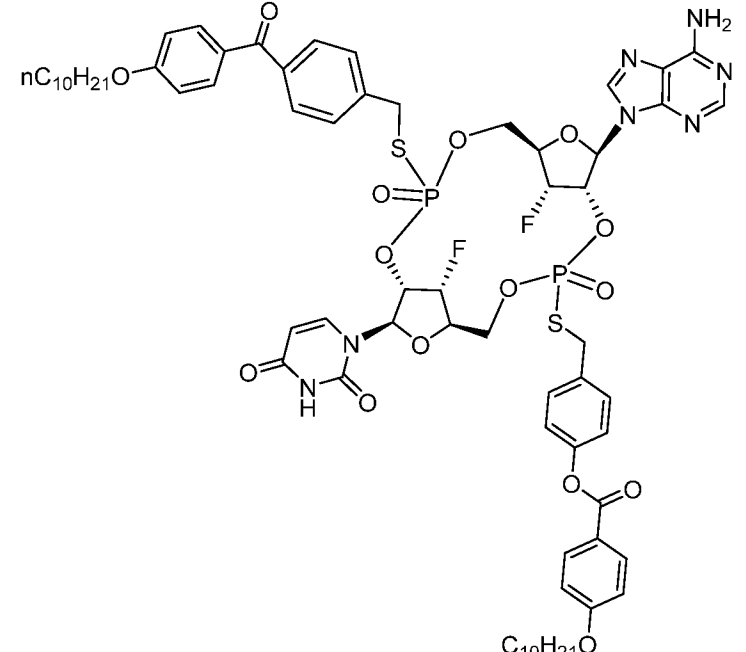
105	 Chemical structure 105: A complex molecule featuring a long alkyl chain ($nC_{10}H_{21}O$) attached to a benzene ring, which is linked via an ester to another benzene ring. This second benzene ring is connected to a sulfur atom, which is part of a cyclic phosphorus-containing structure. The structure includes a pyrimidine ring, a sugar moiety, and a phosphate group.
106	 Chemical structure 106: Similar to structure 105, but with a different alkyl chain ($nC_{11}H_{23}O$) and a different sugar moiety.
107	 Chemical structure 107: A complex molecule featuring a long alkyl chain ($nC_{10}H_{21}O$) attached to a benzene ring, which is linked via an ester to another benzene ring. This second benzene ring is connected to a sulfur atom, which is part of a cyclic phosphorus-containing structure. The structure includes a pyrimidine ring, a sugar moiety, and a phosphate group.

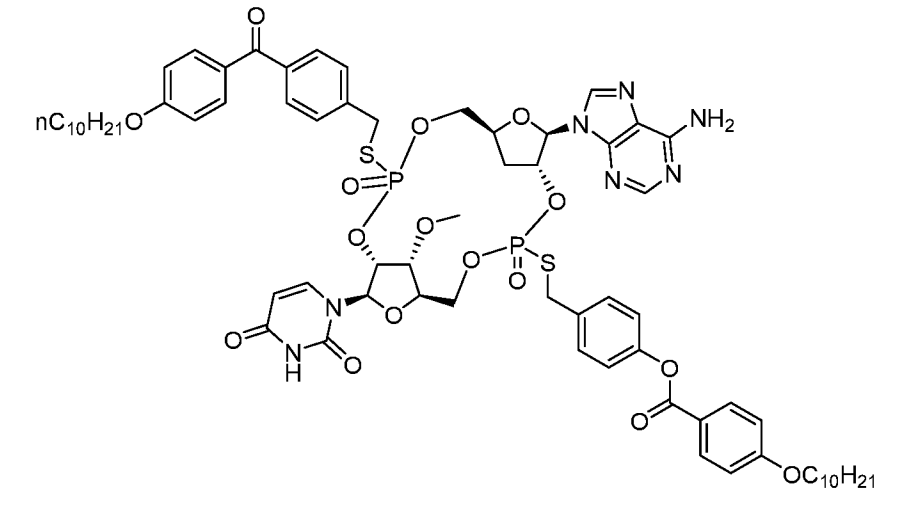
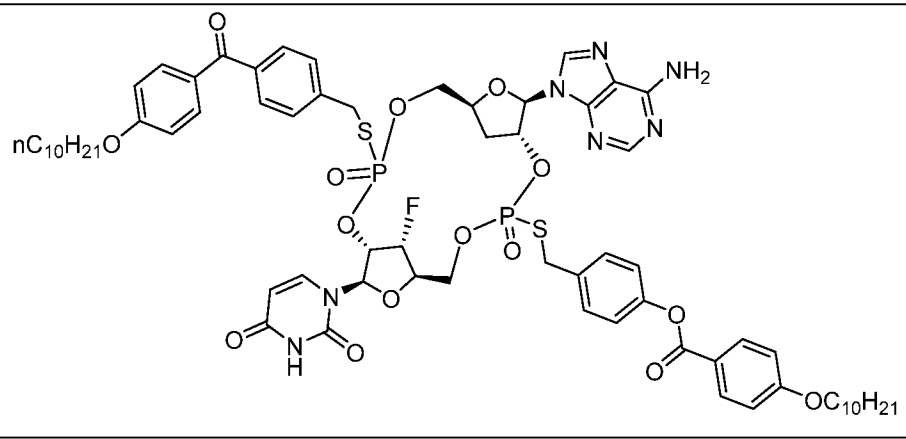
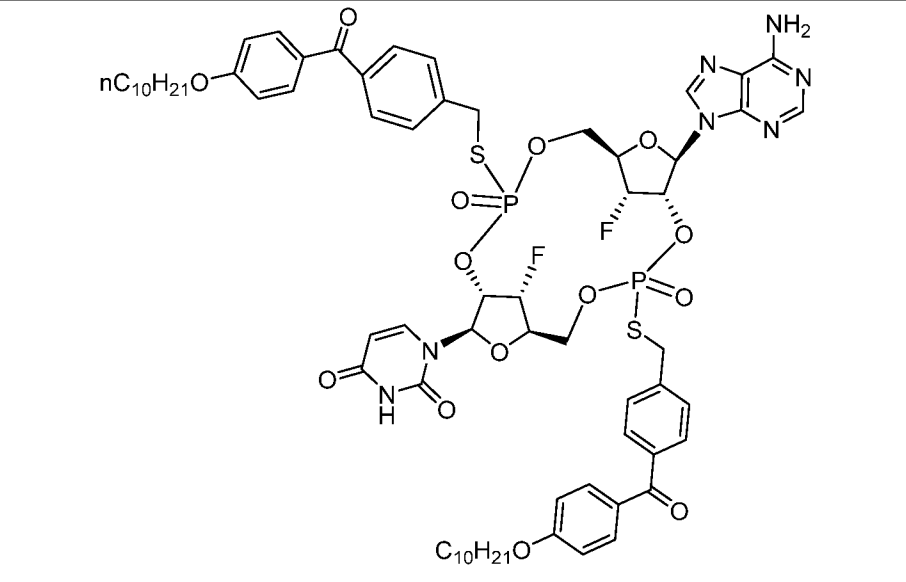
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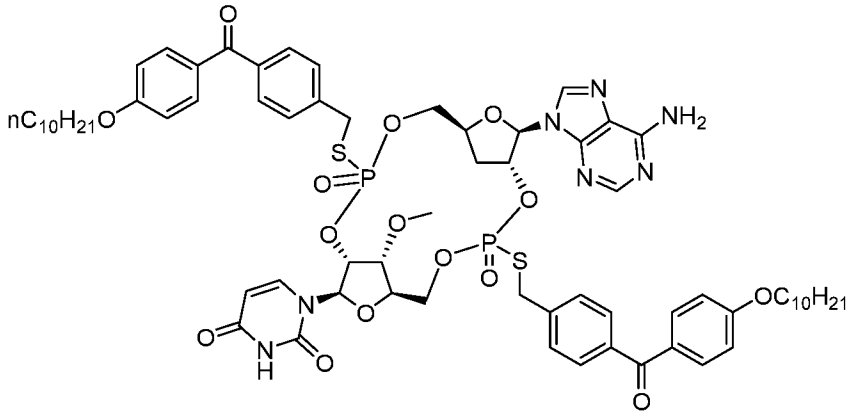
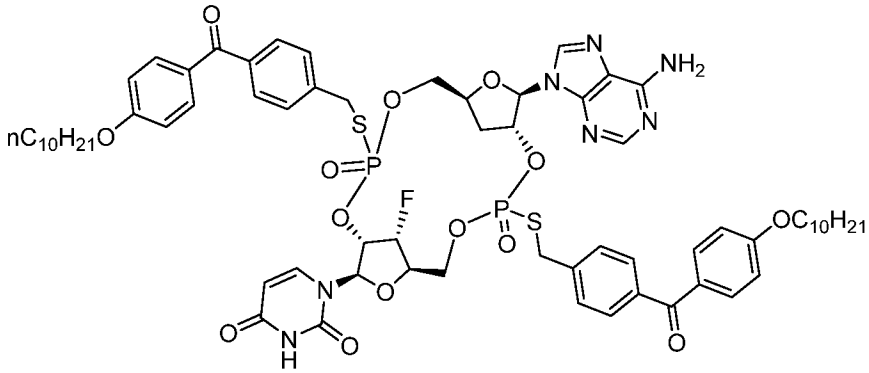
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wherein X is any pharmaceutically acceptable counterion, e.g., lithium, sodium, potassium, calcium, magnesium, aluminum, ammonium, ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like (see, for example, Berge et al., *supra*); and the label “n” indicates that the associated alkyl chain is “normal” (i.e., unbranched). In some embodiments, the compound of Table 1 is not a salt (i.e., the compound is a free acid or free base).

In an embodiment, a compound described herein is in the form of a pharmaceutically acceptable salt. Exemplary salts are described herein, such as ammonium salts. In some embodiments, the compound is a mono-salt. In some embodiments, the compound is a di-salt. In some embodiments, a compound described herein (e.g., a compound in Table 1) is not a salt (e.g., is a free acid or free base).

Without wishing to be bound by theory, a compound of Formula (I) is a small molecule nucleic acid hybrid (cyclic dinucleotide) compound that combines both antiviral and immune modulating activities. The latter activity mediates, for example, controlled apoptosis of virus-infected hepatocytes via stimulation of the innate immune response, similar to what is also achieved by IFN- α therapy in patients suffering from a viral infection. The mechanism of action

of a compound of Formula (I) entails its host immune stimulating activity, which may induce endogenous IFNs via the activation of a PRR, e.g., RIG-I, NOD2, and STING. Activation may occur by binding of a compound of Formula (I) to the nucleotide binding domain of a PRR (e.g., STING), as described previously, and may further result in the induction of PRR expression (e.g., STING expression).

The compounds provided herein may contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereomers, and diastereomeric mixtures. All such isomeric forms of these compounds are expressly included within the scope. Unless otherwise indicated when a compound is named or depicted by a structure without specifying the stereochemistry and has one or more chiral centers, it is understood to represent all possible stereoisomers of the compound. The compounds provided herewith may also contain linkages (e.g., carbon-carbon bonds, phosphorus-oxygen bonds, or phosphorus-sulfur bonds) or substituents that can restrict bond rotation, e.g., restriction resulting from the presence of a ring or double bond. In some embodiments, the compound of Formula (I) comprises an isomer (e.g., an Rp-isomer or Sp isomer) or a mixture of isomers (e.g., Rp-isomers or Sp isomers) of a compound of Formula (I).

Exemplary Methods of Use

The present disclosure relates to methods for inducing the expression of a PRR (e.g., STING) in a subject through administration of an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, the subject may be suffering from a condition described below, e.g., a viral infection (e.g., viral latency), a bacterial infection, a cancer (e.g., a proliferative disease).

Treatment of Viral Infections

Pattern recognition receptors such as STING, RIG-I, and NOD2, have been shown to be an important factor in host recognition of a large number of RNA viruses from a variety of different viral families. In some embodiments, the methods of inducing expression of PRRs (e.g., STING) disclosed herein comprise administration of an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt or stereoisomer thereof to a subject infected with a microbial infection. In some embodiments, the microbial infection is a virus. In some embodiments, the virus is a RNA virus (e.g., a double-stranded RNA (dsRNA) virus, a single-stranded RNA

(ssRNA) virus (e.g., a positive-strand (sense) ssRNA virus or a negative-strand (antisense) ssRNA virus), or a ssRNA retrovirus) or a DNA virus (e.g., a dsDNA virus, ssDNA virus, or a dsDNA retrovirus). In some embodiments, the virus may be a Group I, Group II, Group III, Group IV, Group V, Group VI, or Group VII class of virus, e.g., according to the Baltimore classification system.

In some embodiments, the virus is dsRNA virus, e.g., a Group III virus. In some embodiments, expression of a PRR (e.g., STING) is induced through host-produced or viral-derived RNA. In some embodiments, the virus is a dsRNA virus, and is a member of the *Birnaviridae*, *Chrysoviridae*, *Cystoviridae*, *Endornaviridae*, *Hypoviridae*, *Megabirnaviridae*, *Partitiviridae*, *Picobirnaviridae*, *Reoviridae*, or *Totiviridae* families, or other family of dsRNA virus. Exemplary dsRNA viruses and virus genera include, but are not limited to, *Picobirnavirus*, *Rotavirus*, *Seadornavirus*, *Coltivirus*, *Orbivirus*, and *Orthoreovirus*, or a subtype, species, or variant thereof.

In some embodiments, the virus is ssRNA virus, e.g., a positive-strand (sense) ssRNA virus, e.g., a Group IV virus. In some embodiments, expression of a PRR (e.g., STING) is induced through host-produced or viral-derived RNA. In some embodiments, the virus is a positive-strand (sense) ssRNA virus, and is a member of the *Arteriviridae*, *Coronaviridae*, *Mesoniviridae*, *Roniviridae*, *Dicistroviridae*, *Iflaviridae*, *Marnaviridae*, *Piconaviridae*, *Secoviridae*, *Alphaflexiviridae*, *Betaflexiviridae*, *Gammapflexiviridae*, *Tymoviridae*, *Alphatetraviridae*, *Alvernaviridae*, *Astroviridae*, *Barnaviridae*, *Bromoviridae*, *Caliciviridae*, *Carmotetraviridae*, *Closteroviridae*, *Flaviviridae*, *Leviviridae*, *Luteoviridae*, *Narnaviridae*, *Nodaviridae*, *Permutotetraviridae*, *Potyviridae*, *Togaviridae*, or *Virgaviridae* families, or other family of positive-strand (sense) ssRNA virus. Exemplary positive-strand (sense) ssRNA viruses and virus genera include, but are not limited to, Yellow fever virus, West Nile virus, Hepatitis C virus, Dengue fever virus, Rubella virus, Ross River virus, Sindbis virus, Chikungya virus, Norwalk virus, Japanese encephalitis virus, Tick-borne encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, Kyasanur Forest disease virus (e.g., Monkey disease virus), Western Equine encephalitis virus, Eastern Equine encephalitis virus, Venezuelan Equine encephalitis virus, Sapporo virus, *Norovirus*, *Sapovirus*, *Calicivirus*, *Parechovirus*, Hepatitis A virus, *Rhinovirus* (e.g., Rhinovirus A, Rhinovirus B, and Rhinovirus C), *Enterovirus* (e.g., Enterovirus A, Enterovirus B, Enterovirus C (e.g., poliovirus), Enterovirus D, Enterovirus E,

Enterovirus F, Enterovirus G, or Enterovirus H), *Aphovirus* (e.g., Foot and mouth disease virus), *Nidovirales* (e.g., Cavally virus, Nam Dinh virus, Middle East respiratory syndrome coronavirus (MERS-CoV), Coronavirus HKU1, Coronavirus NL63, SARS-CoV, Coronavirus OC43, and Coronavirus 229E), *Benyvirus*, *Blunavirus*, *Cilevirus*, *Hepevirus* (e.g., Hepatitis E virus), *Higrevirus*, *Idaeovirus*, *Negevirus*, *Ourmiavirus*, *Polemavirus*, *Sobemovirus*, or *Umbravirus*, or a subtype, species, or variant thereof.

In some embodiments, the virus is a member of the genus *Norovirus*, or a subtype, species, or variant thereof. In some embodiments, the virus is the Norwalk virus, Hawaii virus, Snow Mountain virus, Mexico virus, Desert Shield virus, Southampton virus, Lordsdale virus, or Wilkinson virus, or a subtype or variant thereof. In some embodiments, the virus is a member of the genus *Norovirus* and can be classified as genogroup GI, genogroup GII, genogroup GIII, genogroup GIV, or genogroup GV.

In some embodiments, the virus is ssRNA virus, e.g., a negative-strand (antisense) ssRNA virus, e.g., a Group V virus. In some embodiments, expression of a PRR (e.g., STING) is induced through host-produced or viral-derived RNA. In some embodiments, the virus is a negative-strand (antisense) ssRNA virus, and is a member of the *Bornaviridae*, *Filoviridae*, *Paramyxoviridae*, *Rhabdoviridae*, *Nyamiviridae*, *Arenaviridae*, *Bunyaviridae*, *Ophioviridae*, or *Orthomyxoviridae* families, or other family of negative-strand (antisense) ssRNA virus. Exemplary negative-strand (antisense) ssRNA viruses and virus genera include, but are not limited to, Brona disease virus, Ebola virus, Marburg virus, Measles virus, Mumps virus, Nipah virus, Hendra virus, Respiratory syncytial virus, Influenza and Parainfluenza viruses, Metapneumovirus, Newcastle disease virus, *Deltavirus* (e.g., Hepatitis D virus), *Dichohavirus*, *Emaravirus*, *Nyavirus*, *Tenuivirus*, *Varicosavirus*, or a subtype, species, or variant thereof.

In some embodiments, the virus is an ssRNA retrovirus (ssRNA RT virus), e.g., a Group VI virus. In some embodiments, expression of a PRR (e.g., STING) is induced through host-produced or viral-derived RNA. In some embodiments, the virus is an ssRNA RT virus and is a member of the *Metaviridae*, *Pseudoviridae*, or *Retroviridae* families, or other family of ssRNA RT virus. Exemplary ssRNA RT viruses and virus genera include, but are not limited to, *Metavirus*, *Errantivirus*, *Alpharetrovirus* (e.g., Avian leukosis virus, Rous sarcoma virus), *Betaretrovirus* (e.g., Mouse mammary tumor virus), *Gammaretrovirus* (e.g., Murine leukemia virus, Feline leukemia virus), *Deltaretrovirus* (e.g., human T-lymphotropic virus),

Epsilonretrovirus (e.g., Walleye dermal sarcoma virus), *Lentivirus* (e.g., Human immunodeficiency virus 1 (HIV)), or a subtype, species, or variant thereof.

In some embodiments, the virus is a DNA virus, e.g., a dsDNA virus or an ssDNA virus. In some embodiments, the virus is a dsDNA virus, e.g., a Group I virus, and expression of a PRR (e.g., STING) is induced through host-produced or viral-derived RNA. In some embodiments, the virus is a dsDNA virus and is a member of the *Myoviridae*, *Podoviridae*, *Siphoviridae*, *Alloherpesviridae*, *Herpesviridae*, *Malacoherpesviridae*, *Lipothrixviridae*, *Rudiviridae*, *Adenoviridae*, *Ampullaviridae*, *Ascoviridae*, *Asfarviridae*, *Baculoviridae*, *Bicaudaviridae*, *Clavaviridae*, *Corticoviridae*, *Fuselloviridae*, *Globuloviridae*, *Guttaviridae*, *Hytrosaviridae*, *Iridoviridae*, *Marseilleviridae*, *Nimaviridae*, *Pandoraviridae*, *Papillomaviridae*, *Phycodnaviridae*, *Polydnaviruses*, *Polymaviridae*, *Poxviridae*, *Sphaerolipoviridae*, *Tectiviridae*, or *Turriviridae* families, or other family of dsDNA virus. Exemplary dsDNA viruses and virus genera include, but are not limited to, *Dinodnavirus*, *Nudivirus*, smallpox, human herpes virus, Varicella Zoster virus, polyomavirus 6, polyomavirus 7, polyomavirus 9, polyomavirus 10, JC virus, BK virus, KI virus, WU virus, Merkel cell polyomavirus, Trichodysplasia spinulosa-associated polyomavirus, MX polyomavirus, Simian virus 40, or a subtype, species, or variant thereof.

In some embodiments, the virus is an ssDNA virus, e.g., a Group II virus, and expression of a PRR (e.g., STING) is induced through host-produced or viral-derived RNA. In some embodiments, the virus is an ssDNA virus and is a member of the *Anelloviridae*, *Bacillariodnaviridae*, *Bidnaviridae*, *Circoviridae*, *Geminiviridae*, *Inoviridae*, *Microviridae*, *Nanoviridae*, *Parvoviridae*, or *Spiraviridae* families, or other family of ssDNA virus. Exemplary ssDNA viruses and virus genera include, but are not limited to, Torque teno virus, Torque teno midi virus, Torque teno mini virus, *Gyrovirus*, *Circovirus*, Parvovirus B19, *Bocaparvovirus*, *Dependoparvovirus*, *Erythroparvovirus*, *Protoparvovirus*, *Tetraparvovirus*, Bombyx mori densovirus type 2, lymphoid parvo-like virus, Hepatopancreatic parvo-like virus, or a subtype, species, or variant thereof.

In some embodiments, the virus is a dsDNA reverse transcriptase (RT) virus, e.g., a Group VII virus, and expression of a PRR (e.g., STING) is induced through host-produced or viral-derived RNA. In some embodiments, the virus is a dsDNA RT virus and is a member of the *Hepadnaviridae*, or *Caulimoviridae* families, or other family of dsDNA RT virus. Exemplary

dsDNA RT viruses and virus genera include, but are not limited to, Hepatitis B virus, or a subtype, species, or variant thereof.

In some embodiments, the virus (e.g., a virus described herein) is latent, e.g., within a cell. In some embodiments, the virus is an RNA virus (e.g., a double-stranded RNA (dsRNA) virus, a single-stranded RNA (ssRNA) virus (e.g., a positive-strand (sense) ssRNA virus or a negative-strand (antisense) ssRNA virus), or a ssRNA retrovirus) or a DNA virus (e.g., a dsDNA virus, ssDNA virus, or a dsDNA retrovirus) and is latent, e.g., within a cell. In some embodiments, the virus is a Group I, Group II, Group III, Group IV, Group V, Group VI, or Group VII class of virus, e.g., according to the Baltimore classification system, and is latent, e.g., within a cell.

In some embodiments, the virus is an RNA virus (e.g., an RNA virus described herein) and is latent, e.g., within a cell. In some embodiments, the virus is an ssRNA retrovirus (ssRNA RT virus), e.g., a Group VI virus, and is latent, e.g., within a cell. In some embodiments, the virus is the human immunodeficiency virus 1 (HIV)), or a subtype, species, or variant thereof, and is latent, e.g., within a cell.

In some embodiments, the methods of inducing expression of a PRR (e.g., STING) in a subject suffering from a viral infection disclosed herein result in an increase in PRR expression (e.g., STING expression). In some embodiments, expression of a PRR (e.g., STING) is induced by a factor of about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2, about 2.5, about 3, about 4, about 5, about 7.5, about 10, about 15, about 20, about 25, about 30, about 40, about 50, about 75, about 100, about 150, about 200, about 250, about 500, about 1000, about 1500, about 2500, about 5000, about 10,000, or more. In some embodiments, induction of expression of a PRR (e.g., STING) occurs within about 5 minutes of administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In some embodiments, induction of expression of a PRR (e.g., STING) occurs within about 10 minutes, about 15 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 45 minutes, about 1 hour, about 1.5 hours, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 10 hours, about 12 hours or more following administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof to a subject.

Treatment of Bacterial Infections

Recent studies have shown that PRRs (e.g., STING) play a critical role in host recognition of bacterial infections stemming from a variety of species (Dixit, E. and Kagan, J.C. *Adv Immunol* (2013) 117:99-125). In some cases, bacteria may secrete nucleic acids during the exponential growth phase (e.g., *Listeria monocytogenes*; Abdullah, Z. et al, *EMBO J* (2012) 31:4153-4164), which in turn are detected by PRRs such as RIG-I and thus promote the induction of further PRR expression. In other cases, such as for *Legionella pneumophila*, bacterial DNA enters into the cytosol over the course of infection and is transcribed into an RNA ligand for RIG-I (Chiu, Y. H. et al, *Cell* (2009) 138:576-591), thus triggering downstream PRR-mediated signaling events. PRR expression (e.g., STING expression) may further be induced upon recognition of RNA released during phagocytotic uptake of bacteria. Additionally, bacterial cell wall components such as peptidoglycans (e.g., muramyl dipeptide, i.e., MDP) may serve as ligands for activation and induction of PRRs, namely NOD2, and bacterial-derived nucleic acids such as cyclic dinucleotides (e.g., cyclic di-GMP) may bind to and activate PRRs, in particular STING. In some embodiments, the expression of one or more PRRs may be induced through other means not explicitly recited herein.

In some embodiments, the methods of inducing expression of a PRR (e.g., STING) disclosed herein comprise administration of an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof to a subject infected with a microbial infection, e.g., a bacterial infection.

In some embodiments, the bacterium is a Gram-negative bacterium or a Gram-positive bacterium. Exemplary bacteria include, but are not limited to, *Listeria* (e.g., *Listeria monocytogenes*), *Francisella* (e.g., *Francisella tularensis*), *Mycobacteria* (e.g., *Mycobacteria tuberculosis*), *Brucella* (e.g., *Brucella abortus*), *Streptococcus* (e.g., group B *Streptococcus*), *Legionella* (e.g., *Legionella pneumophila*), *Escherichia* (e.g., *Escherichia coli*), *Pseudomonas* (e.g., *Pseudomonas aeruginosa*), *Salmonella* (e.g., *Salmonella typhi*), *Shigella* (e.g., *Shigella flexneri*), *Campylobacter* (e.g., *Campylobacter jejuni*), *Clostridium* (e.g., *Clostridium botulinum*), *Enterococcus* (e.g., *Enterococcus faecalis*), *Vibrio* (e.g., *Vibrio cholera*), *Yersinia* (e.g., *Yersinia pestis*), *Staphylococcus* (e.g., *Staphylococcus aureus*), or other genera, species, subtypes, or variants thereof.

In some embodiments, the methods of inducing expression of a PRR (e.g., STING) in a subject suffering from a bacterial infection disclosed herein result in an increase in PRR expression

(e.g., STING expression). In some embodiments, expression of a PRR (e.g., STING) is induced by a factor of about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2, about 2.5, about 3, about 4, about 5, about 7.5, about 10, about 15, about 20, about 25, about 30, about 40, about 50, about 75, about 100, about 150, about 200, about 250, about 500, about 1000, about 1500, about 2500, about 5000, about 10,000, or more. In some embodiments, induction of expression of a PRR (e.g., STING) occurs within about 5 minutes of administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In some embodiments, induction of expression of a PRR (e.g., STING) occurs within about 10 minutes, about 15 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 45 minutes, about 1 hour, about 1.5 hours, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 10 hours, about 12 hours or more following administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

Treatment of Cancer

Many patients with advanced solid tumors show a spontaneous T cell-inflamed tumor microenvironment, which is predictive of prognosis and clinical response to immunotherapies. Recent findings suggest the STING pathway of cytosolic DNA sensing is an important innate immune sensing mechanism driving type I IFN production in the tumor context. Knowledge of this pathway is guiding the further development of novel immunotherapeutic strategies.

In early-stage colorectal cancer, the presence of activated CD8⁺ T cells within the tumor microenvironment is prognostic of a positive outcome. Patients with other solid tumor histology also appear to have a spontaneous T cell infiltrate that may have similar positive prognostic value. These include breast cancer, renal cell carcinoma, melanoma, ovarian cancer, and gastrointestinal tumors. It is believed that T cell infiltrate includes tumor antigen-specific T cells that have been activated spontaneously in response to the growing tumor, perhaps through immune surveillance mechanisms. This attempted host immune response, even if it does not eliminate the tumor completely, is thought to delay tumor progression and thus yield improved clinical outcome. Furthermore, the innate immune mechanisms can lead to adaptive T cell response against tumor antigens even in the absence of exogenous infection. In this regard, human cancer gene expression profiling studies reveal an association between a type I IFN signature, T cell infiltration, and clinical outcome. Thus, innate immune sensing pathways that trigger type I IFN production might represent crucial intermediate mechanistic step. In gene expression profiling of melanoma, two

major subsets of tumor microenvironment have been found that represent either the presence or absence of a transcriptional profile indicative of T cell infiltrate. In fact, CD8⁺ T cells, macrophages, as well as of some B cells and plasma cells in these lesions in melanoma metastases is similar to the phenotype described in early-stage colon cancer and other tumors in which activated T cells have been associated with favorable prognosis. CD8⁺ T cells were required for the up-regulation of all immune factors within the tumor micro-environment. Studies indicate that IFN production is necessary for optimal T cell priming against tumor antigens. There are many PRRs that trigger IFN- β production by host DCs in response to a growing tumor in vivo including STING. STING is an adapter protein that is activated by cyclic dinucleotides generated by cyclic GMP-AMP synthase (cGAS), which in turn is directly activated by cytosolic DNA. In the presence of these cyclic dinucleotides and/or DNA, STING is translocated from the endoplasmic reticulum to various perinuclear components; for example, palmitoylation of STING at the Golgi has been shown to be essential for STING activation (Mukai, K. et al (2016) *Nat Commun* doi:10.1038/ncomms11932).

Activated STING forms aggregates, activates TBK1, which in turn phosphorylates interferon regulatory factor 3 (IRF3) that directly contributes to type I IFN gene transcription. This pathway has been implicated in the sensing of DNA viruses, and also in selected autoimmune models. Moreover, activating mutations of STING have recently been identified in human patients with a vasculitis/pulmonary inflammation syndrome that is characterized by increased type I IFN production. Mechanistic studies using mouse transplantable tumor models revealed that STING-knockout mice, and IRF3-knockout mice showed defective spontaneous T cell priming against tumor antigens in vivo, and rejection of immunogenic tumors was ablated. Similarly, tumor-derived DNA was found within the cytosol of a major population of tumor-infiltrating DCs, and this was associated with STING pathway activation and IFN- β production. Therefore, the host STING pathway appears to be an important innate immune sensing pathway that detects the presence of a tumor and to drive DC activation and subsequent T cell priming against tumor-associated antigens in vivo. A functional role for the STING pathway in vivo has also been reported in other mouse-tumor systems. An inducible glioma model was shown to result in induction of a type I IFN gene signature as part of the host response. This induction was substantially reduced in STING-knockout mice, and tumors grew more aggressively, leading to shorter mouse survival. Exogenous delivery of cyclic dinucleotides as STING agonists exerted a

therapeutic effect in vivo. A crucial role for host type I IFNs and the host STING pathway was also confirmed in the B16.OVA and EL4.OVA models in response to cryo-ablation. Interestingly, the mechanisms involved paralleled what was observed in the Bm12 mouse model of lupus because host STING was also required for maximal production of anti-DNA antibodies. Thus, the antitumor immune response triggered in part by tumor DNA has overlap with the mechanisms involved in autoimmunity driven by extracellular DNA. A role for STING also has been explored in an inducible colon cancer model. It seems likely that the ability of a cancer in an individual patient to support STING pathway activation is linked to the spontaneous generation of a T cell-inflamed tumor microenvironment. Because this phenotype is associated with improved prognosis of early-stage cancer patients, and also with clinical response to immunotherapies in the metastatic setting, failed STING activation may therefore represent an early functional block, and thus itself may have prognostic/predictive value as a biomarker. Second, strategies that activate or mimic the output of the host STING pathway should have immunotherapeutic potential in the clinic. In as much as non-T cell-inflamed tumors appear to lack evidence of a type I IFN transcriptional signature, strategies to promote robust innate signaling via APCs in the tumor microenvironment might facilitate improved cross-priming of tumor antigen-specific CD8⁺ T cells, and also augment chemokine production for subsequent oncolytic activity.

Recognition of nucleic acid ligands by a PRRs such as cGAS, RIG-I and/STING stimulates the production of type I interferons (e.g., IFN- α or IFN- β), thus triggering a series of downstream signaling events that may lead to apoptosis in susceptible cells. In recent years, a connection between the induction of PRR expression and a number of cancers has been discovered. For example, RIG-I expression has been shown to be significantly downregulated in hepatocellular carcinoma, and patients exhibiting low RIG-I expression in tumors had shorter survival and poorer responses to IFN- α therapy (Hou, J. et al, *Cancer Cell* (2014) 25:49-63). As such, it has been suggested that the level of RIG-I expression may be useful as a biomarker for prediction of prognosis and response to immunotherapy. In other cases, induction of RIG-I expression has been shown to induce immunogenic cell death of pancreatic cancer cells, prostate cancer cells, breast cancer cells, skin cancer cells, and lung cancer cells (Duewell, P. et al, *Cell Death Differ* (2014) 21:1825-1837; Besch, R. et al, *J Clin Invest* (2009) 119:2399-2411; Kaneda, Y. *Oncoimmunology* (2013) 2:e23566; Li, X.Y. et al, *Mol Cell Oncol* (2014) 1:e968016), highlighting a new approach in immune-mediated cancer treatment.

STING is recognized as the key adapter protein in the cGAS-STING-IFN cascade, although it is also reported to be a sensor for DNA. A role for STING in the stimulation of innate immunity in response to cancer has also been identified. Recent studies have revealed the presence of tumor-derived DNA in the cytosol of certain antigen-presenting cells, such as tumor-infiltrating dendritic cells, likely generated through tumor cell stress or cell death. This tumor-derived DNA is known to activate cGAS which causes the production of cyclic nucleotides that have been shown to activate STING, resulting in production of associated type 1 interferons (Woo, S.R. et al, *Immunity* (2014) 41:830-842). Stimulation of STING and resulting downstream signaling pathways also likely contributes to effector T cell recruitment into the inflamed tumor microenvironment (Woo, S. R. *Trends in Immunol* (2015) 36:250-256). STING activation in the tumor microenvironment can induce adaptive immune response leading to anti-tumor activity. Hence, in those tumors that are STING-deficient, the described herein can still have anti-tumor activity through activation of antigen-presenting cells and dendritic cells, (APCs and DCs) and induction of adaptive immune response.

In some embodiments, the methods of inducing expression of a PRR (e.g., a PRR described herein) comprise administration of an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof to a subject suffering from cancer. In some embodiments, the methods of inducing expression of STING disclosed herein comprise administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof to a subject suffering from cancer. In some embodiments, the methods of inducing expression of RIG-I disclosed herein comprise administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof to a subject suffering from cancer. In some embodiments, the methods of inducing expression of NOD2 disclosed herein comprise administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof to a subject suffering from cancer. In some embodiments, the cancer is selected from a cancer of the breast, bone, brain, cervix, colon, gastrointestinal tract, eye, gall bladder, lymph nodes, blood, lung, liver, skin, mouth, prostate, ovary, penis, pancreas, uterus, testicles, stomach, thymus, thyroid, or other part of the body. In some embodiments, the cancer comprises a solid tumor (e.g., a carcinoma, a sarcoma, or a lymphoma). In some embodiments, the cancer is a hepatocellular carcinoma or other cancer of the liver. In some embodiments, the cancer is a leukemia or other cancer of the blood. In some embodiments, the cancer comprises breast cancer, renal cell carcinoma, colon cancer, melanoma, ovarian cancer,

head and neck squamous cell carcinoma, pancreatic cancer, prostate cancer, lung cancer, brain cancer, thyroid cancer, renal cancer, testis cancer, stomach cancer, urothelial cancer, skin cancer, cervical cancer, endometrial cancer, liver cancer, lung cancer, lymphoma or gastrointestinal stromal cancer and solid tumors. In some embodiments, the cancer cells (e.g., tumor cells) comprise specific cancer-associated antigens that induce a T-cell-mediated anti-tumor response.

In some embodiments, the methods of inducing expression of a PRR (e.g., STING, RIG-I, MDA5, LGP2) in a subject suffering from a cancer disclosed herein result in an increase in PRR expression (e.g., STING expression). In some embodiments, expression of a PRR (e.g., STING) is induced by a factor of about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2, about 2.5, about 3, about 4, about 5, about 7.5, about 10, about 15, about 20, about 25, about 30, about 40, about 50, about 75, about 100, about 150, about 200, about 250, about 500, about 1000, about 1500, about 2500, about 5000, about 10,000, or more. In some embodiments, induction of expression of a PRRs (e.g., STING) occurs within about 5 minutes of administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In some embodiments, induction of expression of a PRRs (e.g., STING) occurs within about 5 minutes of administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In some embodiments, induction of expression of a PRR (e.g., STING) occurs within about 10 minutes, about 15 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 45 minutes, about 1 hour, about 1.5 hours, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 10 hours, about 12 hours or more following administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. It is recognized that activation of STING by compounds may lead to induction of expression of other PRRs such as RIG-I, MDA5, NOD2 etc. which may further amplify IFN production in the tumor microenvironment and prime T-cells for enhanced anti-tumor activity.

In some embodiments, the methods of inducing expression of a PRR (e.g., STING) in a subject suffering from a cancer disclosed herein result in an increase in PRR expression (e.g., STING expression). In some embodiments, expression of a PRR (e.g., STING) is induced by a factor of about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2, about 2.5, about 3, about 4, about 5, about 7.5, about 10, about 15, about 20, about 25, about 30, about 40, about 50, about 75, about 100, about 150, about 200, about 250, about 500, about 1000, about 1500, about 2500, about 5000, about 10,000, or more. In some embodiments,

induction of expression of a PRR (e.g., STING) occurs within about 5 minutes of administration of a compound of Formula (I) or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, induction of expression of a PRR (e.g., STING) occurs within about 10 minutes, about 15 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 45 minutes, about 1 hour, about 1.5 hours, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 10 hours, about 12 hours or more following administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

Pharmaceutical Compositions

While a compound of the present disclosure (e.g., a compound of Formula (I)) may be administered alone, it is preferable to administer said compound as a pharmaceutical composition or formulation, where the compounds are combined with one or more pharmaceutically acceptable diluents, excipients or carriers. The compounds according to the disclosure may be formulated for administration in any convenient way for use in human or veterinary medicine. In certain embodiments, the compounds included in the pharmaceutical preparation may be active itself, or may be a prodrug, *e.g.*, capable of being converted to an active compound in a physiological setting. Regardless of the route of administration selected, the compounds of the present disclosure, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present disclosure, are formulated into a pharmaceutically acceptable dosage form such as described below or by other conventional methods known to those of skill in the art.

The amount and concentration of compounds of the present disclosure (e.g., a compound of Formula (I)) in the pharmaceutical compositions, as well as the quantity of the pharmaceutical composition administered to a subject, can be selected based on clinically relevant factors, such as medically relevant characteristics of the subject (*e.g.*, age, weight, gender, other medical conditions, and the like), the solubility of compounds in the pharmaceutical compositions, the potency and activity of the compounds, and the manner of administration of the pharmaceutical compositions. For further information on Routes of Administration and Dosage Regimes the reader is referred to Chapter 25.3 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

Thus, another aspect of the present disclosure provides pharmaceutically acceptable compositions comprising a therapeutically effective amount or prophylactically effective amount of a compound described herein (e.g., a compound of Formula (I)), formulated together with one

or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the present disclosure may be specially formulated for administration in solid or liquid form, including those adapted for oral, intratumoral, parenteral administration, for example, by subcutaneous, intramuscular, intraperitoneal, or intravenous injection as, for example, a sterile solution or suspension. However, in certain embodiments the subject compounds may be simply dissolved or suspended in sterile water. In certain embodiments, the pharmaceutical preparation is non-pyrogenic, i.e., does not elevate the body temperature of a patient.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of the compound other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, stabilizing agent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject antagonists from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13)

agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) ascorbic acid; (17) pyrogen-free water; (18) isotonic saline; (19) Ringer's solution; (20) ethyl alcohol; (21) phosphate buffer solutions; (22) cyclodextrins such as Captisol®; and (23) other non-toxic compatible substances such as antioxidants and antimicrobial agents employed in pharmaceutical formulations.

As set out above, certain embodiments of the compounds described herein may contain a basic functional group, such as an amine, and are thus capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. The term "pharmaceutically acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present disclosure. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the disclosure, or by separately reacting a purified compound of the disclosure in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like (see, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

In other cases, the compounds of the present disclosure may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of the compound of the present disclosure (e.g., a compound of Formula (I)). These salts can likewise be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like (see, for example, Berge et al., *supra*).

Wetting agents, emulsifiers, and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

The pharmaceutically acceptable carriers, as well as wetting agents, emulsifiers, lubricants, coloring agents, release agents, coating agents, sweetening, flavoring agents, perfuming agents, preservatives, antioxidants, and other additional components may be present in an amount between about 0.001% and 99% of the composition described herein. For example, said pharmaceutically acceptable carriers, as well as wetting agents, emulsifiers, lubricants, coloring agents, release agents, coating agents, sweetening, flavoring agents, perfuming agents, preservatives, antioxidants, and other additional components may be present from about 0.005%, about 0.01%, about 0.05%, about 0.1%, about 0.25%, about 0.5%, about 0.75%, about 1%, about 1.5%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 85%, about 90%, about 95%, or about 99% of the composition described herein.

Pharmaceutical compositions of the present disclosure may be in a form suitable for oral administration, e.g., a liquid or solid oral dosage form. In some embodiments, the liquid dosage form comprises a suspension, a solution, a linctus, an emulsion, a drink, an elixir, or a syrup. In some embodiments, the solid dosage form comprises a capsule, tablet, powder, dragée, or powder. The pharmaceutical composition may be in unit dosage forms suitable for single administration of precise dosages. Pharmaceutical compositions may comprise, in addition to the compound described herein (e.g., a compound of Formula (I)) or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable carrier, and may optionally further comprise one or more pharmaceutically acceptable excipients, such as, for example, stabilizers (e.g., a binder, e.g., polymer, e.g., a precipitation inhibitor, diluents, binders, and lubricants.

In some embodiments, the composition described herein comprises a liquid dosage form for oral administration, e.g., a solution or suspension. In other embodiments, the composition described herein comprises a solid dosage form for oral administration capable of being directly compressed into a tablet. In addition, said tablet may include other medicinal or pharmaceutical agents, carriers, and or adjuvants. Exemplary pharmaceutical compositions include compressed tablets (e.g., directly compressed tablets), e.g., comprising a compound of the present disclosure (e.g., a compound of Formula (I)) or a pharmaceutically acceptable salt thereof.

Formulations of the present disclosure include those suitable for parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about 99 percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent. Pharmaceutical compositions of this disclosure suitable for parenteral administration comprise compounds of the disclosure in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben,

chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a compound of the present disclosure (e.g., a compound of Formula (I)), it may be desirable to slow the absorption of the drug from subcutaneous, intraperitoneal, or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered form of the compound of the present disclosure is accomplished by dissolving or suspending compound in an oil vehicle.

In some embodiments, it may be advantageous to administer the compound of the present disclosure (e.g., a compound of Formula (I)) in a sustained fashion. It will be appreciated that any formulation that provides a sustained absorption profile may be used. In certain embodiments, sustained absorption may be achieved by combining a compound of the present disclosure with other pharmaceutically acceptable ingredients, diluents, or carriers that slow its release properties into systemic circulation.

Routes of Administration

The compounds and compositions used in the methods described herein may be administered to a subject in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. Exemplary routes of administration of the compositions used in the methods described herein include topical, enteral, or parenteral applications. Topical applications include but are not limited to epicutaneous, inhalation, enema, eye drops, ear drops, and applications through mucous membranes in the body. Enteral applications include oral administration, rectal administration, vaginal administration, and gastric feeding tubes. Parenteral administration includes intravenous, intraarterial, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intrasternal, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal, and topical modes of administration. Parenteral administration may be by continuous infusion over a selected period of time. In certain

embodiments of the disclosure, a composition described herein comprising a compound of Formula (I) is administered orally. In other embodiments of the disclosure, a composition described herein comprising a compound of Formula (I) is administered parenterally (e.g., intraperitoneally). It is recognized that for treatment of solid tumors, direct injection of the compounds into the tumor may also be carried out (e.g., intratumoral administration). It is recognized that for treatment of solid tumors, direct injection of the compounds into the tumor may also be carried out (e.g., intratumoral administration).

For intravenous, intraperitoneal, or intrathecal delivery or direct injection (e.g., intratumoral), the composition must be sterile and fluid to the extent that the composition is deliverable by syringe. In addition to water, the carrier can be an isotonic buffered saline solution, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition. Long-term absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

The choice of the route of administration will depend on whether a local or systemic effect is to be achieved. For example, for local effects, the composition can be formulated for topical administration and applied directly where its action is desired. For systemic, long term effects, the composition can be formulated for enteral administration and given via the digestive tract. For systemic, immediate and/or short term effects, the composition can be formulated for parenteral administration and given by routes other than through the digestive tract.

Dosages

The compositions of the present disclosure are formulated into acceptable dosage forms by conventional methods known to those of skill in the art. Actual dosage levels of the active ingredients in the compositions of the present disclosure (e.g., a compound of Formula (I)) may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, without being toxic to the subject. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed,

the route of administration, the time of administration, the rate of absorption of the particular agent being employed, the duration of the treatment, other drugs, substances, and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors well known in the medical arts. A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the composition required. For example, the physician or veterinarian can start doses of the substances of the disclosure employed in the composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a composition of the disclosure will be that amount of the substance which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Preferably, the effective daily dose of a therapeutic composition may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

Preferred therapeutic dosage levels are between about 0.1 mg/kg to about 1000 mg/kg (*e.g.*, about 0.2 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 350 mg/kg, 400 mg/kg, 450 mg/kg, 500 mg/kg, 600 mg/kg, 700 mg/kg, 800 mg/kg, 900 mg/kg, or 1000 mg/kg) of the composition per day administered (*e.g.*, orally or intraperitoneally) to a subject afflicted with the disorders described herein (*e.g.*, HBV infection). Preferred prophylactic dosage levels are between about 0.1 mg/kg to about 1000 mg/kg (*e.g.*, about 0.2 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 350 mg/kg, 400 mg/kg, 450 mg/kg, 500 mg/kg, 600 mg/kg, 700 mg/kg, 800 mg/kg, 900 mg/kg, or 1000 mg/kg) of the composition per day administered (*e.g.*, orally or intraperitoneally) to a subject. The dose may also be titrated (*e.g.*, the dose may be escalated gradually until signs of toxicity appear, such as headache, diarrhea, or nausea).

The frequency of treatment may also vary. The subject can be treated one or more times per day (*e.g.*, once, twice, three, four or more times) or every so-many hours (*e.g.*, about every 2,

4, 6, 8, 12, or 24 hours). The composition can be administered 1 or 2 times per 24 hours. The time course of treatment may be of varying duration, *e.g.*, for two, three, four, five, six, seven, eight, nine, ten, or more days, two weeks, 1 month, 2 months, 4 months, 6 months, 8 months, 10 months, or more than one year. For example, the treatment can be twice a day for three days, twice a day for seven days, twice a day for ten days. Treatment cycles can be repeated at intervals, for example weekly, bimonthly or monthly, which are separated by periods in which no treatment is given. The treatment can be a single treatment or can last as long as the life span of the subject (*e.g.*, many years).

Patient Selection and Monitoring

The methods of the present disclosure described herein entail administration of a compound of Formula (I) or a pharmaceutically acceptable salt thereof to a subject to activate the PRR for IFNs, ISGs and cytokines production or additionally induce the expression of PRRs (*e.g.*, RIG-I, STING etc.). In some embodiments, the subject is suffering from or is diagnosed with a condition, *e.g.*, a proliferative disease, *e.g.*, cancer. Accordingly, a patient and/or subject can be selected for treatment using a compound of Formula (I) or a pharmaceutically acceptable salt thereof by first evaluating the patient and/or subject to determine whether the subject is infected with a proliferative disease, *e.g.*, cancer. A subject can be evaluated as infected with a proliferative disease (*e.g.*, cancer) using methods known in the art. The subject can also be monitored, for example, subsequent to administration of a compound described herein (*e.g.*, a compound of Formula (I) or a pharmaceutically acceptable salt thereof).

In some embodiments, the subject is a mammal. In some embodiments, the subject is a human. In some embodiments, the subject is an adult. In some embodiments, the subject has a proliferative disease, *e.g.*, cancer. In some embodiments, the subject has a cancer of the of the breast, bone, brain, cervix, colon, gastrointestinal tract, eye, gall bladder, lymph nodes, blood, lung, liver, skin, mouth, prostate, ovary, penis, pancreas, uterus, testicles, stomach, thymus, thyroid, or other part of the body. In some embodiments, the subject has a cancer comprising a solid tumor (*e.g.*, a carcinoma, a sarcoma, or a lymphoma). In some embodiments, the subject has a hepatocellular carcinoma or other cancer of the liver. In some embodiments, the subject has a leukemia or other cancer of the blood. In some embodiments, the subject has a breast cancer, renal cell carcinoma, colon cancer, melanoma, ovarian cancer, head and neck squamous cell carcinoma, pancreatic cancer, prostate cancer, lung cancer, brain cancer, or gastrointestinal stromal cancer. In

some embodiments, the subject has cancer cells (e.g., tumor cells) comprising specific cancer-associated antigens that induce a T-cell response.

In some embodiments, the subject is treatment naïve. In some embodiments, the subject has been previously treated for a proliferative disease (e.g., a cancer). In some embodiments, the subject has relapsed.

Combination Therapies

A compound described herein may be used in combination with other known therapies. Administered “in combination”, as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, e.g., the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as “simultaneous” or “concurrent delivery”. In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

A compound described herein and the at least one additional therapeutic agent can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the compound described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed.

In some embodiments, the combination of a compound of Formula (I) or a pharmaceutically acceptable salt thereof and the additional agent has a synergistic or additive effect. In some embodiments, the term “additive” refers to an outcome wherein when two agents

are used in combination, the combination of the agents acts in a manner equal to but not greater than the sum of the individual activity of each agent.

In some embodiments, the term “additive” refers to an outcome wherein when two agents are used in combination, the combination of the agents acts in a manner equal to but not greater than the sum of the individual activity of each agent. In some embodiments, the terms “synergy” or “synergistic” refer to an outcome wherein when two agents are used in combination, the combination of the agents acts so as to require a lower concentration of each individual agent than the concentration required to be efficacious in the absence of the other agent. In some embodiments, a synergistic effect results in a reduced in a reduced minimum inhibitory concentration of one or both agents, such that the effect is greater than the sum of the effects. A synergistic effect is greater than an additive effect. In some embodiments, the agents in the composition herein may exhibit a synergistic effect, wherein the activity at a particular concentration is greater than at least about 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 10, 12, 15, 20, 25, 50, or 100 times the activity of either agent alone.

For example, any of the methods described herein may further comprise the administration of a therapeutically effective amount of an additional agent. Exemplary additional pharmaceutical agents include, but are not limited to, anti-proliferative agents, anti-cancer agents, anti-diabetic agents, anti-inflammatory agents, immunosuppressant agents, and a pain-relieving agent. Pharmaceutical agents include small organic molecules such as drug compounds (*e.g.*, compounds approved by the U.S. Food and Drug Administration as provided in the Code of Federal Regulations (CFR)), peptides, proteins, carbohydrates, monosaccharides, oligosaccharides, polysaccharides, nucleoproteins, mucoproteins, lipoproteins, synthetic polypeptides or proteins, small molecules linked to proteins, glycoproteins, steroids, nucleic acids, DNAs, RNAs, nucleotides, nucleosides, oligonucleotides, antisense oligonucleotides, lipids, hormones, vitamins, and cells. In some embodiments, the additional agent is an anti-cancer agent, *e.g.*, an alkylating agent (*e.g.*, cyclophosphamide).

In an embodiment, the additional agent is an immunooncology agent, for example, an agent that activate the immune system, *e.g.*, making it able to recognize cancer cells and destroy them. Exemplary immunooncology compounds are compounds that inhibit the immune checkpoint blockade pathway. In an embodiment, the compound is an antibody such as a PD-1 or PD-L1

antibody or a co-stimulatory antibody. In some embodiments, the compound is an anti-CTLA4 antibody. In another embodiment, the agent is a cell based agent such as CAR-t therapy.

EXAMPLES

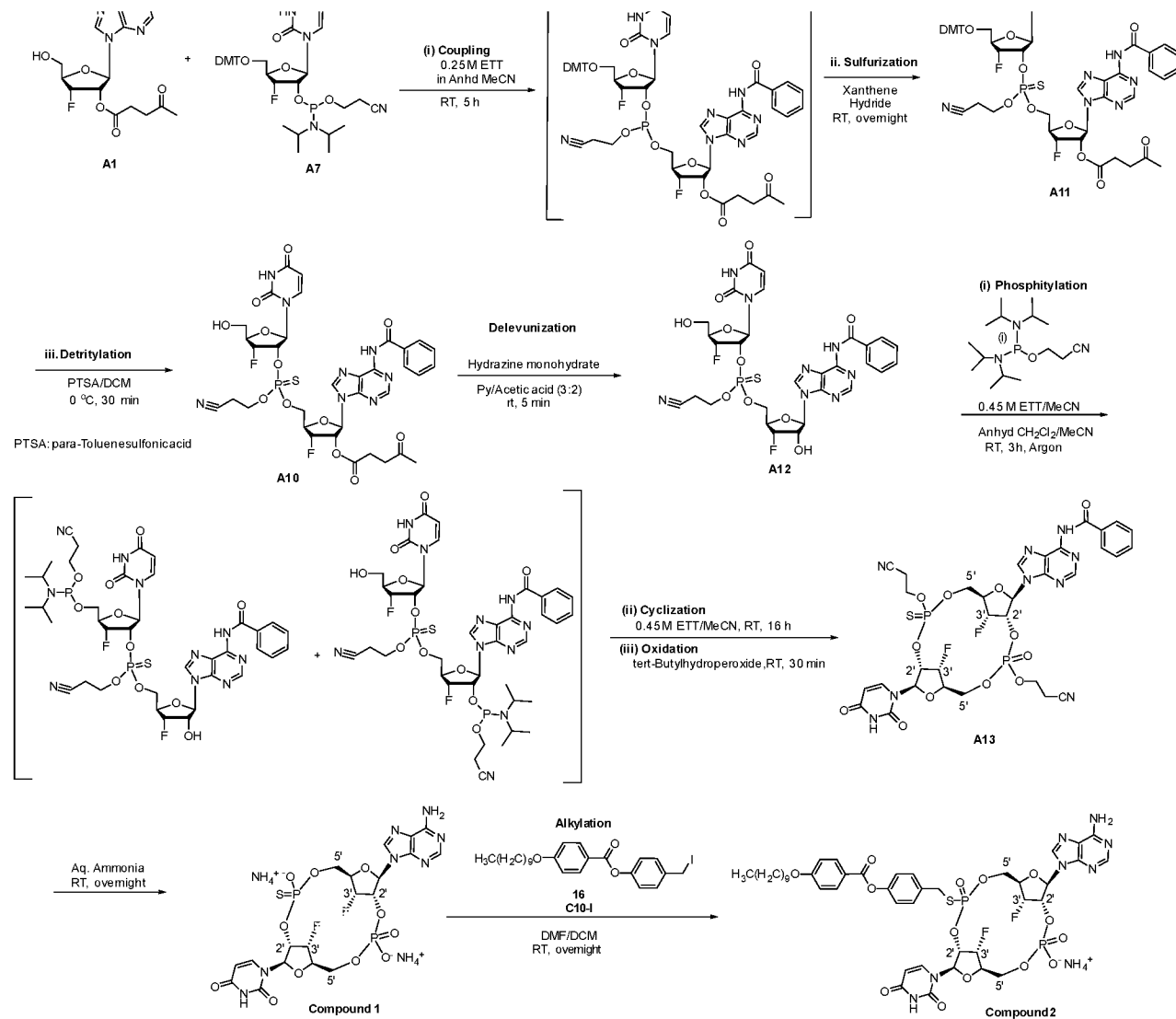
The disclosure is further illustrated by the following examples and synthesis schemes, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby. It is to be further understood that resort may be had to various other embodiments, modifications, and equivalents thereof which may suggest themselves to those skilled in the art without departing from the spirit of the present disclosure and/or scope of the appended claims.

Abbreviations used in the following examples and elsewhere herein are:

3H-BD	Iyer-Beaucage reagent
Ac	Acetyl
DCA	dichloroacetic acid
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMT	Dimethoxytrityl
EtOAc	Ethyl acetate
ETT	5-(ethylthio)-1 <i>H</i> -tetrazole
h	hours
IPA	isopropyl alcohol
LCMS	liquid chromatography-mass spectrometry
MeOH	methanol
MSNT	1-Mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole
PTSA	p-Toluenesulfonic acid
Py	Pyridine
r.t.	room temperature
TBHP	Tert-butyl hydroperoxide
TEA	Triethylamine

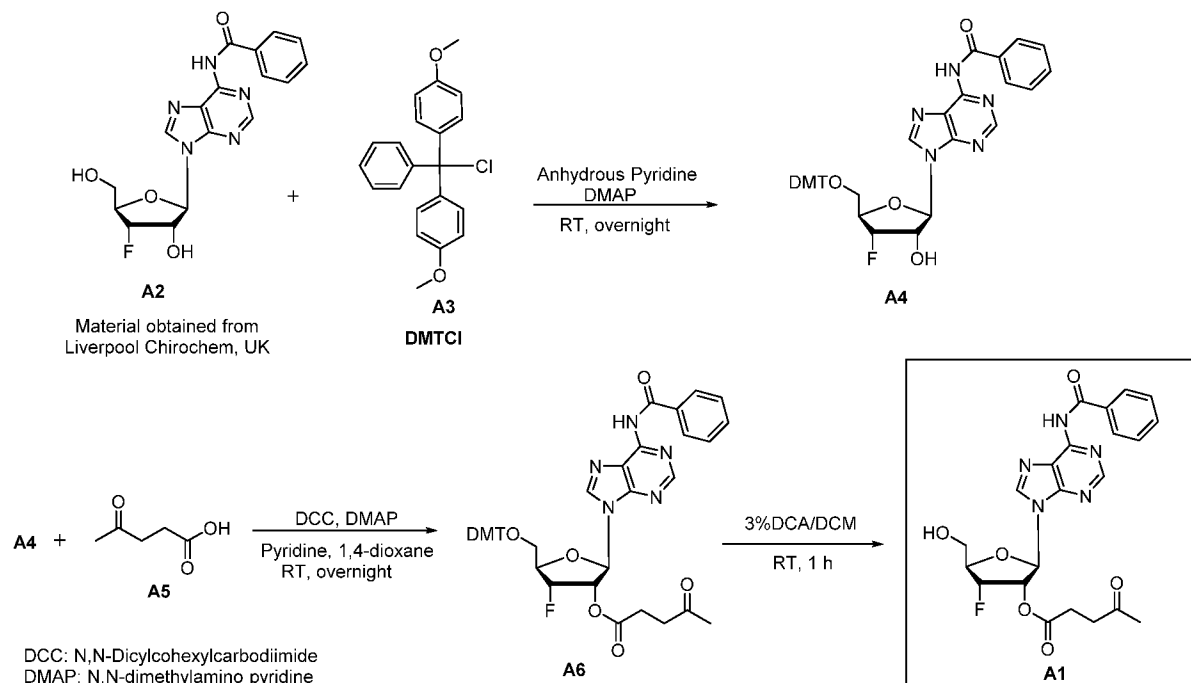
THF	tetrahydrofuran
TLC	thin-layer chromatography

Example 1: Exemplary Preparation of Compounds of the Disclosure



Synthesis of Exemplary Building Blocks

Exemplary Synthesis of Intermediate A1



Exemplary Synthesis of Intermediate A2

DMTCl (**3**, 1.1798 g, 0.0035 mmol, 1.3 equiv.) was added to a solution of nucleoside (**A2**, 1.0000 g, 0.0027 mmol, 1 equiv.) in 20 mL anhydrous pyridine. A catalytic amount of DMAP (0.0160 g, 5 mol%) was added to the reaction mixture and stirred overnight under argon. The reaction mixture was quenched with saturated NaHCO_3 , concentrated to remove pyridine, and coevaporated with toluene. The residue was dissolved in methylene chloride (CH_2Cl_2), and the resulting solution was washed with water three times, followed by brine. The organic extract was dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product thus obtained was absorbed over silica gel in the presence of 3% triethylamine and purified by combiflash from 0–3% CH_2Cl_2 to afford 1.3900 g (77%) of pure tritylated nucleoside **A4**. The Combiflash cartridge was equilibrated with 2% Et_3N in CH_2Cl_2 , prior to loading of compound.

Exemplary Synthesis of Intermediate A1

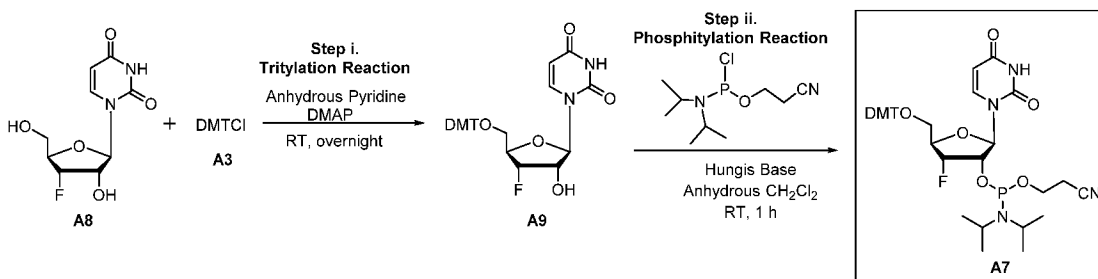
A5 (0.9940 g, 0.0062 mol, 3 equiv.) was dissolved in anhydrous dioxane (15 mL) in a pre-dried 100 mL round-bottom flask under argon. To this solution N,N'-dicyclohexylcarbodiimide (DCC, 0.6310 g, 0.0031 mole, 1.5 equiv.) was added. Another portion of dioxane (10 mL) was added, and the reaction mixture was stirred at room temperature for 2 h. The mixture was filtered

through an oven dried and cooled under argon filtration setup, and the precipitate was washed with dry dioxane (10 mL). The filtrate was added to a solution of **A4** (1.3800 g, 0.0020 mol, 1 equiv.) in anhydrous pyridine (10 mL) followed by the addition of a catalytic amount of DMAP (0.0122 g, 5 mol%) under argon. The reaction mixture was stirred overnight at room temperature and monitored by TLC (6% MeOH/CH₂Cl₂) for the completion of esterification. The reaction mixture was concentrated under vacuum and evaporated with toluene (3 × 50 mL). The residue was dissolved in CH₂Cl₂ (100 mL) and washed with water (2 × 30 mL), followed by brine (30 mL), filtered, and concentrated to obtain **A6**.

Detritylation

A6 was taken forward for the detritylation reaction. Approximately 50 mL of 3% dichloroacetic acid (DCA)/CH₂Cl₂ was added to the crude residue, followed by adding 0.8 mL of triethylsilane. The reaction mixture was stirred for 1 h. The progress of the reaction was monitored by TLC using 6% MeOH/CH₂Cl₂. At the end of the reaction, water (50 mL) was added and the solution was stirred. The organic and aqueous layers separated, and the organic layer was washed with water (125 mL), NaHCO₃ (5%, 3 × 50 mL), brine (50 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue after workup was purified by combiflash (0–10% MeOH/CH₂Cl₂), affording 0.3602 g (37%) of **A1**.

Exemplary Synthesis of Intermediate **A7**



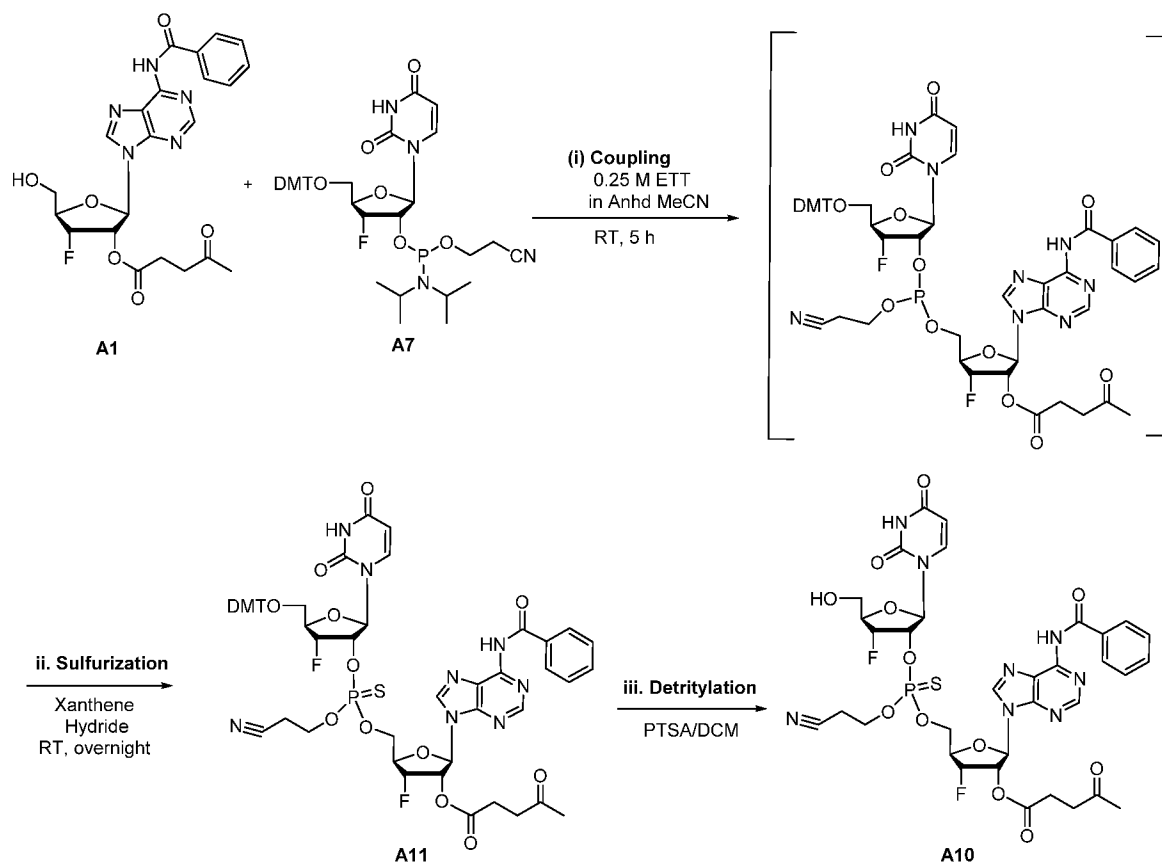
Tritylation

A3 (1.6942g, 0.0050 mol, 1.3 equiv.) was added to a solution of U-nucleoside (**A8**, 0.9500 g, 0.0039 mmol, 1 equiv.) in 20 mL anhydrous pyridine. DMAP (0.0100 g, 2 mol%) was added to the reaction mixture and stirred overnight under argon. The reaction mixture was quenched with saturated NaHCO₃ and concentrated to remove pyridine and coevaporated with toluene. The residue thus obtained was dissolved in methylene chloride, and the resulting solution was washed with water three times, followed by brine. The organic extract was dried over anhydrous sodium sulfate, filtered, and concentrated. The product thus obtained was absorbed over silica gel in the

presence of 3% triethylamine and purified by combiflash from 0-8% CH₂Cl₂ to afford **A9** (1.5293 g, 73%). Combiflash cartridge was equilibrated with 3% Et₃N in CH₂Cl₂ before loading the compound.

Phosphitylation

Phosphitylation reaction must be carried under strict anhydrous conditions. **A9** (1.5100 g, 0.0027 mmol, 1 equiv.) was dissolved in anhydrous methylene chloride and coevaporated three times with anhydrous toluene and put under high vacuum overnight. The vacuum was released under argon, and the compound was further dissolved in 25 mL anhydrous CH₂Cl₂, followed by the addition of Hunigs base (1.5 mL, 0.0083 mol, 3 equiv). The reaction mixture was cooled in an ice bath, and Cl-phosphitylating reagent (1.0 g, 0.0045 mol, 1.5 equiv.) was added and stirred at room temperature for 1 h. The reaction mixture was quenched with sat NaHCO₃ solution and extracted with 100 mL CH₂Cl₂. The aqueous layer was further extracted with 2 x 50 mL CH₂Cl₂. The combined organic extract was washed twice (2 x 200 mL) with water, followed by brine, dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The material was purified by flash silica gel column chromatography in the presence of 3% Et₃N. The silica column was equilibrated in 50% EA/hexanes in the presence of 3% Et₃N. The product was dissolved in a minimum amount of 50% EA/hexanes and eluted in 60% EA/hexanes, **A7** (1.2000 g, 50%).

Exemplary Synthesis of Intermediate A10*Coupling*

A1 (0.3536 g, 0.0011 mol, 1 equiv.) and **A7** (0.7860 g, 0.0011 mmol, 1.3 equiv.) were coevaporated three times with acetonitrile and CH_2Cl_2 and kept under high vacuum overnight. Vacuum was always released under argon. A 1:1 mixture of 40 mL anhydrous CH_2Cl_2 and acetonitrile were added to make a clear solution, followed by adding 5-ethylthiotetrazole (ETT) solution (4.2 mL, 0.25 M, 1.3 equiv). The coupling reaction was carried out for 5 h, and the progress of the reaction was monitored by TLC (5% MeOH/ CH_2Cl_2).

Sulfurization

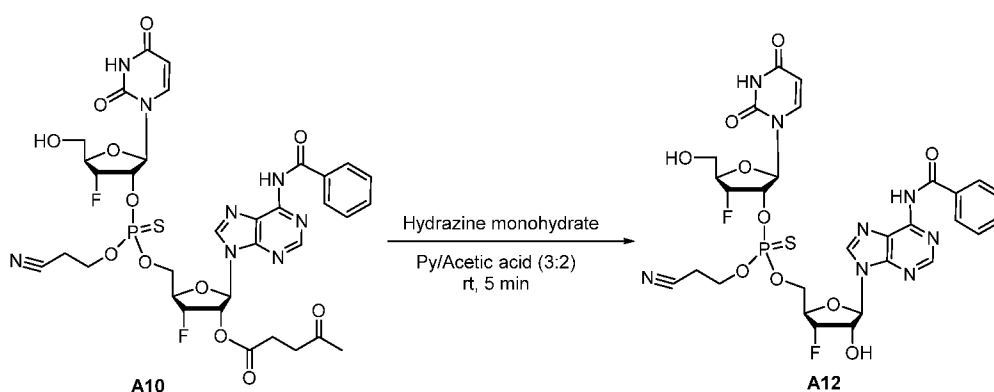
Xanthene hydride (0.2250 g, 0.0015 mol, 2 equiv.) was added to the coupling reaction mixture and stirred overnight. The reaction mixture was centrifuged to remove the solid. The solution thus obtained was concentrated to remove acetonitrile and partitioned between water and CH_2Cl_2 . The organic extract was washed with saturated sodium bicarbonate solution, followed by

water and brine, dried over anhydrous sodium sulfate, and concentrated under vacuum to afford **A11**.

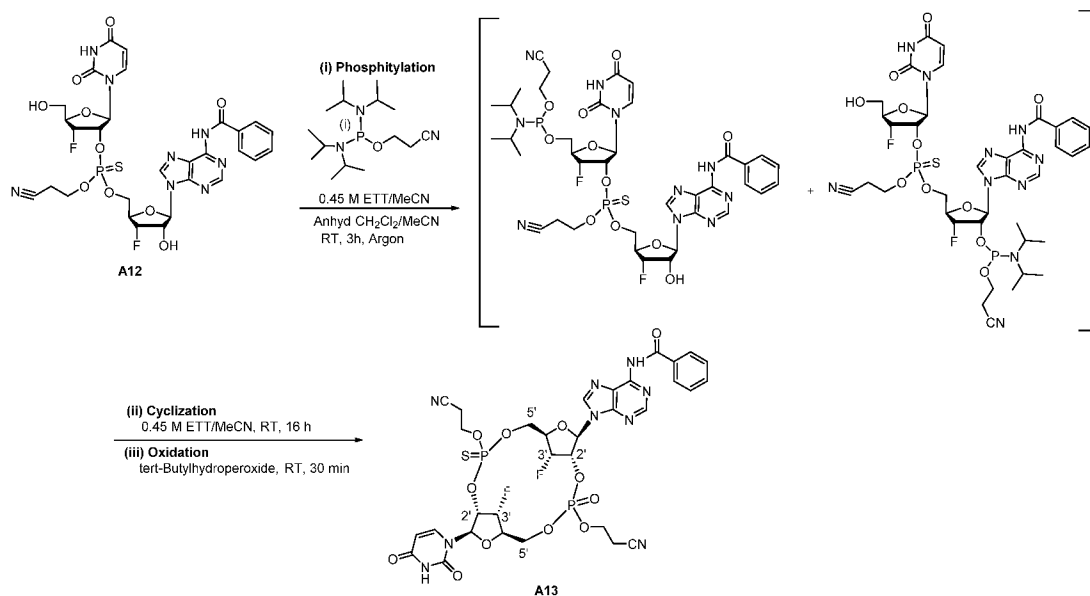
Detritylation

A11 was taken in 30 mL DCM, and to that solution, 5% p-toluene sulfonic acid dissolved in 2 mL MeOH was added and stirred for 45 min at 0 °C. To the reaction mixture, water was added and stirred for 20 min at room temperature. The reaction mixture was extracted with CH₂Cl₂. The CH₂Cl₂ layer was further washed with saturated sodium bicarbonate solution, followed by water and brine, dried over anhydrous MgSO₄, filtered, and concentrated to ~3 mL volume. Hexanes was added to the solution at room temperature for precipitation. The solution was decanted, and the solid thus obtained was further washed twice with hexanes to afford 0.4142 g (65%) of dinucleotide **A10** as a white solid. **A10** was taken forward for the next delevunization step without any further purification.

Synthesis of Intermediate **A12**



Hydrazine hydrate (95 uL) was added to 5 mL (3:2) pyridine/acetic acid. The resulting solution was added to the **A10** (0.3830 g). The reaction mixture was stirred at room temperature for 5 min and quenched with 2,5-pentanedione and concentrated under reduced pressure. The residue was partitioned between water and 30% isopropanol (IPA)/DCM. The organic extract was dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. Acetonitrile was added to the residue and concentrated to ensure complete removal of isopropanol. The crude material thus obtained was further purified by Combiflash using 0-10% MeOH/CH₂Cl₂. The product eluted in 8% MeOH/CH₂Cl₂, affording 0.2040 g (60%) of dinucleotide **A12**.

Synthesis of Intermediate A13*Coevaporation*

Dinucleotide (**A12**, 0.2000 g, 0.0003 mol, 1 equiv.) was coevaporated with anhydrous acetonitrile (3×40 mL) adding some CH₂Cl₂ for a clear solution, dried under high vacuum, flushed three times with argon, and dissolved in a mixture of anhydrous acetonitrile and anhydrous CH₂Cl₂ (4:1, 25 mL).

Phosphoramidite formation

2-Cyanoethyl tetra-isopropyl phosphorodiamidite (0.084 mL, 0.0003 mol, 1 equiv.) was added to the solution of **A12**, followed by adding 0.45 M ETT (0.6 mL, 0.0003 mol, 1 equiv.) in anhydrous acetonitrile in 4 portions over a period of 1 h under an inert atmosphere. The solution was stirred for 4 h at room temperature.

Cyclization

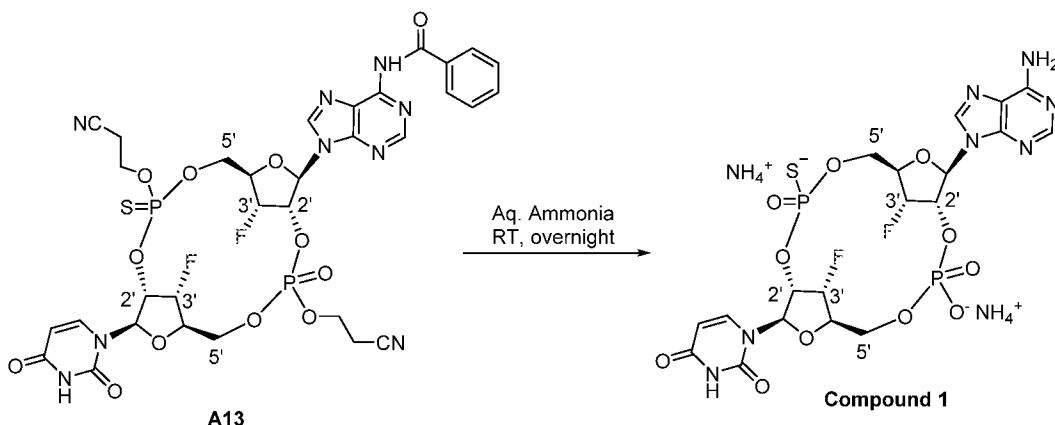
0.45 M ETT in anhydrous acetonitrile (1.2 mL, 0.0006 mol, 2 equiv.) was added to the mixture of phosphoramidite and stirred for 17 h at room temperature.

Oxidation

Tert-butyl hydroperoxide (5–6 M in nonane, 0.13 mL, 0.0006 mol, 2 equiv.) was added dropwise to the stirred solution of the cyclized product at room temperature, and the reaction mixture was stirred for 30 min. The reaction mixture was evaporated under reduced pressure to remove acetonitrile, absorbed over silica gel by dissolving the residue in CH₂Cl₂/MeOH, and

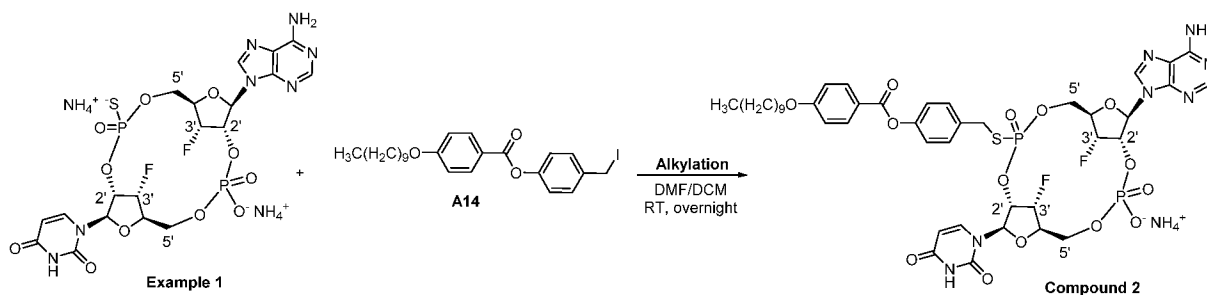
purified by Combiflash using 0–10% MeOH/CH₂Cl₂, affording **A13** (0.0520 g, 60%) as a white solid.

Exemplary Synthesis of Compound 1



A13 (0.0370 g) was incubated with 5 mL aq. NH₃ at room temperature for overnight. The reaction mixture was concentrated to dryness to remove ammonia, further taken up in 5 mL water, and extracted with 3 x 5 mL ethyl acetate. The organic extract was discarded. The aqueous extract was analyzed by RP-HPLC, LCMS, and ³¹P NMR, and concentrated to dryness and kept in vacuum to get quantitative yield (0.0290 g) of **Compound 1**.

Exemplary Synthesis of Compound 2



Compound 1 (0.0226 g, 0.00003 mol, 1 equiv.) was dissolved in 200 μ L of DMF. The C10-I alkylating reagent **A14** (0.0194 g, 0.00004 mol, 1.2 equiv.) was dissolved in 200 μ L DCM. The iodo solution was added to the solution of nucleoside. Additional DMF (100 μ L) was added to the reaction mixture. The reaction mixture was stirred overnight. The reaction mixture was then concentrated to remove DCM. To that solution, 6 times volumes of *t*-butyl methyl ether was added. The suspension thus formed was centrifuged. The supernatant was taken out, and the solid was dissolved in 1:1 IPA/DCM and washed with water. The IPA/DCM layer was collected,

concentrated, and co-evaporated multiple times with acetonitrile, affording 0.0200 g (59%) of **Compound 2** as a white solid.

EQUIVALENTS

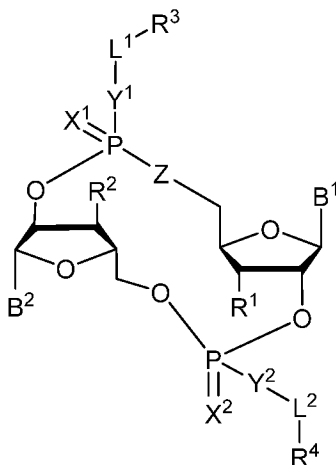
The disclosures of each patent, patent application, and publication cited herein are hereby incorporated by reference in their entirety. While this disclosure has been described with reference to specific aspects, it is apparent that other aspects and variations may be devised by others skilled in the art without departing from the true spirit and scope of the disclosure. The appended claims are intended to be construed to include all such aspects and equivalent variations. Any patent, publication, or other disclosure material, in whole or in part, that is said to be incorporated by reference herein is incorporated herein only to the extent that the incorporated material does not conflict with existing definitions, statements, or other disclosure material set forth in this disclosure. As such, and to the extent necessary, the disclosure as explicitly set forth herein supersedes any conflicting material incorporated herein by reference.

While this disclosure has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the disclosure encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A compound of Formula (I):



Formula (I)

or a pharmaceutically acceptable salt or stereoisomer thereof, wherein:

Z is either S or O;

each of B¹ and B² is independently a purinyl nucleobase or pyrimidinyl nucleobase;

each of X¹ and X² is independently O or S;

each of Y¹ and Y² is independently O, S, or NR⁵;

each of L¹ and L² is independently absent, C₁-C₆ alkyl or C₁-C₆ heteroalkyl, wherein each alkyl and heteroalkyl is optionally substituted with R⁶;

each of R¹ and R² is independently hydrogen, halo, -CN, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), or OR⁷;

each of R³ and R⁴ is independently hydrogen, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), C₁-C₂₀ heteroalkyl (e.g., C₁-C₆ heteroalkyl), OC(O)OC₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), cycloalkyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, and heteroaryl is optionally substituted with one or more R⁸;

R⁵ is hydrogen or C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl);

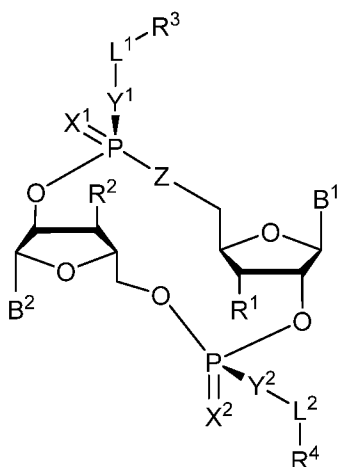
R⁶ is halo, -CN, C₁-C₂₀ alkyl (e.g., C₁-C₆ alkyl), OR⁷, oxo, cycloalkyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, cycloalkyl, heterocyclyl, aryl, and heteroaryl is optionally substituted with one or more R⁹;

R^7 is hydrogen, C_1 - C_{20} alkyl (e.g., C_1 - C_6 alkyl), cycloalkyl, heterocyclyl, aryl, or heteroaryl, wherein each alkyl, cycloalkyl, heterocyclyl, aryl, and heteroaryl is optionally substituted with one or more R^9 ;

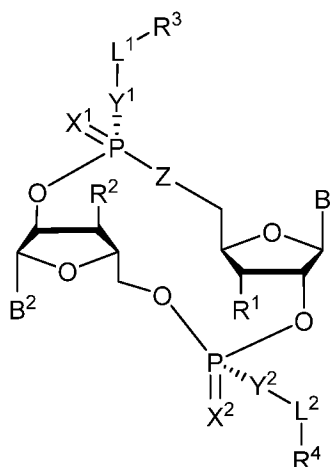
each R^8 is independently C_1 - C_{20} alkyl (e.g., C_1 - C_6 alkyl), C_1 - C_{20} heteroalkyl, $C(O)$ - C_1 - C_{20} alkyl, $OC(O)$ - C_1 - C_{20} alkyl (e.g., C_1 - C_6 alkyl), $C(O)O$ - C_1 - C_{20} alkyl (e.g., C_1 - C_6 alkyl), $OC(O)O$ - C_1 - C_{20} alkyl (e.g., C_1 - C_6 alkyl), $C(O)N(R^5)$ - C_1 - C_{20} alkyl (e.g., C_1 - C_6 alkyl), $N(R^5)C(O)$ - C_1 - C_{20} alkyl (e.g., C_1 - C_6 alkyl), $OC(O)N(R^5)$ - C_1 - C_{20} alkyl (e.g., C_1 - C_6 alkyl), O -aryl, O -heteroaryl, $C(O)$ -aryl, $C(O)$ -heteroaryl, $OC(O)$ -aryl, $C(O)O$ -aryl, $OC(O)$ -heteroaryl, $C(O)O$ -heteroaryl, $C(O)O$ -aryl, $C(O)O$ -heteroaryl, $C(O)N(R^5)$ -aryl, $C(O)N(R^5)$ -heteroaryl, $N(R^5)C(O)$ -aryl, $N(R^5)_2C(O)$ -aryl, or $N(R^5)C(O)$ -heteroaryl, $S(O)_2N(R^5)$ -aryl, wherein each alkyl, heteroalkyl, aryl, and heteroaryl is optionally substituted by one or more R^9 ; and

each R^9 is independently C_1 - C_{20} alkyl, O - C_1 - C_{20} alkyl, C_1 - C_{20} heteroalkyl, halo, $-CN$, OH , oxo, aryl, heteroaryl, O -aryl, or O -heteroaryl.

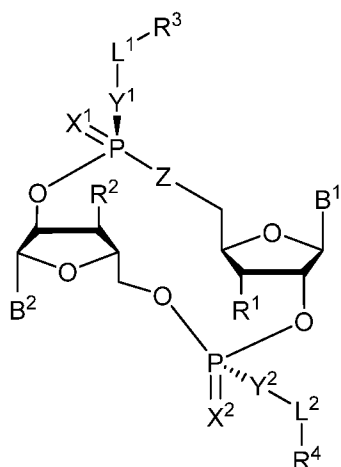
2. The compound of claim 1, wherein the compound is a compound of Formula (I-a), (I-b), (I-c), or (I-d):



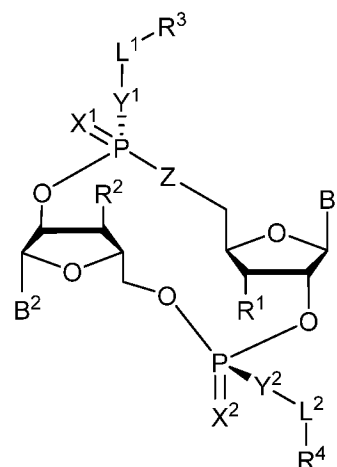
Formula (I-a)



Formula (I-b)



Formula (I-c)



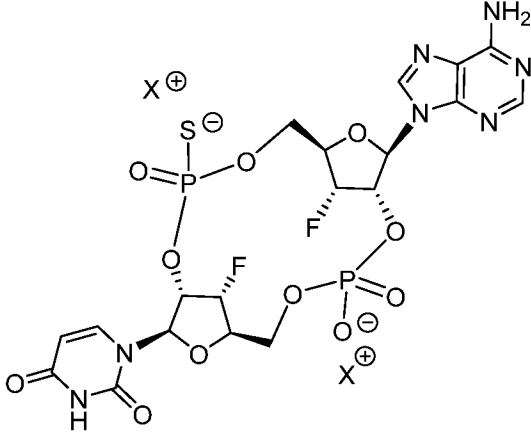
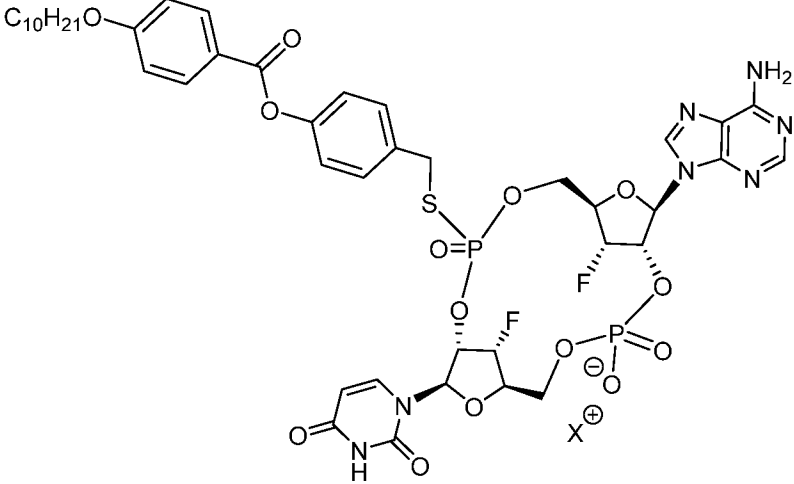
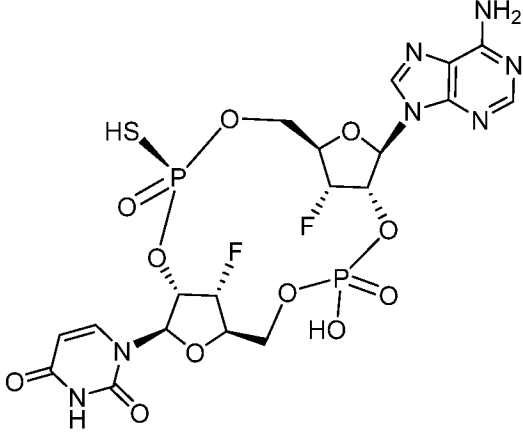
Formula (I-d)

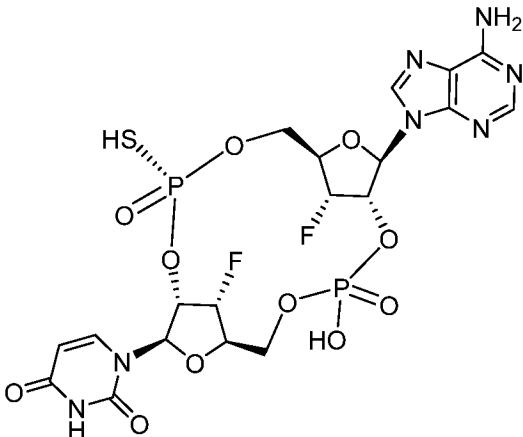
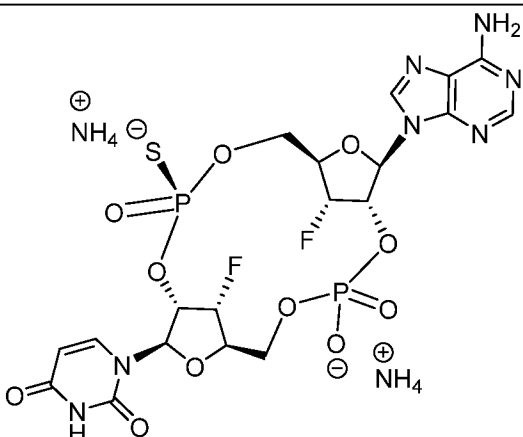
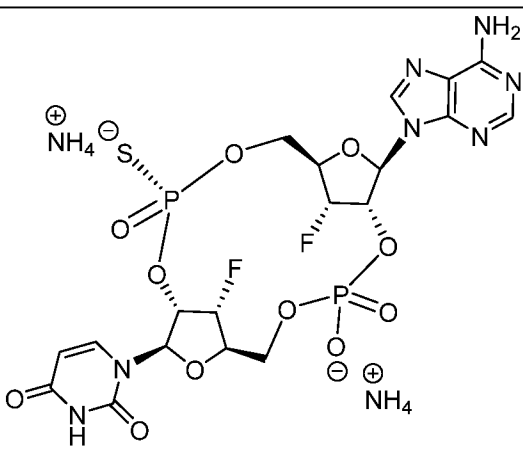
or a pharmaceutically acceptable salt thereof.

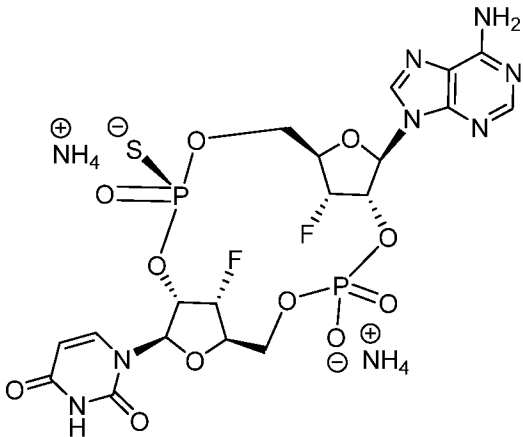
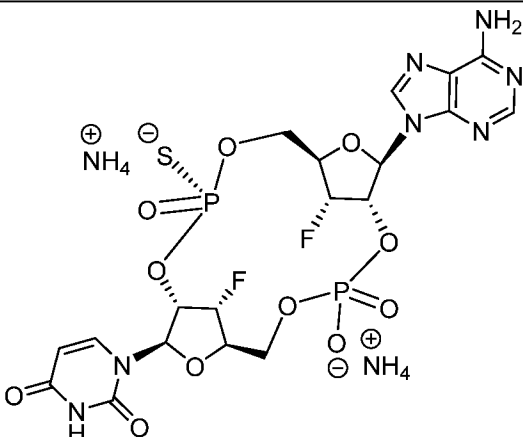
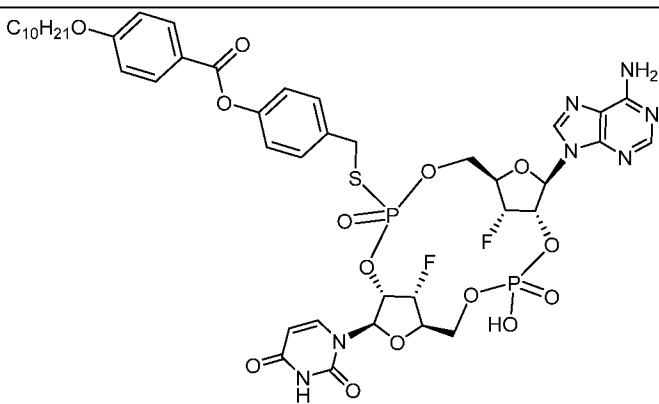
3. The compound of claim 1 or 2, wherein Z is O.
4. The compound of claim 1 or 2, wherein Z is S.
5. The compound of any one of claims 1-4, wherein B¹ is a purinyl nucleobase and B² is a pyrimidinyl nucleobase.
6. The compound of any one of claims 1-4, wherein B¹ is adenosinyl or guanosinyl and B² is cytosinyl, thyminyl, or uracilyl.
7. The compound of any one of claims 1-4, wherein B¹ is adenosinyl, and B² is uracilyl.
8. The compound of any one of claims 1-4, wherein B² is a purinyl nucleobase and B¹ is a pyrimidinyl nucleobase.
9. The compound of any one of claims 1-4 and 8, wherein B² is adenosinyl or guanosinyl and B¹ is cytosinyl, thyminyl, or uracilyl.
10. The compounds of any one of claims 1-4, 8, and 9, wherein B² is adenosinyl, and B¹ is uracilyl.

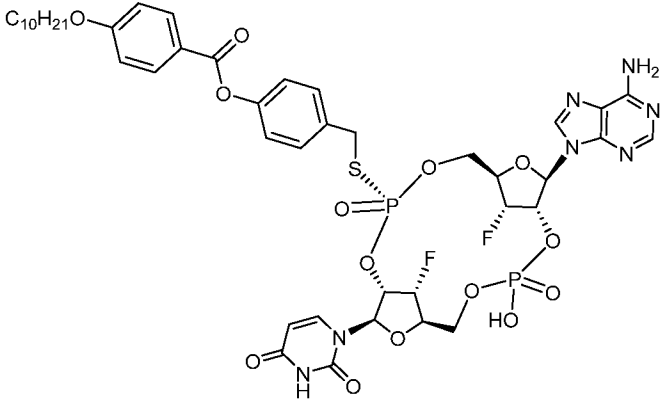
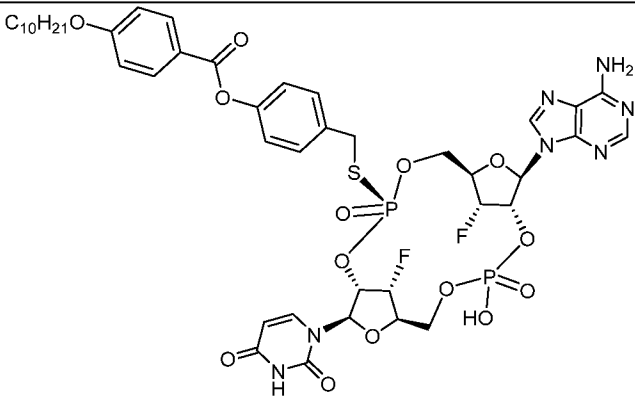
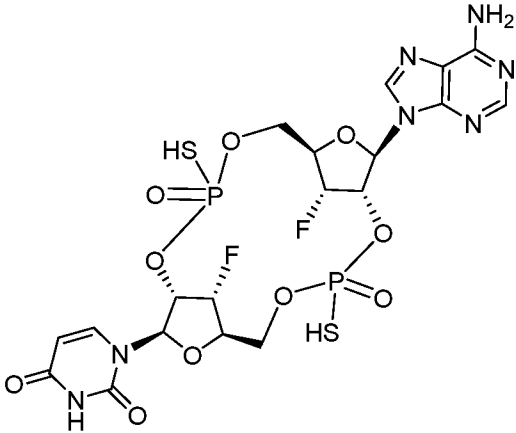
11. The compound of any one of claims 1-10, wherein each of R^1 and R^2 is independently hydrogen, halo, or OR^7 .
12. The compound of any one of claims 1-11, wherein each of R^1 and R^2 is independently halo (e.g., fluoro).
13. The compound of any one of claims 1-12, wherein each of R^1 and R^2 is not hydrogen or OR^7 .
14. The compound of any one of claims 1-13, wherein each of X^1 and X^2 is independently O.
15. The compound of any one of claims 1-14, wherein each of Y^1 and Y^2 is independently O or S.
16. The compound of any one of claims 1-15, wherein one of Y^1 or Y^2 is O and the other of Y^1 or Y^2 is S.
17. The compound of any one of claims 1-15, wherein each of Y^1 or Y^2 is independently S.
18. The compound of any one of claims 1-15, wherein each of Y^1 or Y^2 is independently O.
19. The compound of any one of claims 1-18, wherein each of L^1 and L^2 is independently C_1 - C_6 alkyl (e.g., CH_2).
20. The compound of any one of claims 1-19, wherein each of R^3 and R^4 is independently hydrogen, aryl, or heteroaryl, wherein aryl and heteroaryl is optionally substituted with 1-5 R^8 .
21. The compound of any one of claims 1-20, wherein R^3 is aryl or heteroaryl, each of which is optionally substituted with 1-5 R^8 , and R^4 is hydrogen.
22. The compound of any one of claims 1-21, wherein R^3 is phenyl substituted with 1 R^8 and R^4 is hydrogen.

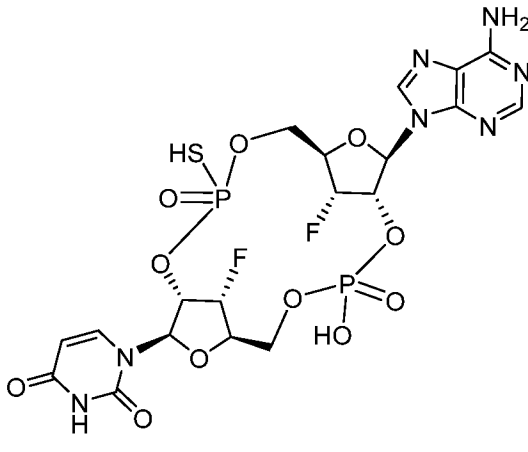
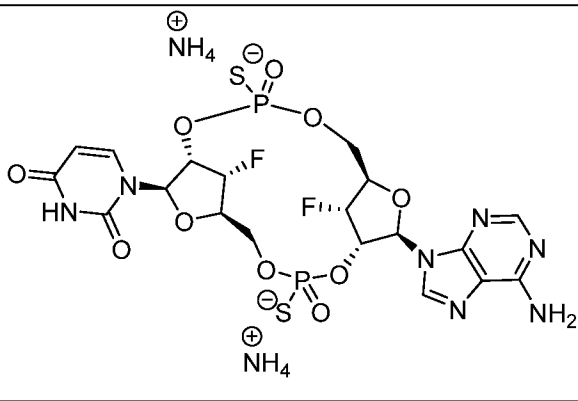
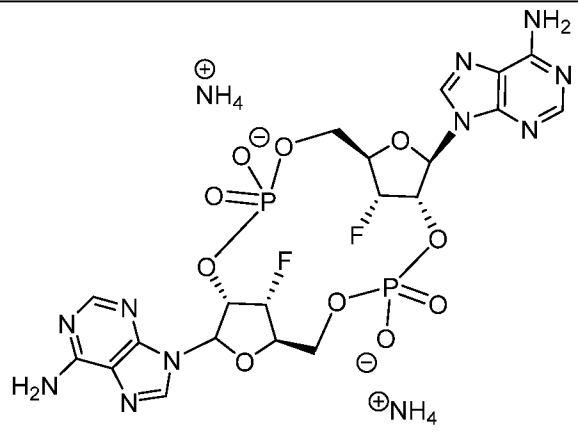
23. The compound of any one of claims 1-20, wherein each of R^3 and R^4 is independently phenyl substituted with 1 R^8 .
24. The compound of any one of claims 1-16, wherein each of Y^1 and Y^2 is O and each of R^3 and R^4 is independently hydrogen.
25. The compound of any one of claims 1-16, wherein Y^2 is O and R^4 is hydrogen.
26. The compound of any one of claims 1-16, wherein each of Y^1 and Y^2 is independently S and each of R^3 and R^4 is independently substituted with 1 R^8 .
27. The compound of any one of claims 1-16, wherein Y^1 is S and R^3 is substituted with 1 R^8 .
28. The compound of any one of claims 1-27, wherein R^8 is OC(O)-aryl optionally substituted by 1-5 R^9 (e.g., 1 R^9).
29. The compound of any one of claims 1-27, wherein R^8 is C(O)-aryl optionally substituted by 1-5 R^9 (e.g., 1 R^9).
30. The compound of claim 28 or 29, wherein R^9 is O- C_{1-12} alkyl (e.g., O-CH₂(CH₂)₈CH₃).
31. The compound of claim 1, wherein the compound is selected from the following table:

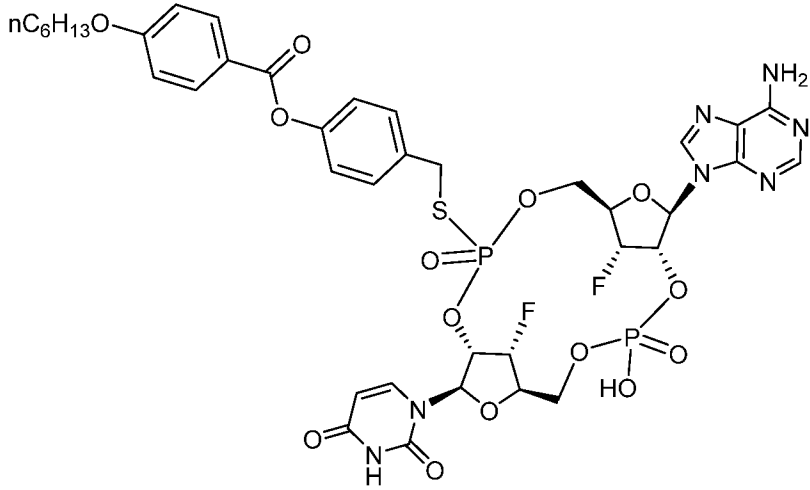
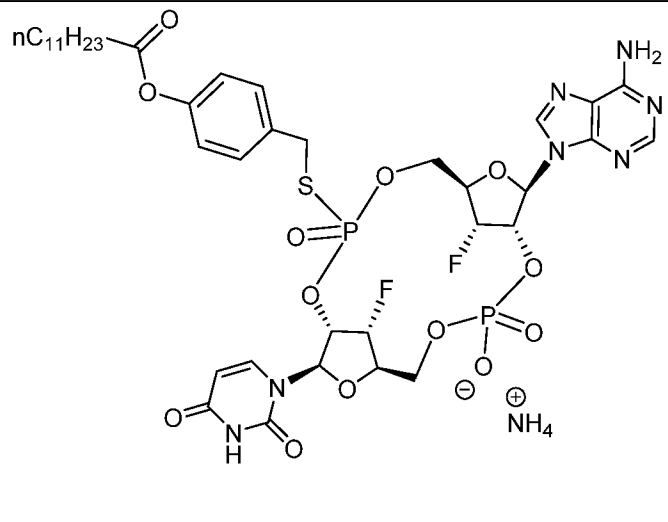
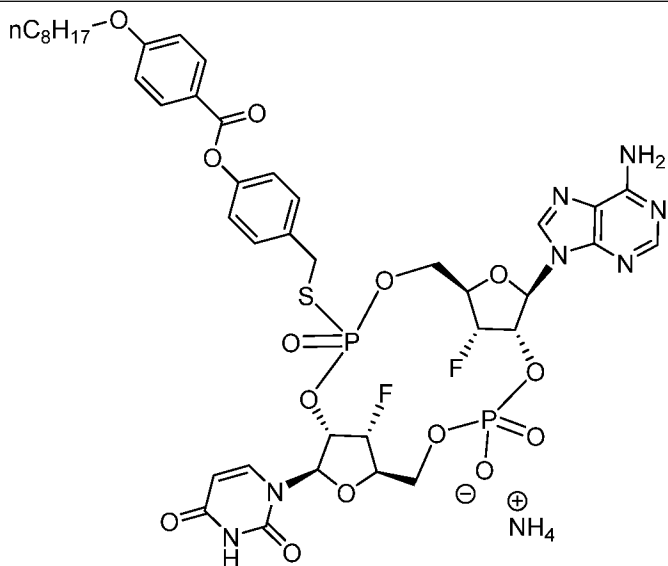
Compound No.	Structure
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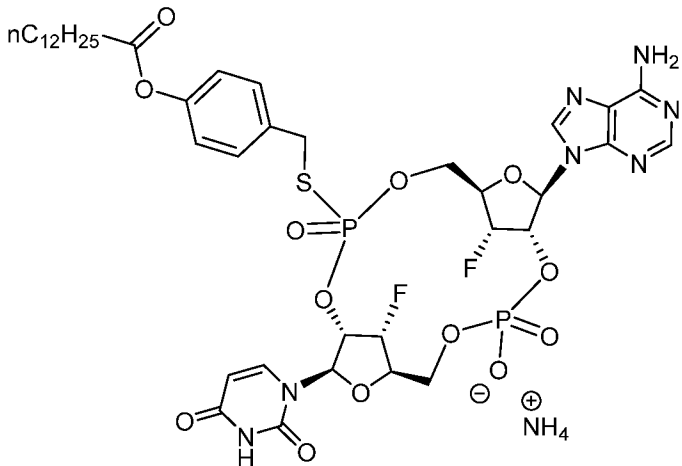
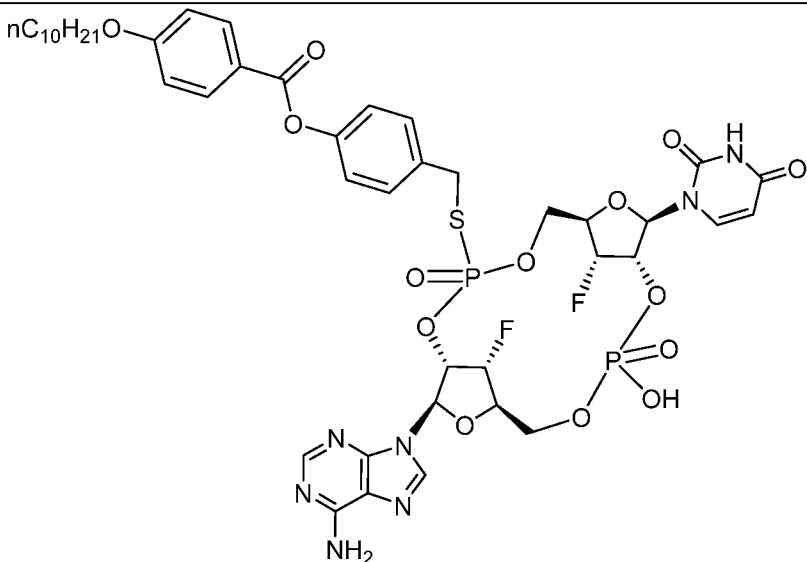
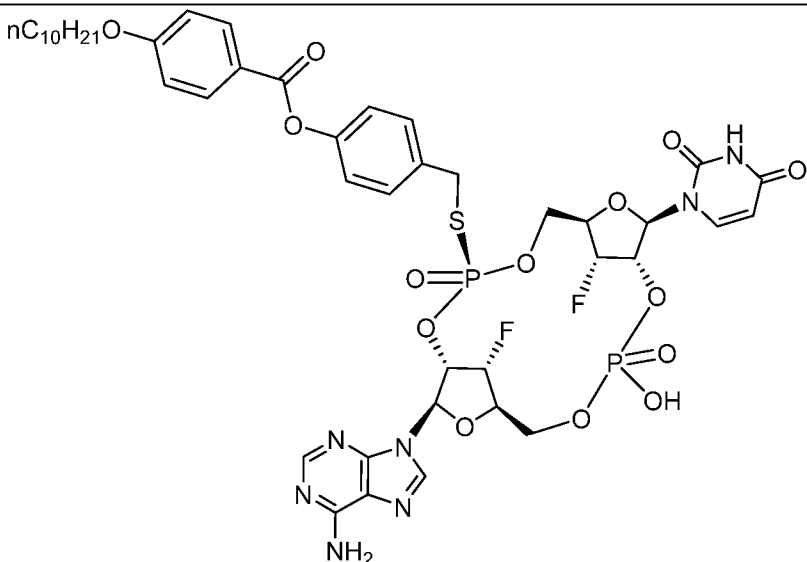
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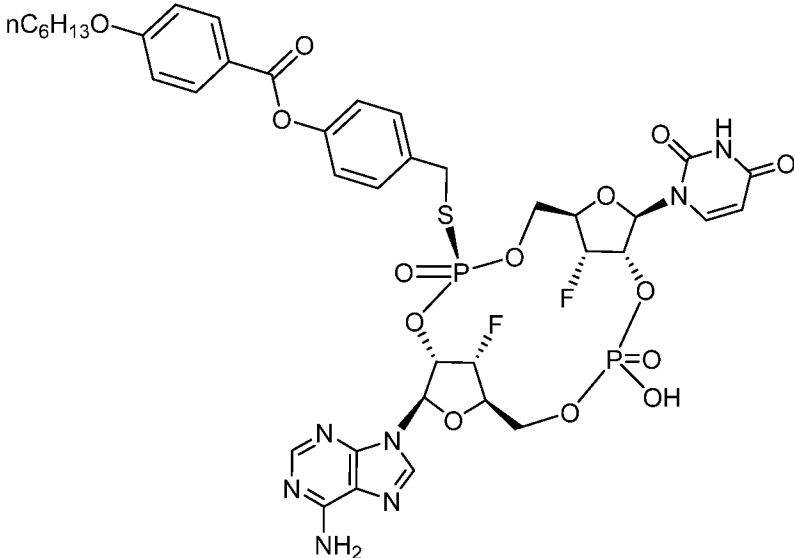
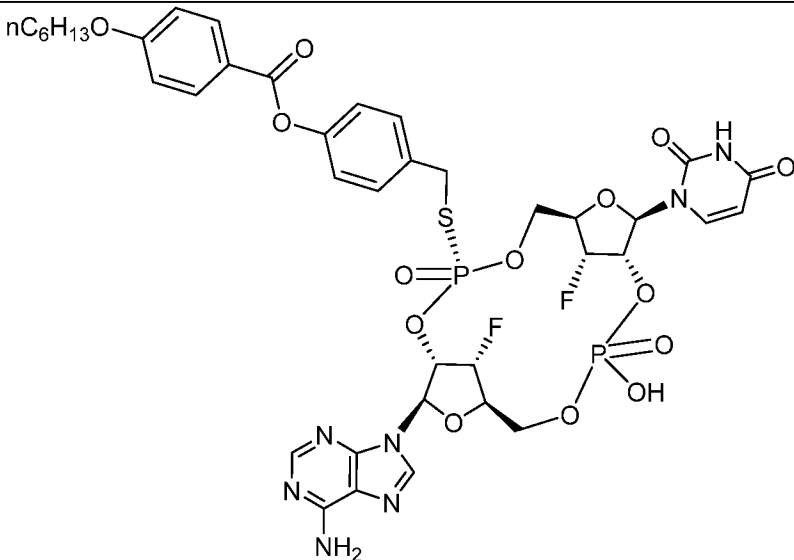
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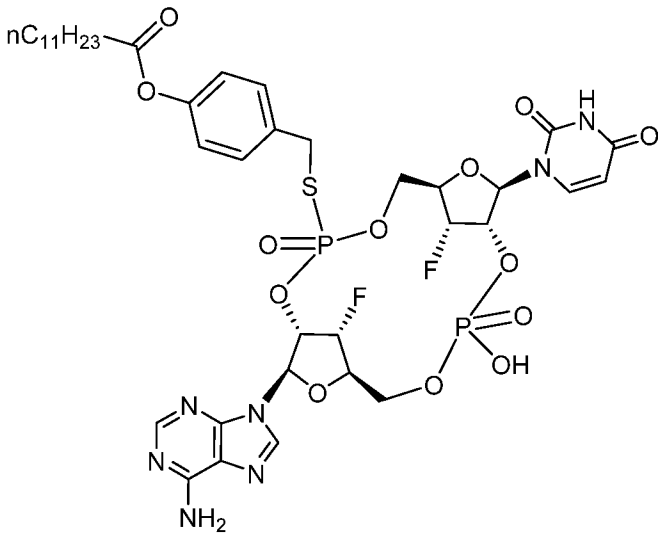
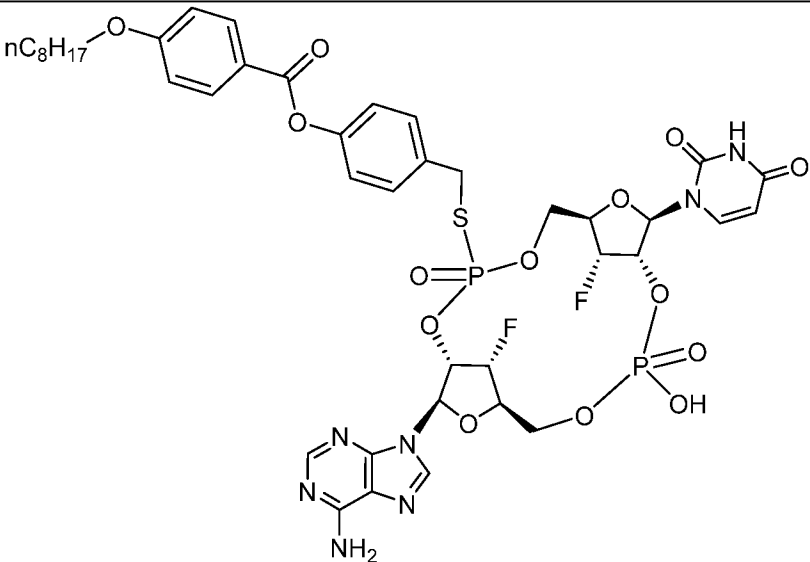
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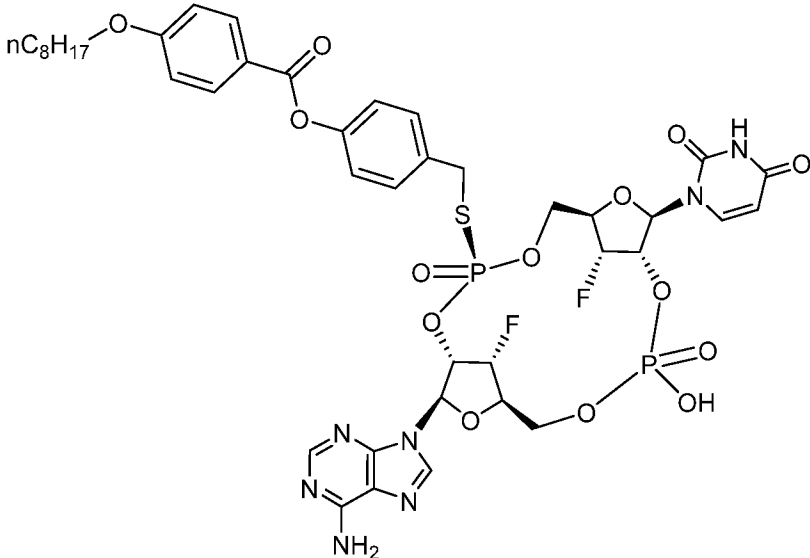
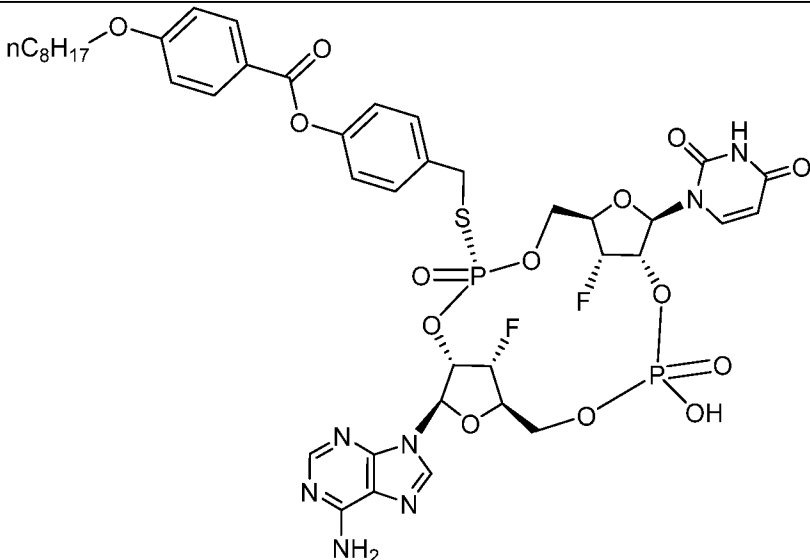
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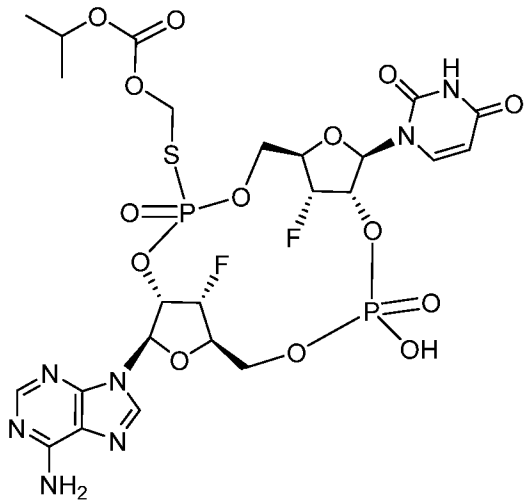
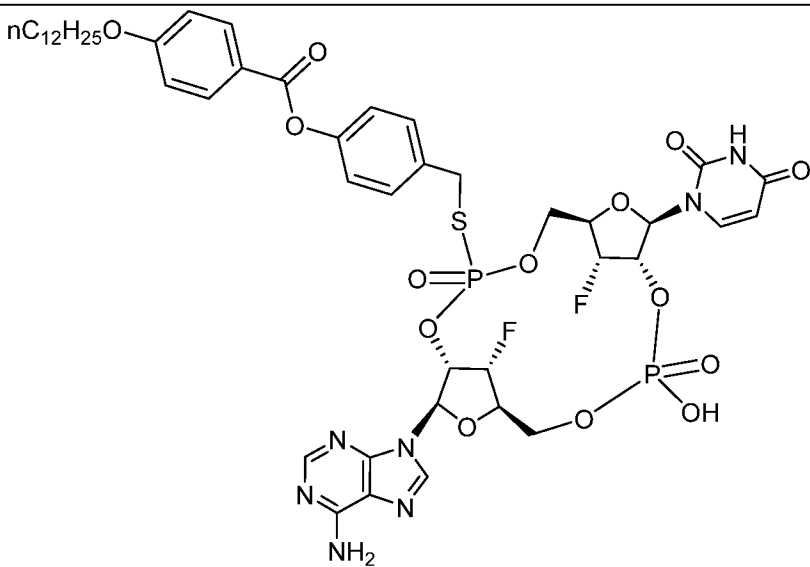
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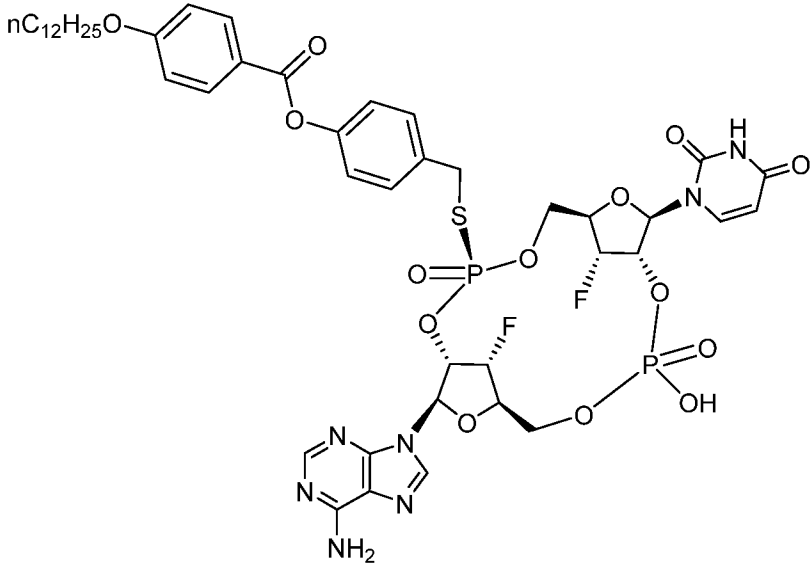
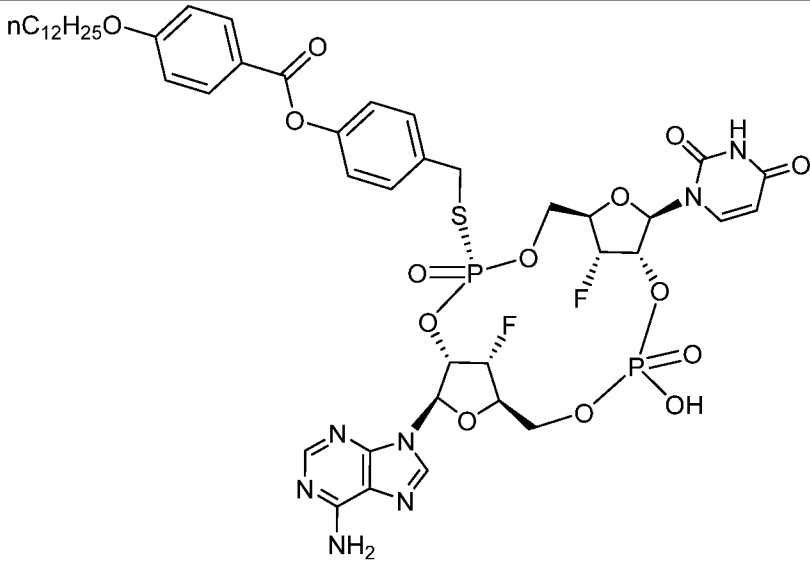
22	Chemical structure 22: A complex molecule featuring a central bicyclic nucleoside core. The core consists of two five-membered rings linked by a sulfur atom (S) and a phosphorus atom (P). One ring is substituted with an amino group (NH₂) and a pyrimidine ring. The other ring is substituted with a thiazine ring and a phosphate group (P=O, OH). The phosphate group is linked to a long chain containing a benzoyl group and a long alkyl chain (nC₁₀H₂₁O).
23	Chemical structure 23: A complex molecule featuring a central bicyclic nucleoside core. The core consists of two five-membered rings linked by a sulfur atom (S) and a phosphorus atom (P). One ring is substituted with an amino group (NH₂) and a pyrimidine ring. The other ring is substituted with a thiazine ring and a phosphate group (P=O, OH). The phosphate group is linked to a long chain containing a benzoyl group and a long alkyl chain (nC₆H₁₃O).

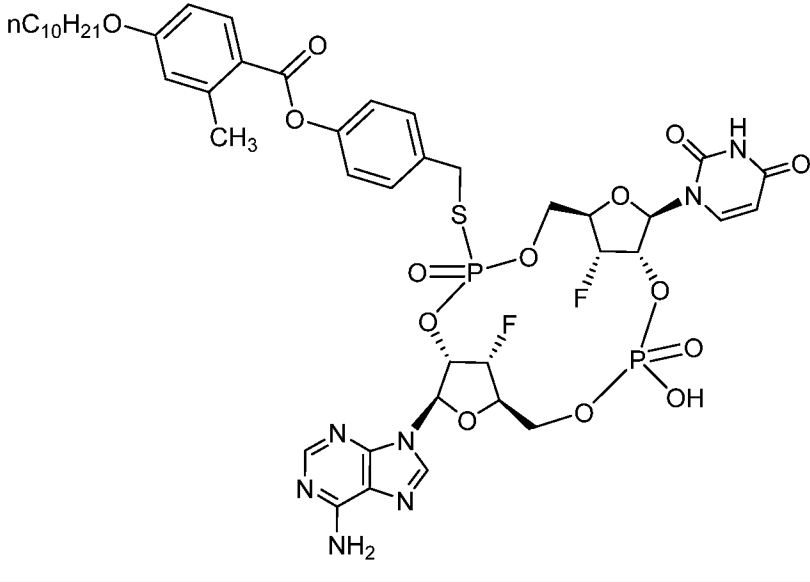
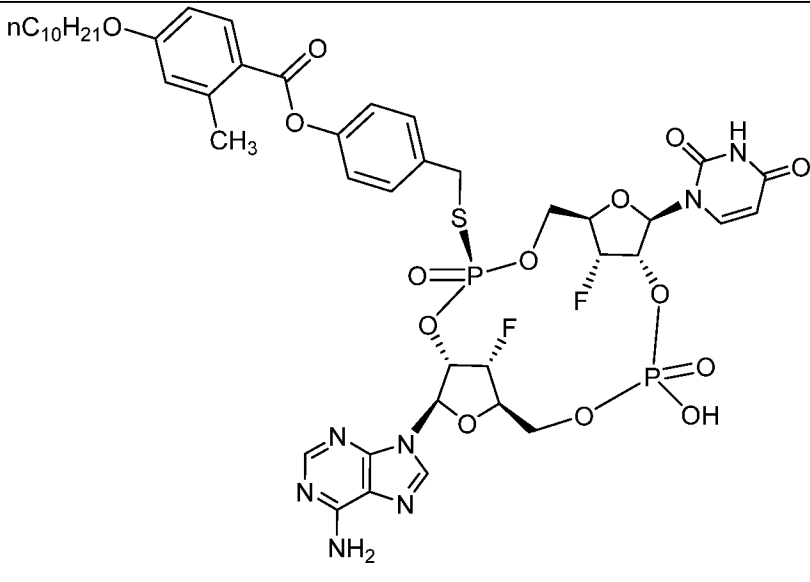
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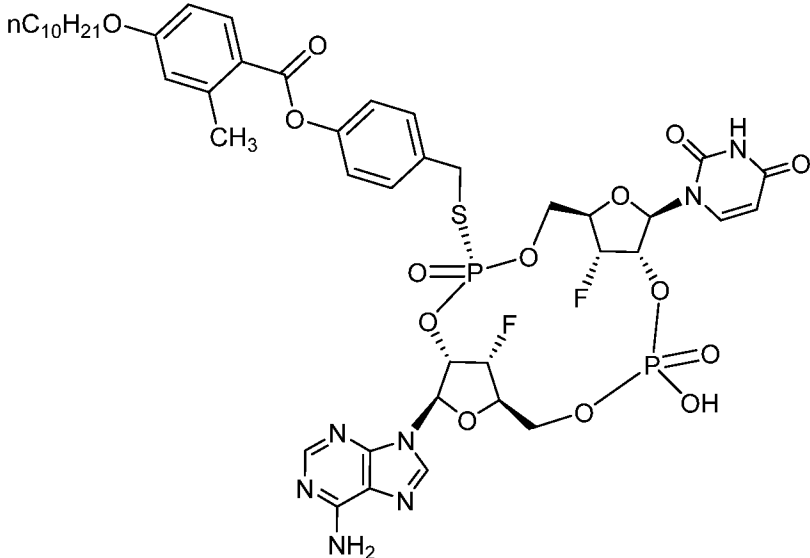
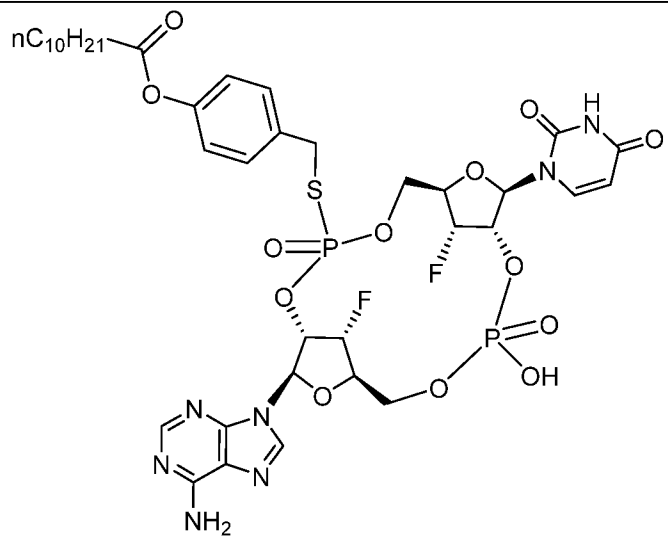
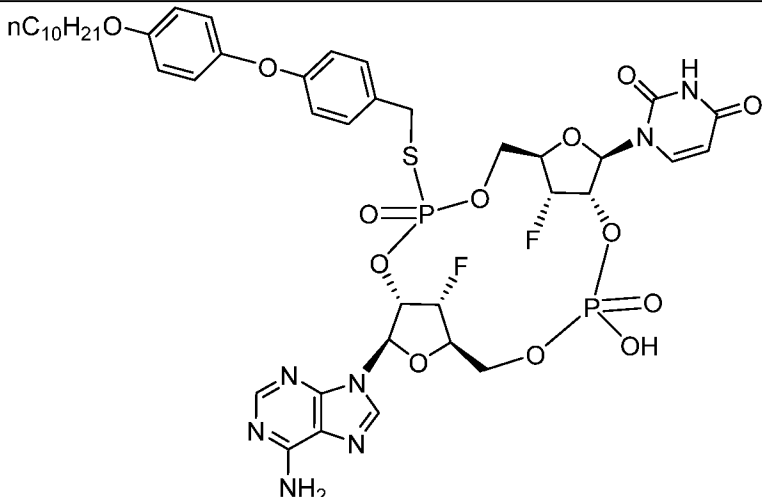
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28	 Chemical structure 28 is a complex molecule. It features a long alkyl chain (n₈H₁₇) attached to a phenyl ring, which is linked via an ester to another phenyl ring. This second phenyl ring is connected to a sulfur atom, which is part of a cyclic phosphite structure. The cyclic phosphite is linked to a ribose sugar, which is further connected to a pyrimidine ring. The ribose sugar also has a fluorine atom and a phosphate group attached.
29	 Chemical structure 29 is a complex molecule. It features a long alkyl chain (n₈H₁₇) attached to a phenyl ring, which is linked via an ester to another phenyl ring. This second phenyl ring is connected to a sulfur atom, which is part of a cyclic phosphite structure. The cyclic phosphite is linked to a ribose sugar, which is further connected to a pyrimidine ring. The ribose sugar also has a fluorine atom and a phosphate group attached.

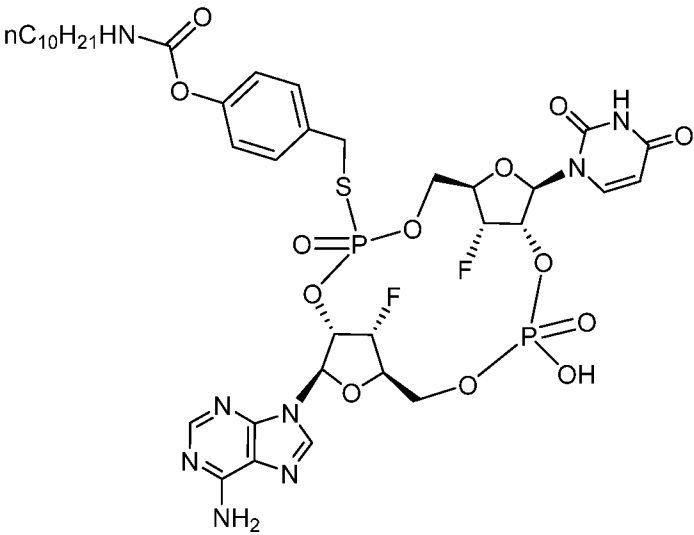
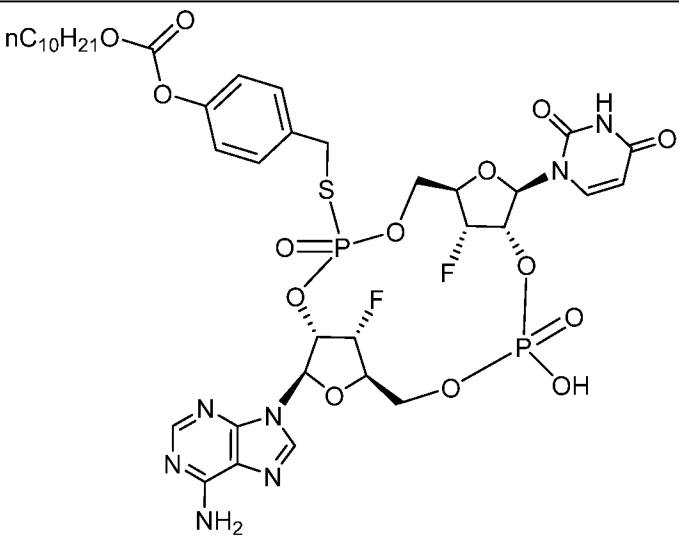
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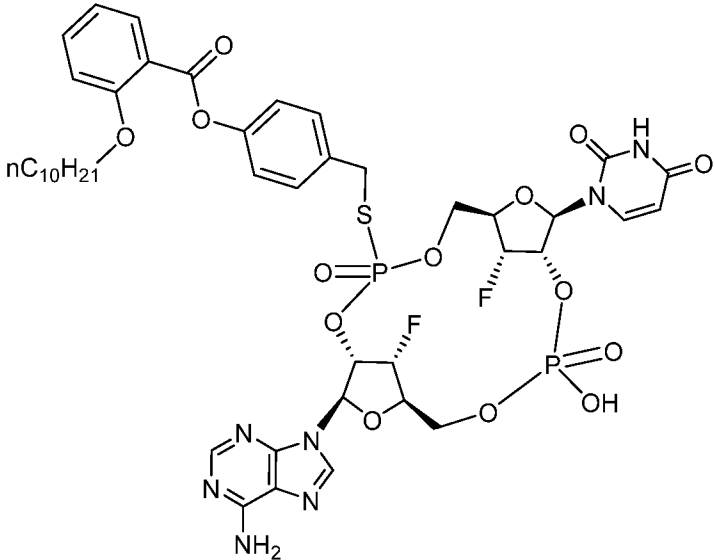
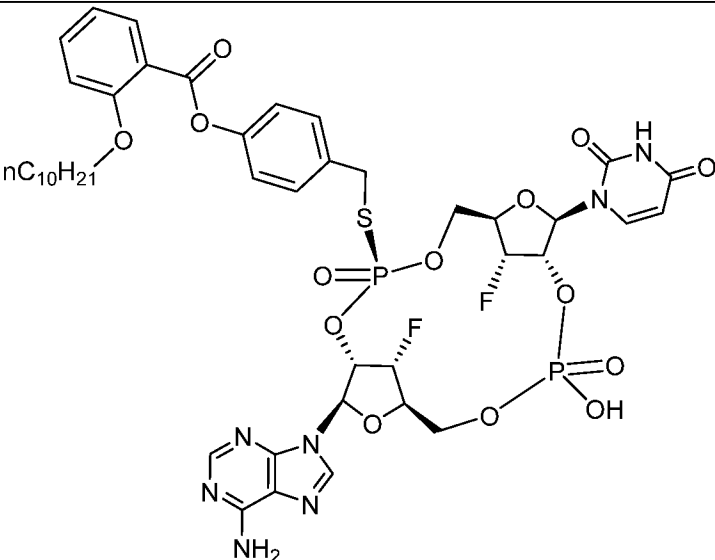
32	 Chemical structure 32 is a complex molecule. It features a central bicyclic core with a fluorine atom. The structure is substituted with a 4-(n-dodecyloxy)benzoyl group, a 2-aminopurine, and a 2-thiopyranose derivative. The structure includes a phosphate group and a pyrimidine ring.
33	 Chemical structure 33 is a complex molecule. It features a central bicyclic core with a fluorine atom. The structure is substituted with a 4-(n-dodecyloxy)benzoyl group, a 2-aminopurine, and a 2-thiopyranose derivative. The structure includes a phosphate group and a pyrimidine ring.

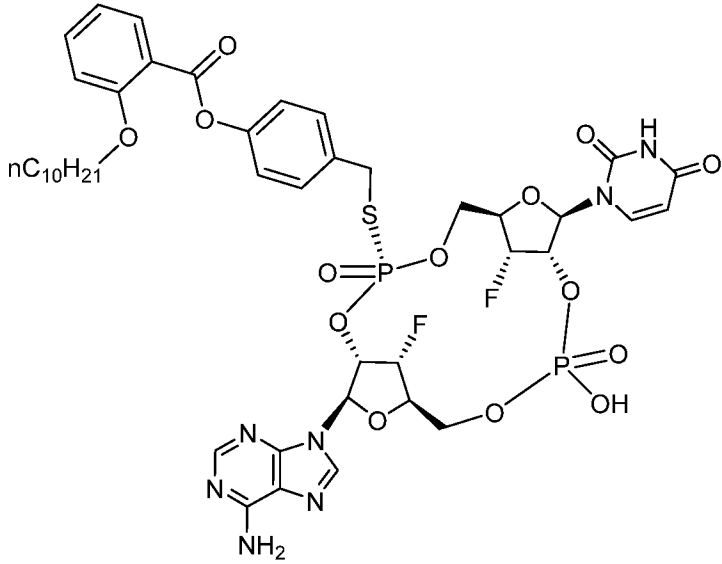
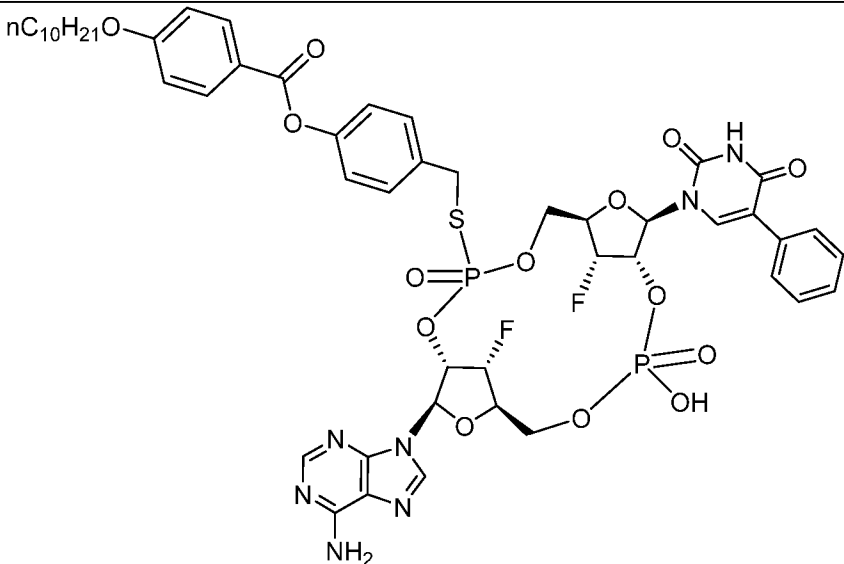
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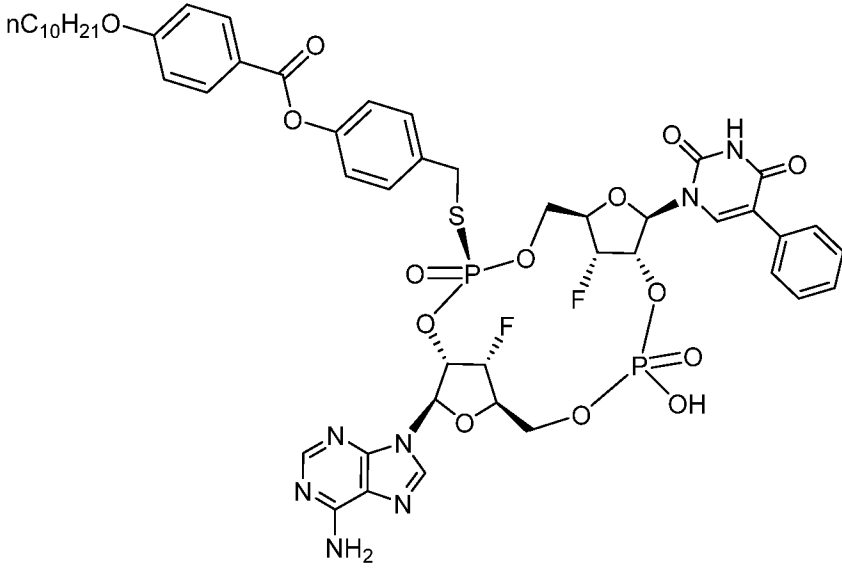
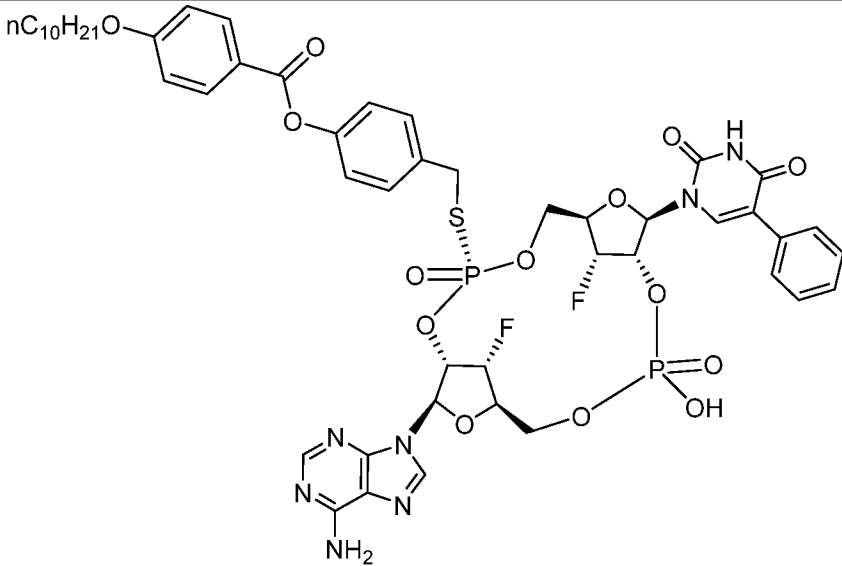
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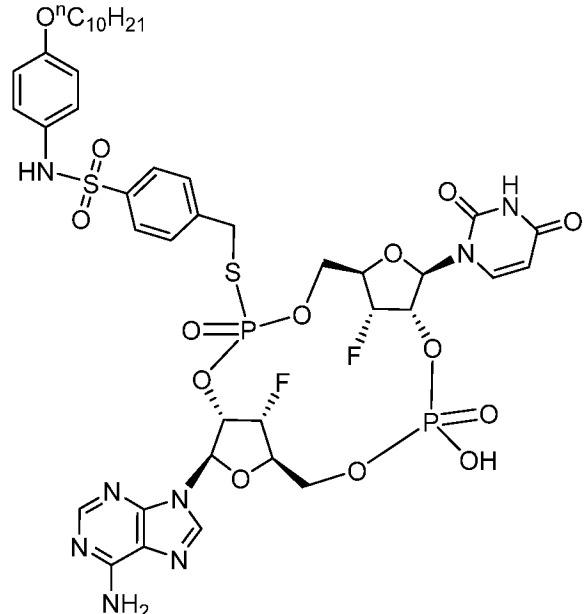
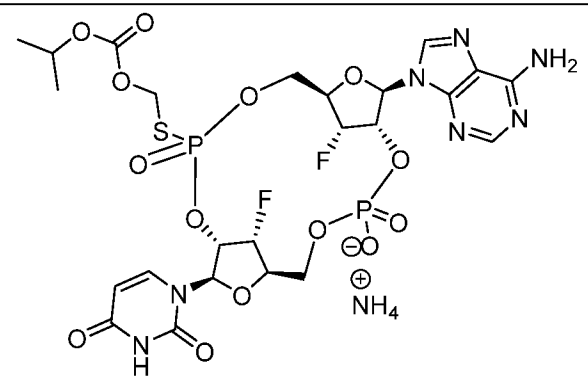
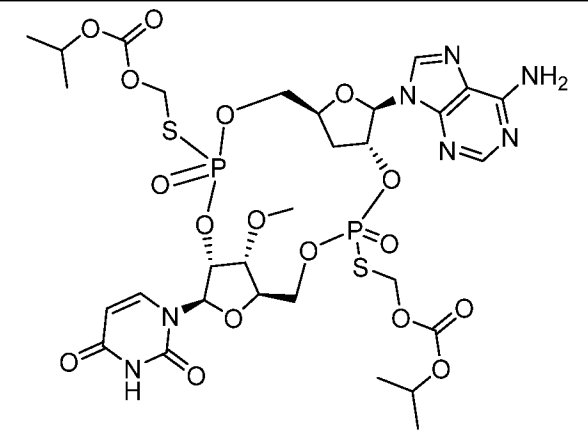
39	Chemical structure 39: A complex molecule featuring a central bicyclic nucleoside core. The core consists of two fused five-membered rings, each containing an oxygen atom. The left ring is substituted with an amino group (NH₂) and a triazole ring. The right ring is substituted with a uracil base. The core is linked via a phosphorus atom to a long chain containing a thioether (S) and a carbonyl group (C=O). The chain is terminated by a long alkyl chain (nC₁₀H₂₁O).
40	Chemical structure 40: A complex molecule featuring a central bicyclic nucleoside core. The core consists of two fused five-membered rings, each containing an oxygen atom. The left ring is substituted with an amino group (NH₂) and a triazole ring. The right ring is substituted with a uracil base. The core is linked via a phosphorus atom to a long chain containing a thioether (S) and a carbonyl group (C=O). The chain is terminated by a long alkyl chain (nC₁₀H₂₁O).

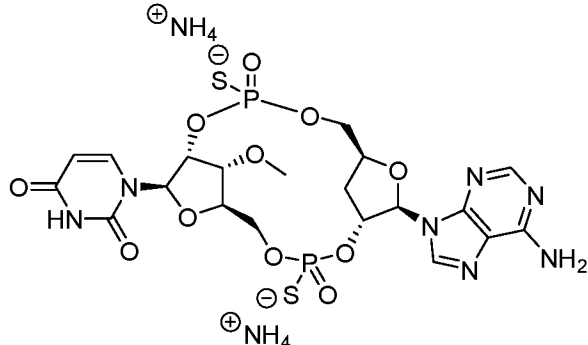
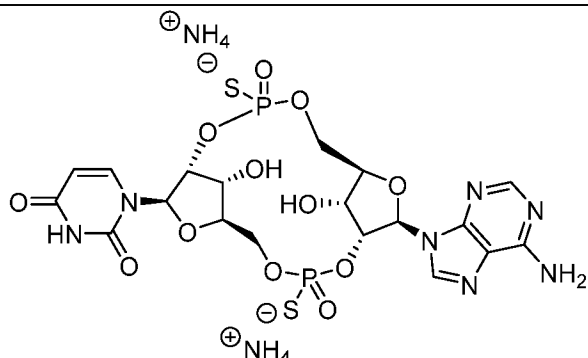
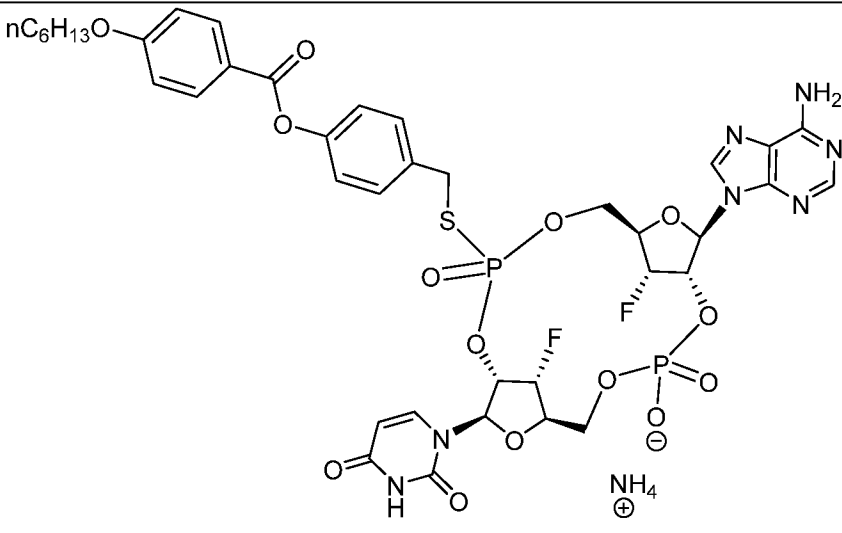
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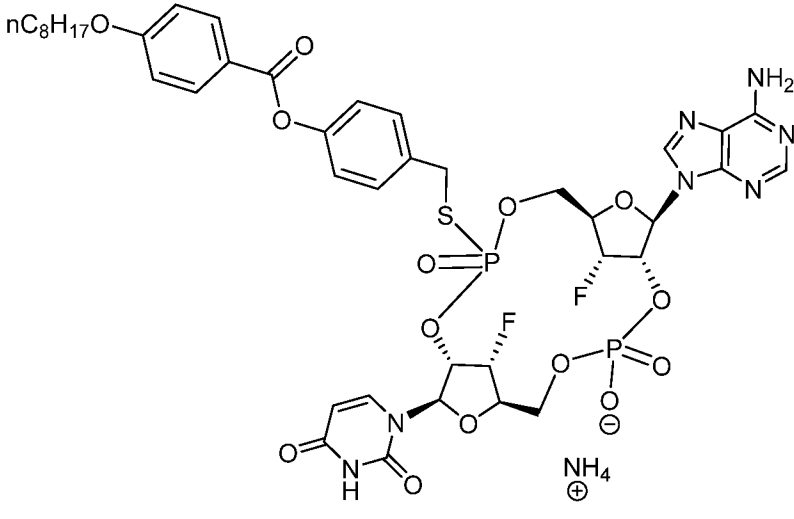
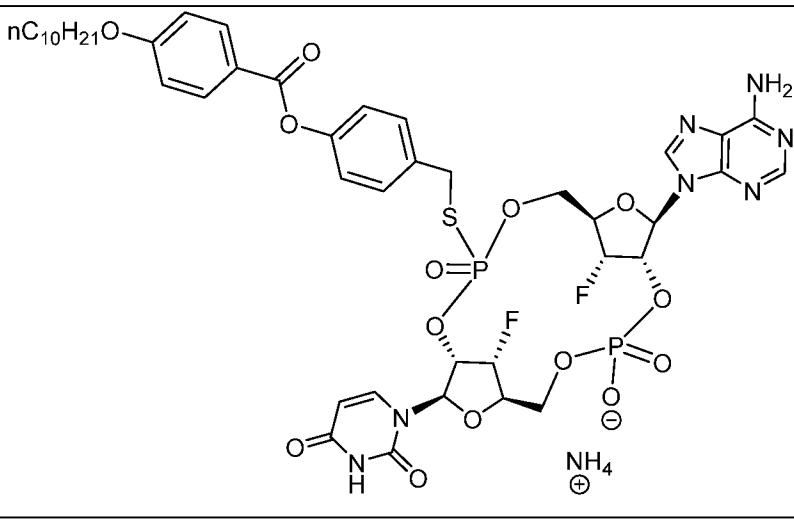
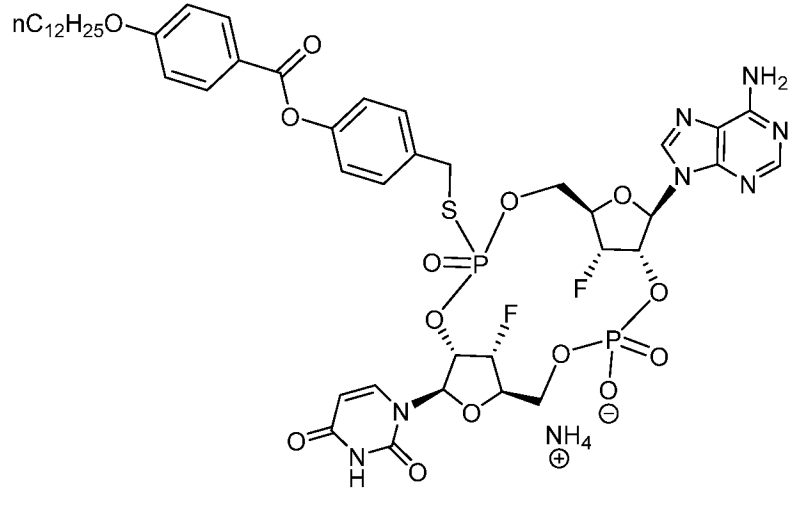
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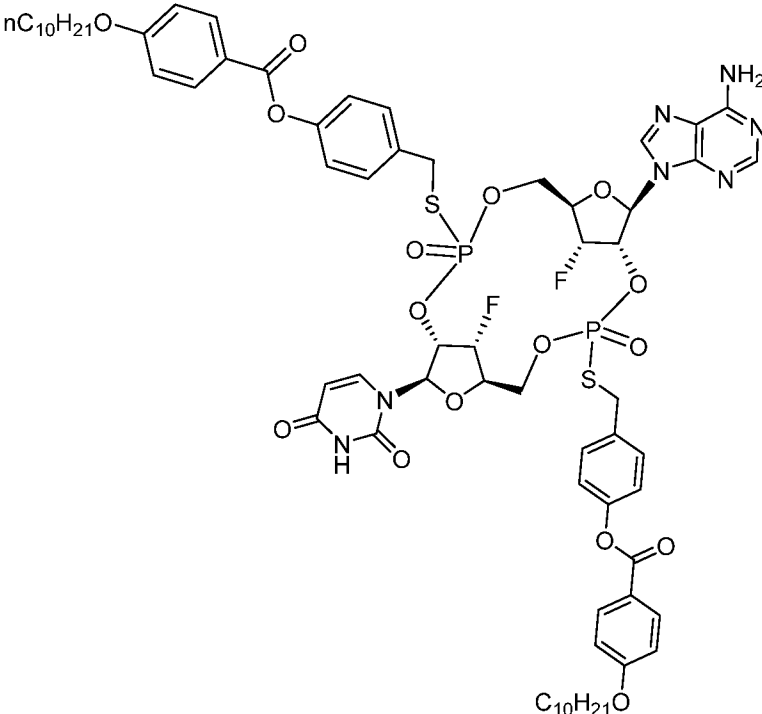
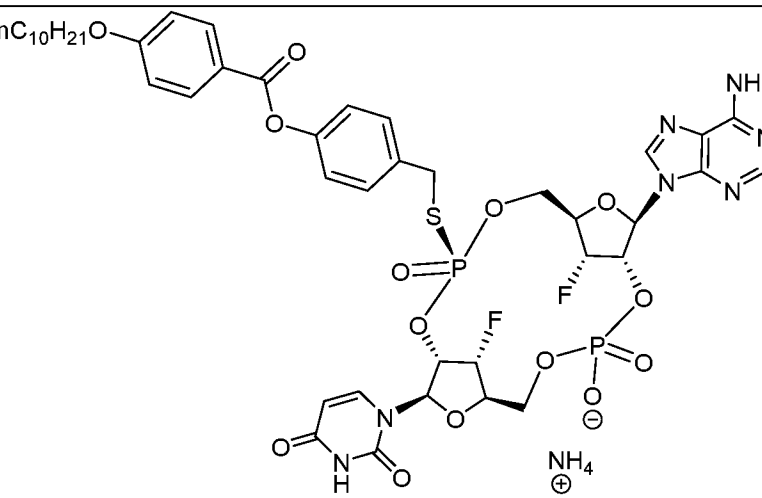
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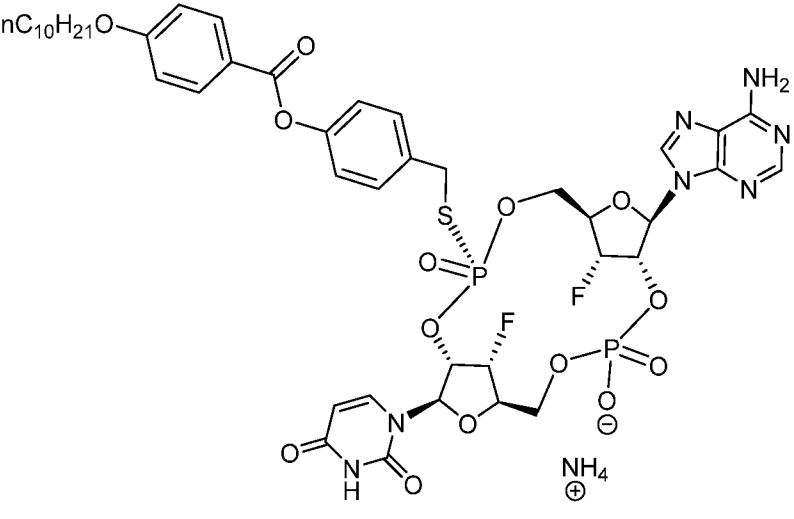
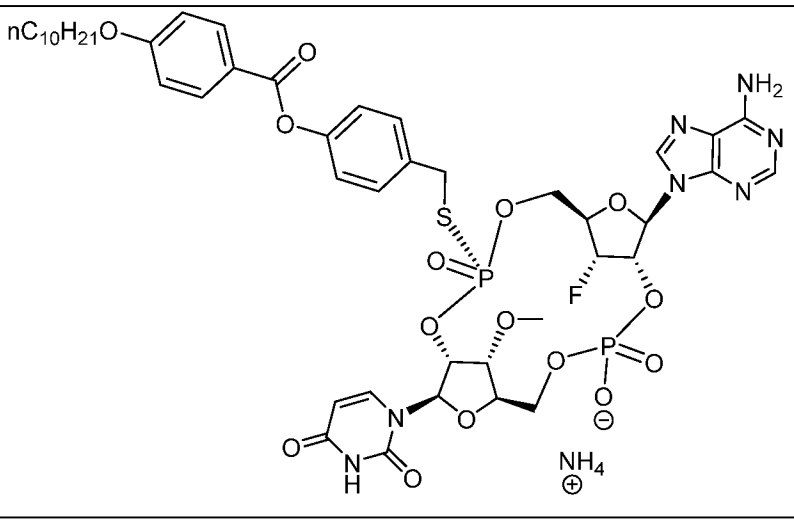
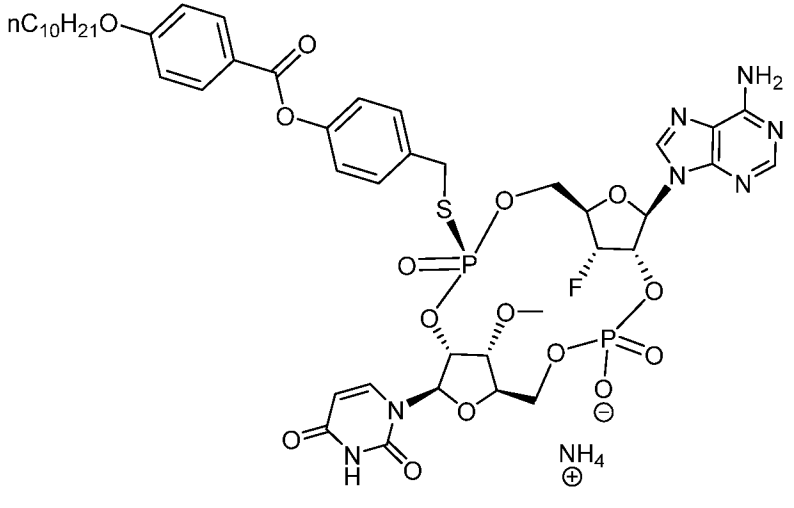
47	 Chemical structure 47: A complex molecule featuring a diphosphate core. One phosphate is linked to a 2-aminopurine-9-yl group. The other phosphate is linked to a 2-phenyl-4H-pyrimidin-5-yl group. A thioether bridge connects the diphosphate to a 4-(n-decyloxybenzoyloxy)benzyl group.
48	 Chemical structure 48: A complex molecule featuring a diphosphate core. One phosphate is linked to a 2-aminopurine-9-yl group. The other phosphate is linked to a 2-phenyl-4H-pyrimidin-5-yl group. A thioether bridge connects the diphosphate to a 4-(n-decyloxybenzoyloxy)benzyl group.

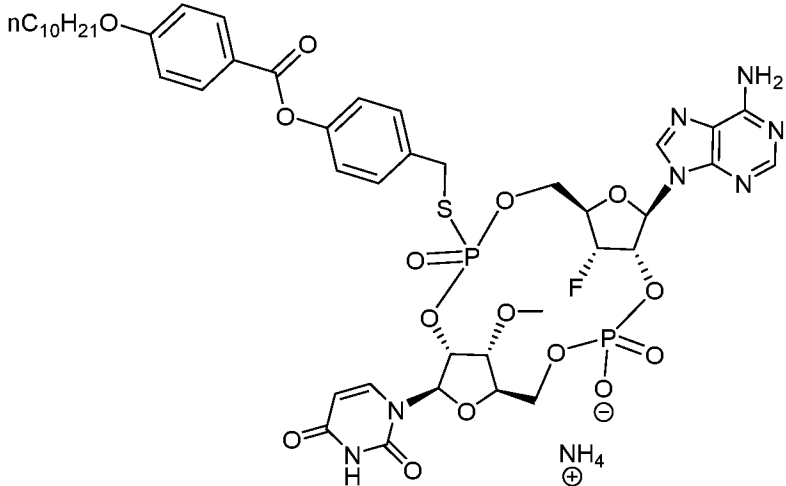
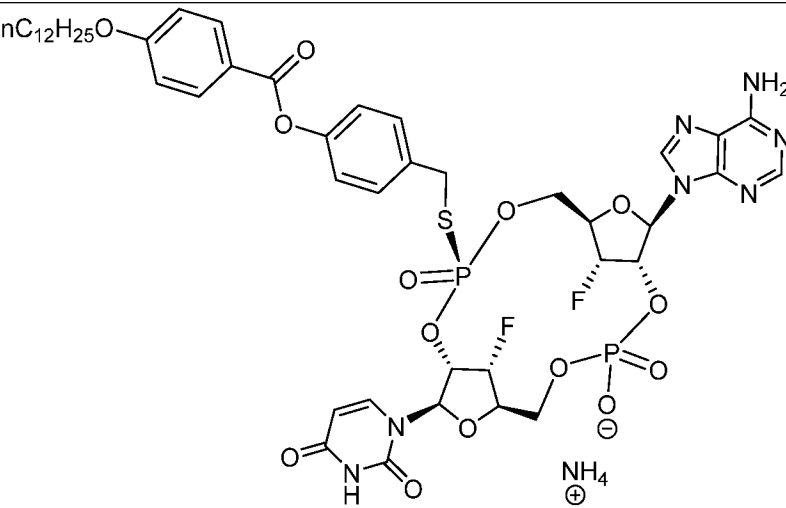
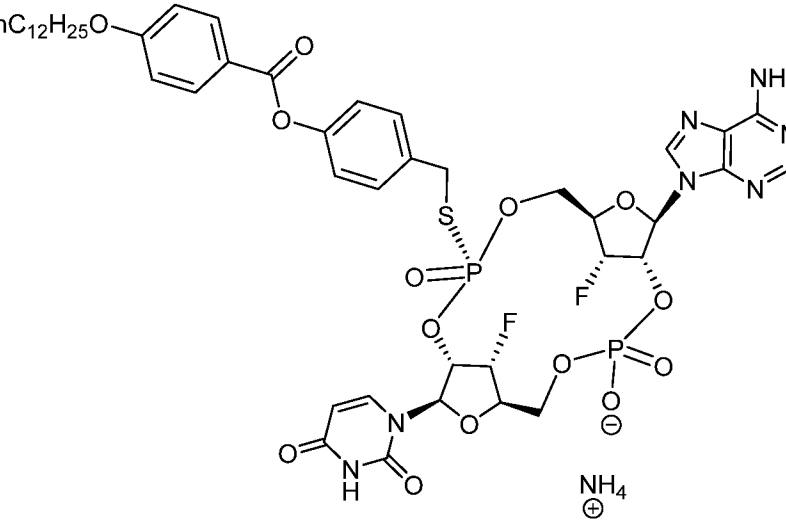
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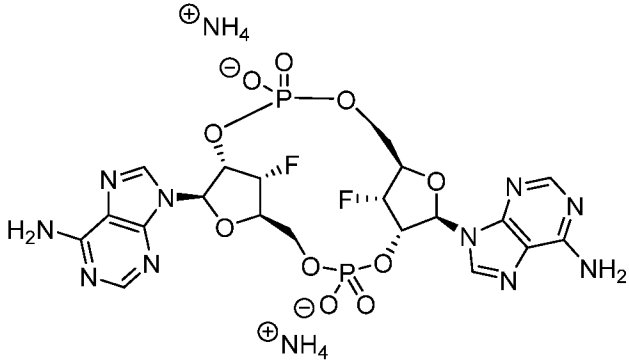
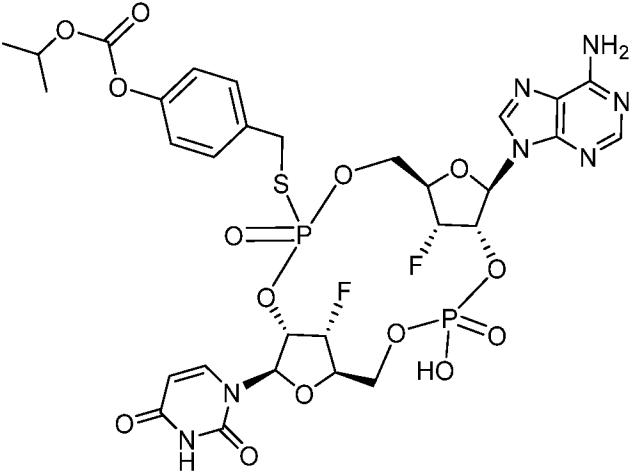
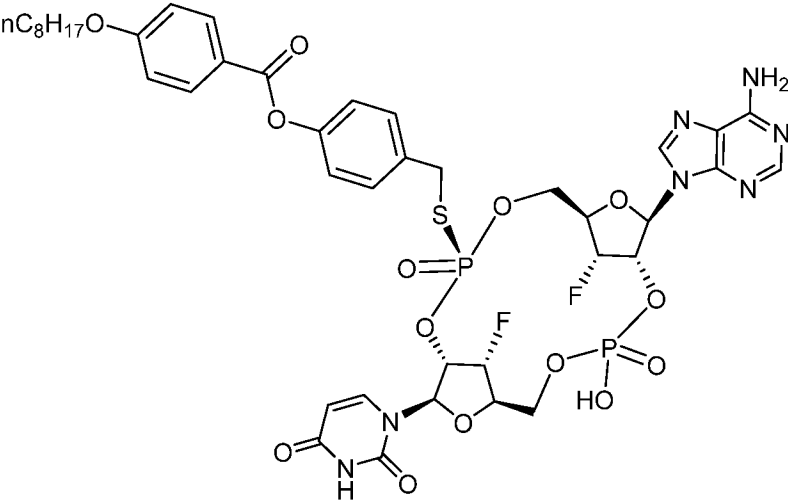
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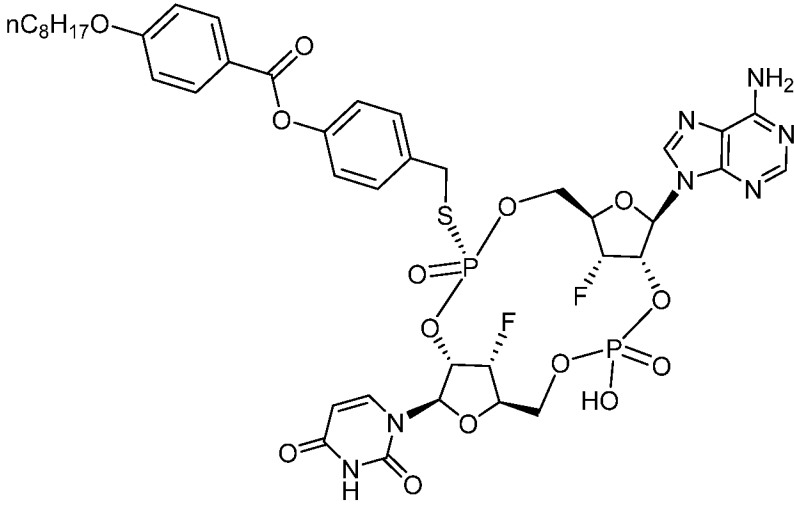
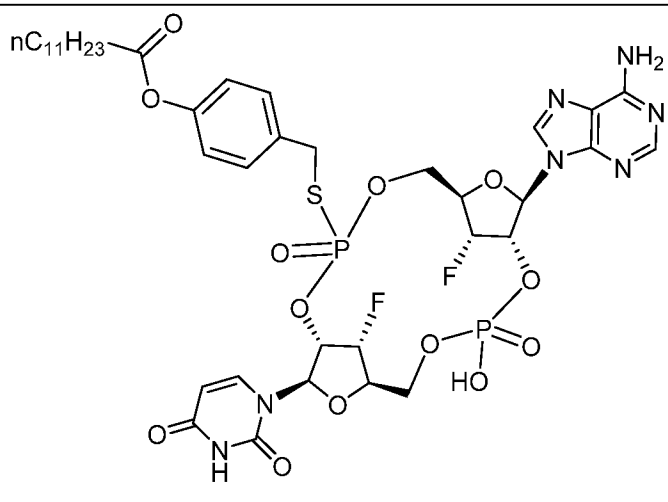
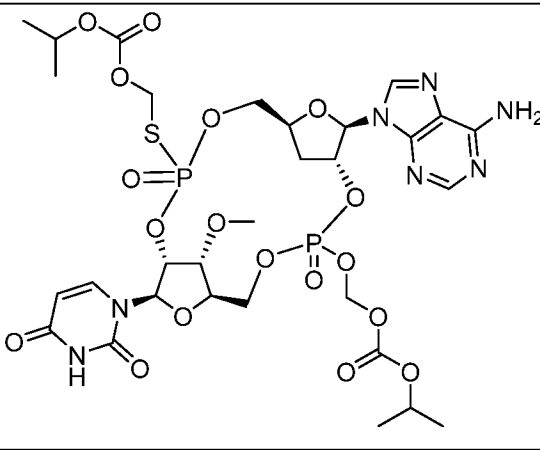
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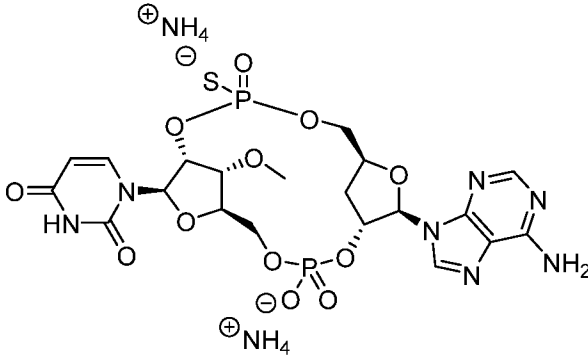
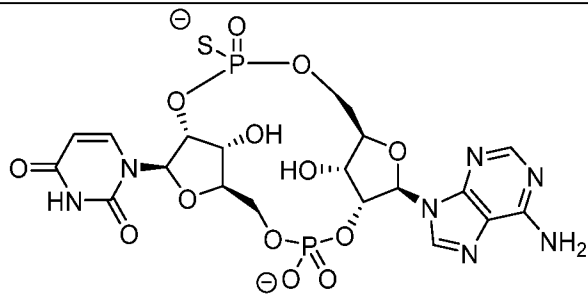
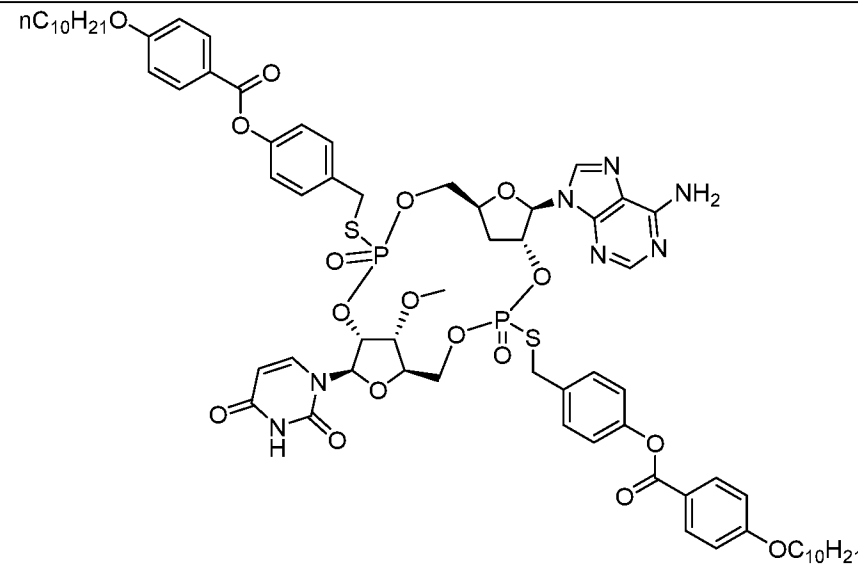
58	 Chemical structure 58 is a complex molecule featuring a central ribose derivative with a thiazine ring at C1, a thymine base at C2, and a 4'-phosphate group at C4'. The phosphate is linked via a triphosphate chain to a 4'-phosphate group on a ribose derivative, which is further linked via a thymine base to a 4'-phosphate group on a ribose derivative, which is finally linked via a thymine base to a 4'-phosphate group on a ribose derivative. The molecule is labeled with $nC_{10}H_{21}O$ and $C_{10}H_{21}O$.
59	 Chemical structure 59 is a complex molecule similar to 58, but with a different phosphate linkage and a thymine base at C2. The molecule is labeled with $nC_{10}H_{21}O$ and NH_4^+.

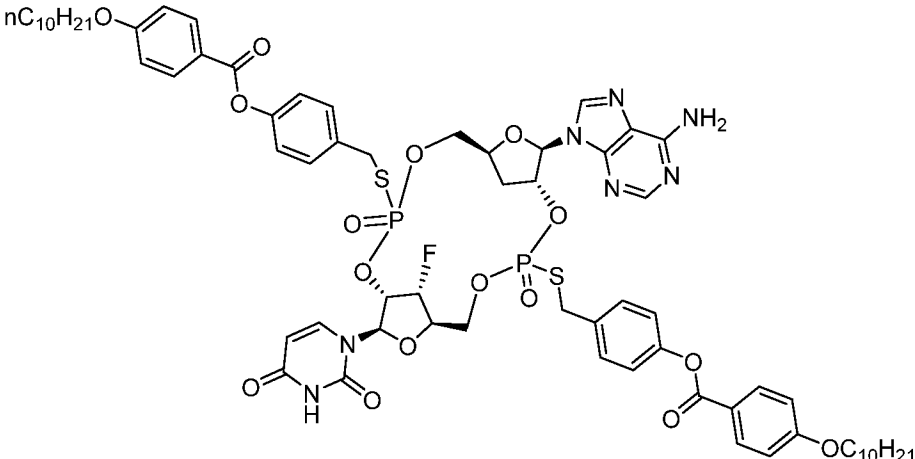
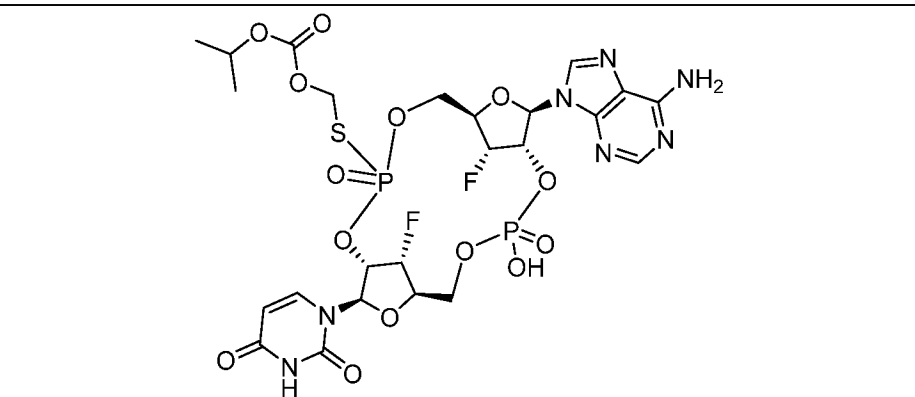
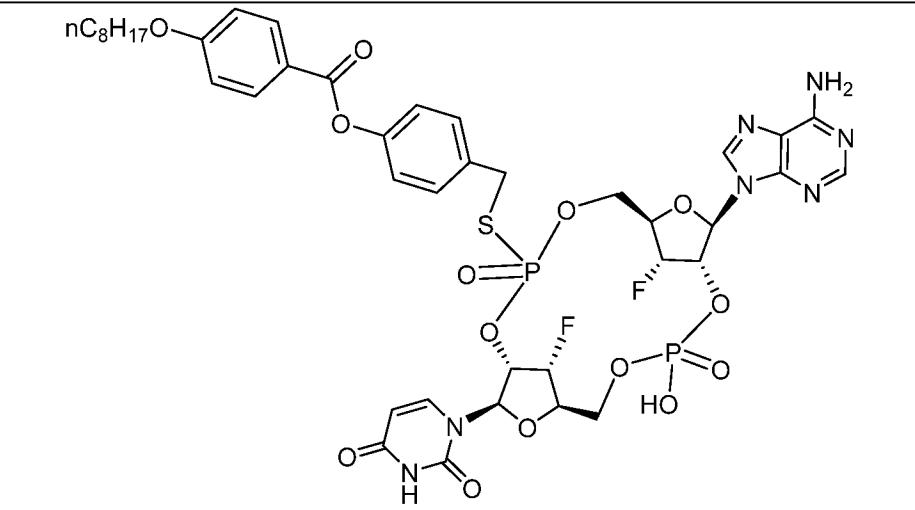
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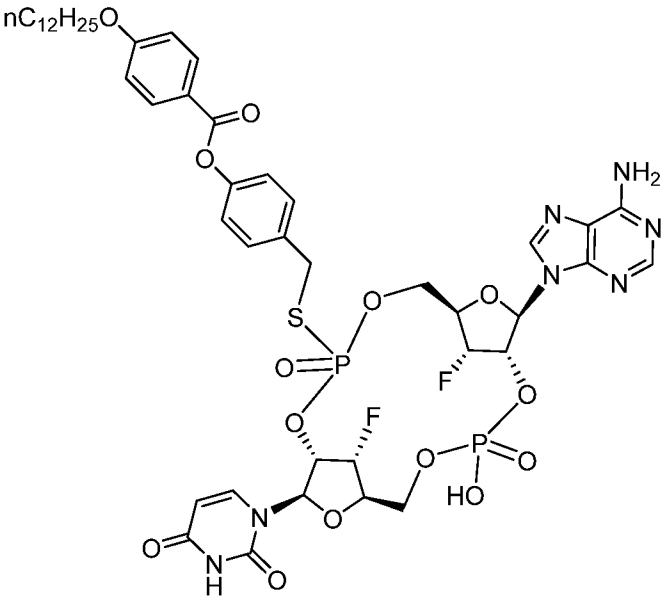
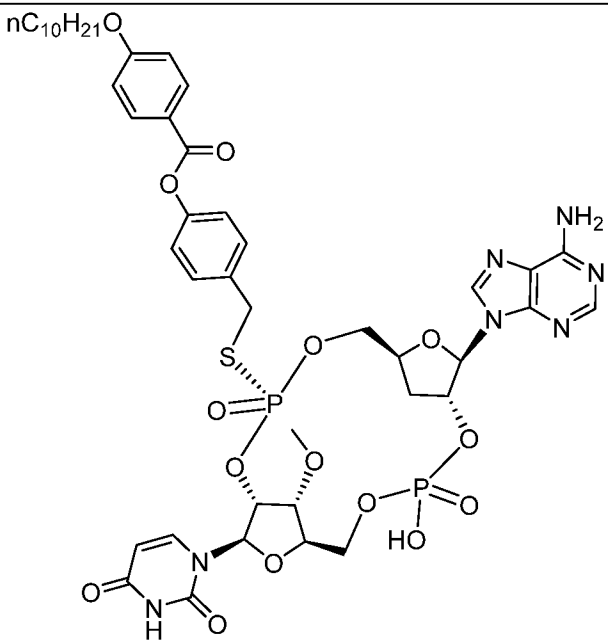
63	 Chemical structure 63: A complex molecule featuring a nucleoside core. The sugar moiety is a ribose derivative with a fluorine atom at the 2' position and a phosphate group at the 3' position. The phosphate group is linked to a thioether bridge, which is connected to a benzyl group. The benzyl group is further linked to a p-tert-butylbenzoate group. The base is an adenine derivative. The counterion is NH_4^+.
64	 Chemical structure 64: A complex molecule featuring a nucleoside core. The sugar moiety is a ribose derivative with a fluorine atom at the 2' position and a phosphate group at the 3' position. The phosphate group is linked to a thioether bridge, which is connected to a benzyl group. The benzyl group is further linked to a p-tert-butylbenzoate group. The base is an adenine derivative. The counterion is NH_4^+.
65	 Chemical structure 65: A complex molecule featuring a nucleoside core. The sugar moiety is a ribose derivative with a fluorine atom at the 2' position and a phosphate group at the 3' position. The phosphate group is linked to a thioether bridge, which is connected to a benzyl group. The benzyl group is further linked to a p-tert-butylbenzoate group. The base is an adenine derivative. The counterion is NH_4^+.

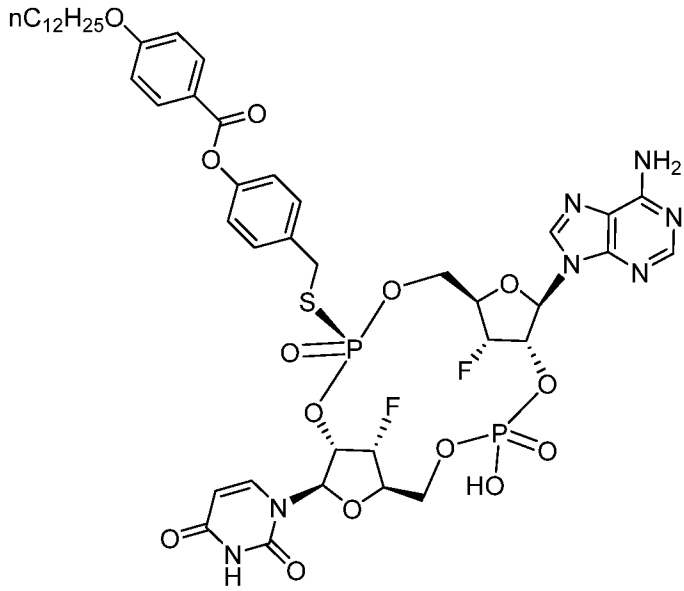
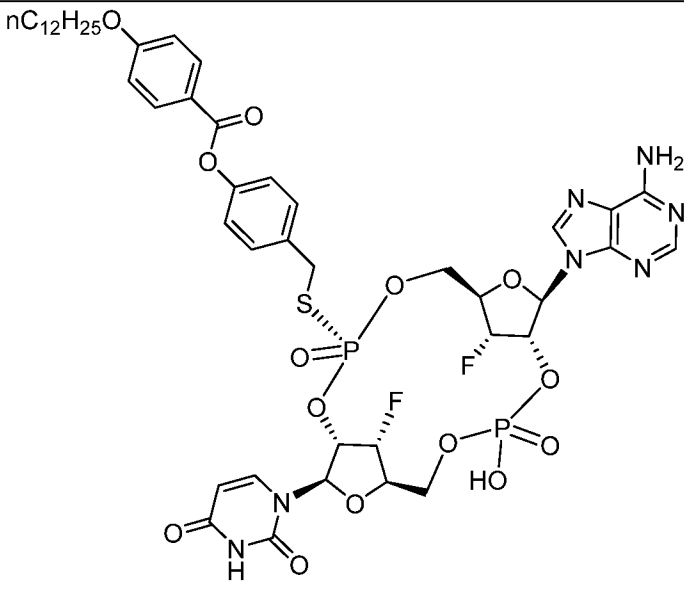
66	 Chemical structure 66 shows a dinucleotide d(GpCpGp) where both phosphate groups are protonated, resulting in two ammonium ions (NH_4^+).
67	 Chemical structure 67 shows a dinucleotide d(GpCpGp) with a 4-((isopropoxy carbonyl)oxy)benzylthio group attached to the 5' phosphate.
68	 Chemical structure 68 shows a dinucleotide d(GpCpGp) with a 4-((n-octyloxy carbonyl)oxy)benzylthio group attached to the 5' phosphate.

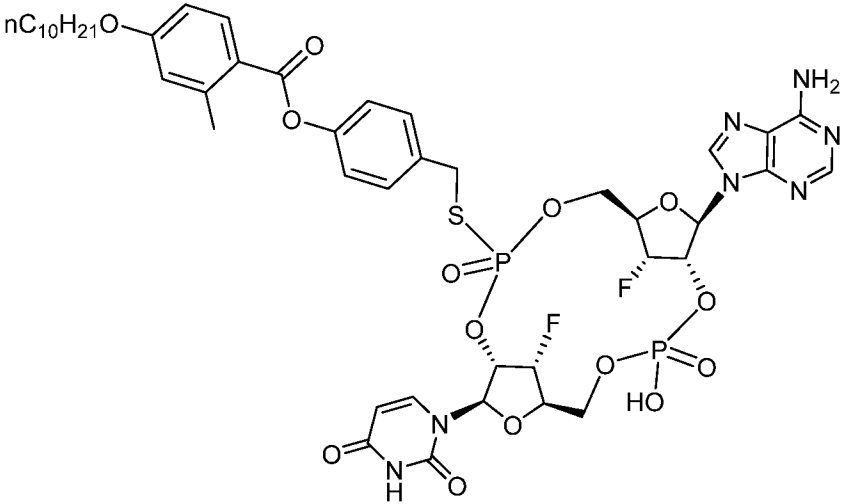
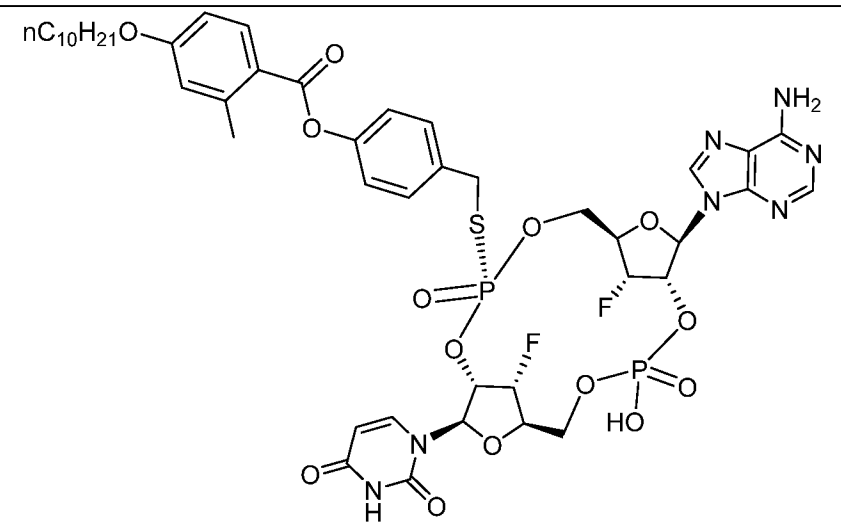
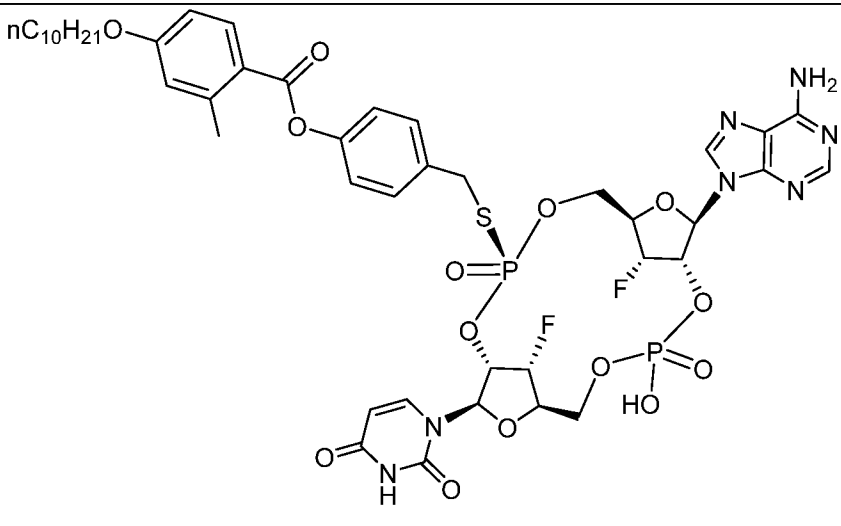
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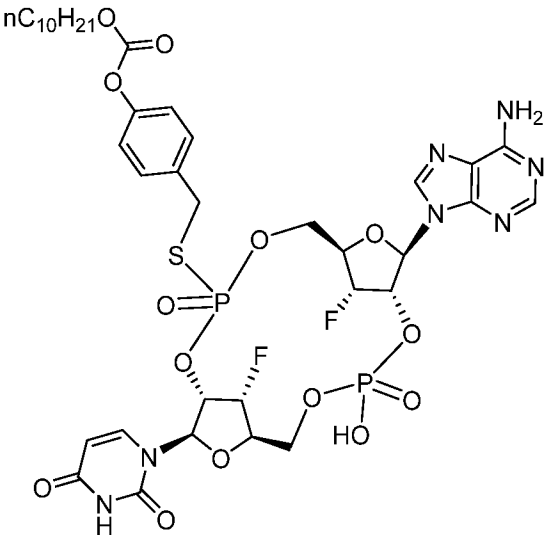
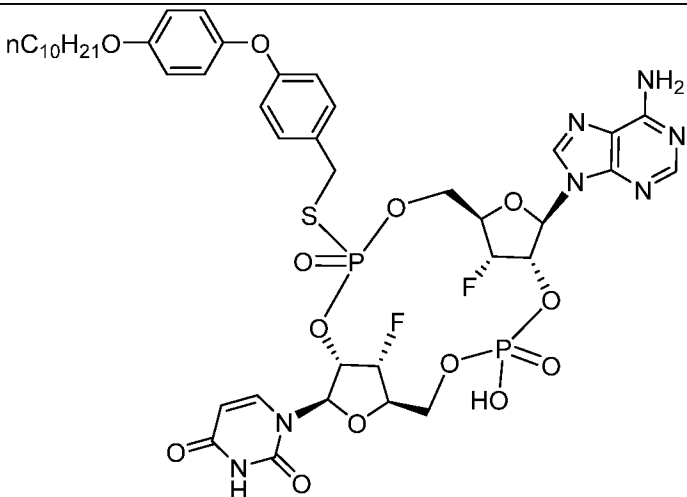
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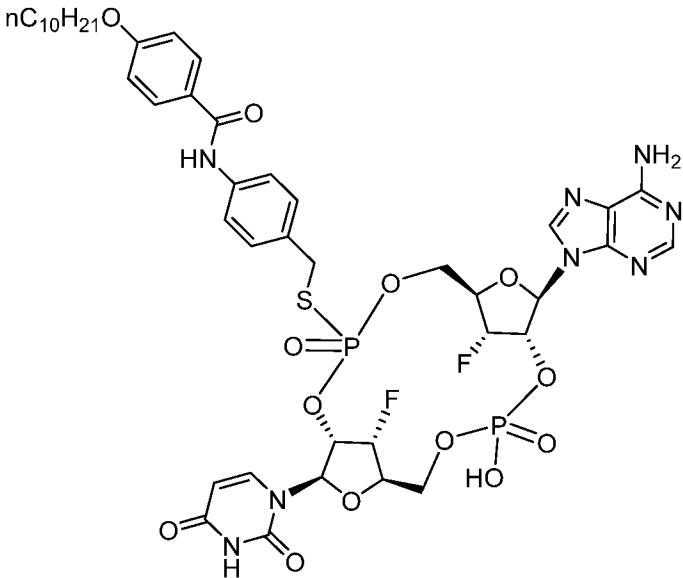
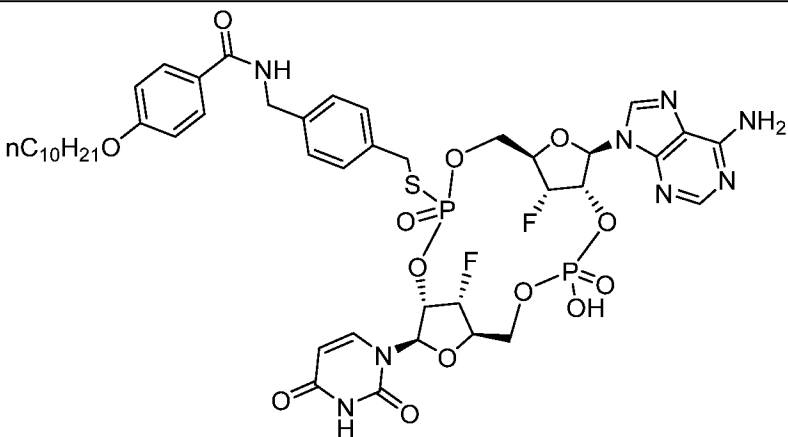
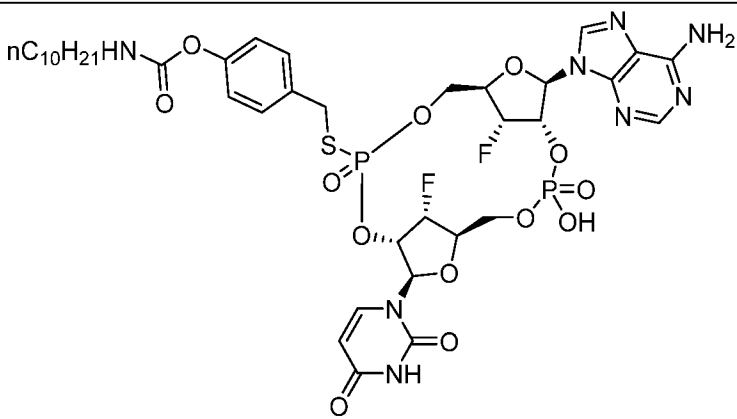
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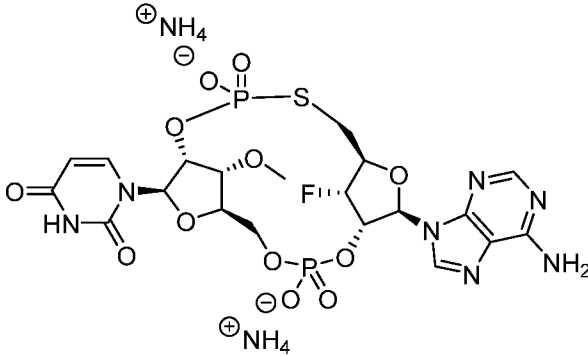
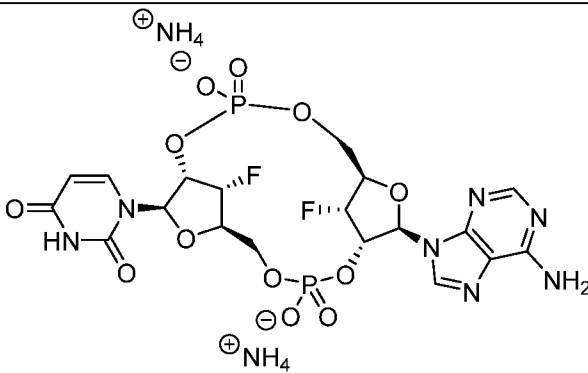
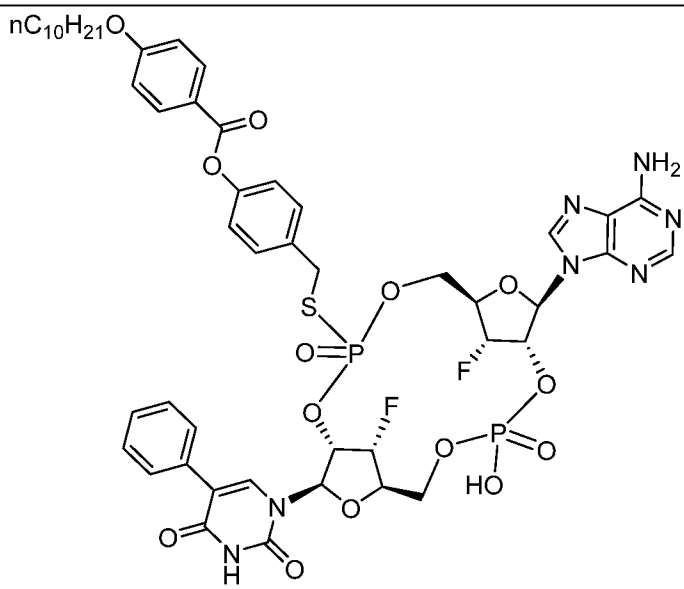
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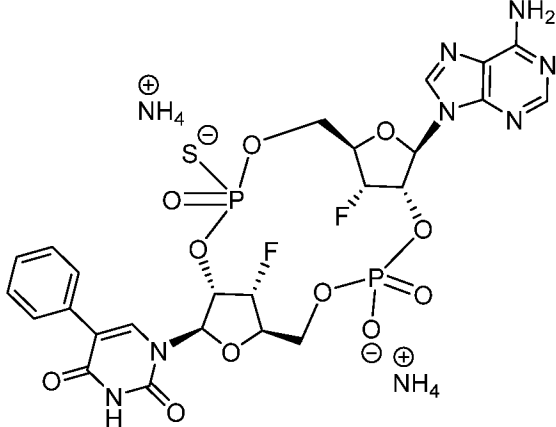
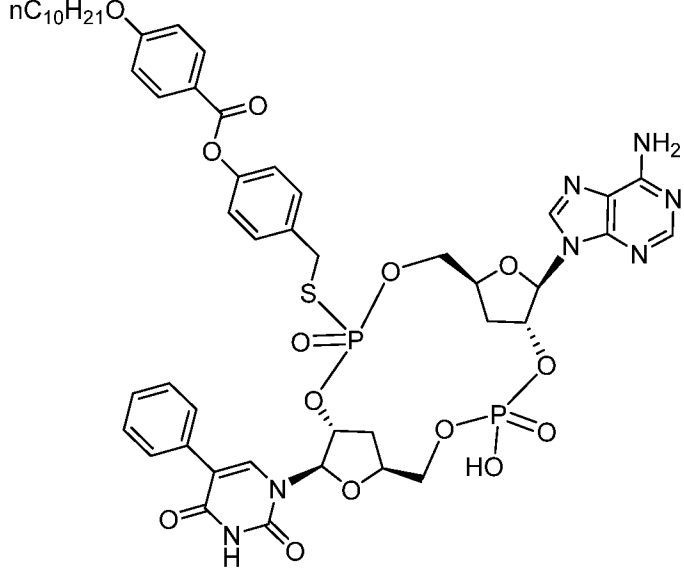
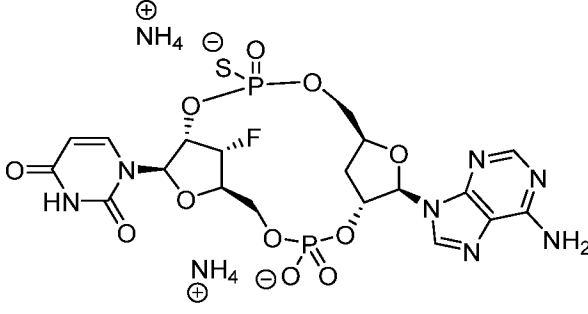
80	 Chemical structure 80: A complex molecule featuring a central phosphorus atom (P) bonded to a sulfur atom (S) and two oxygen atoms (O). The sulfur atom is part of a chain that includes a long alkyl chain (nC12H25O) and a benzene ring. The phosphorus atom is also bonded to a fluorine atom (F) and a hydroxyl group (HO). The molecule includes a pyrimidine ring system and a pyrazole ring system.
81	 Chemical structure 81: A complex molecule featuring a central phosphorus atom (P) bonded to a sulfur atom (S) and two oxygen atoms (O). The sulfur atom is part of a chain that includes a long alkyl chain (nC12H25O) and a benzene ring. The phosphorus atom is also bonded to a fluorine atom (F) and a hydroxyl group (HO). The molecule includes a pyrimidine ring system and a pyrazole ring system.

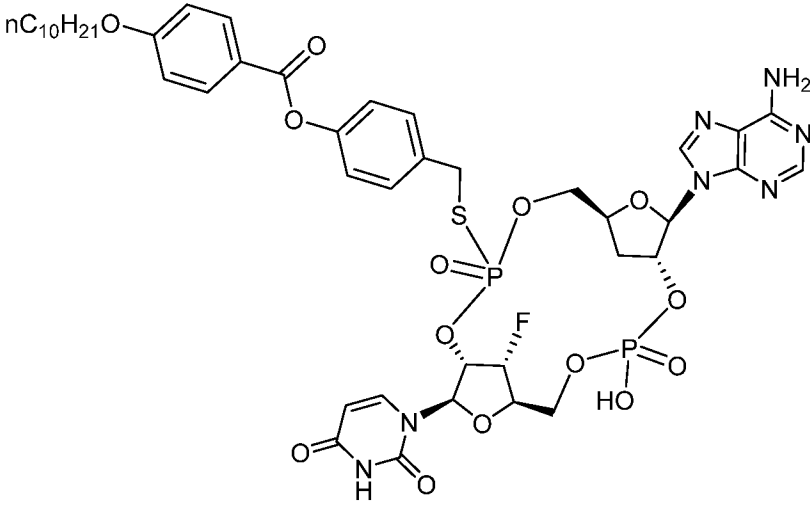
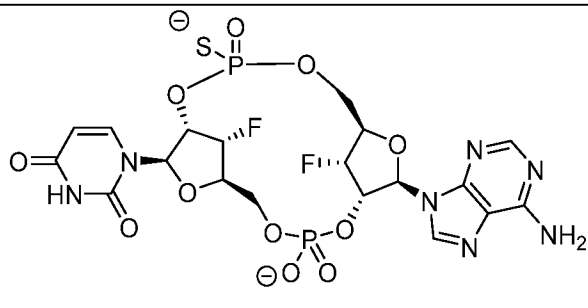
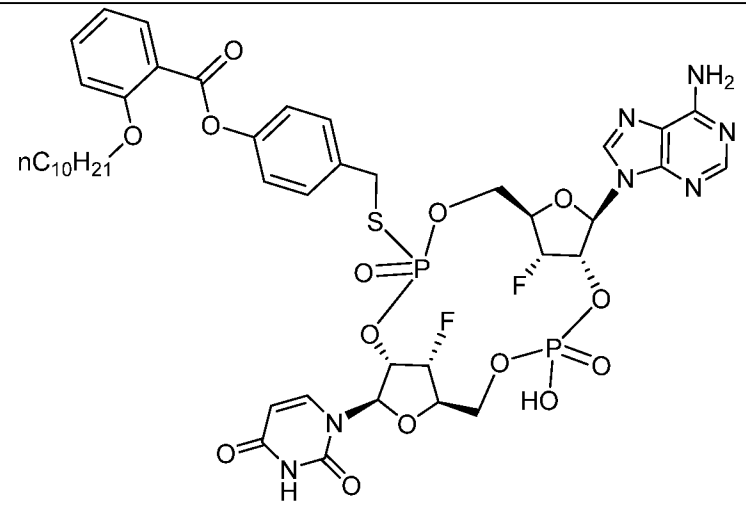
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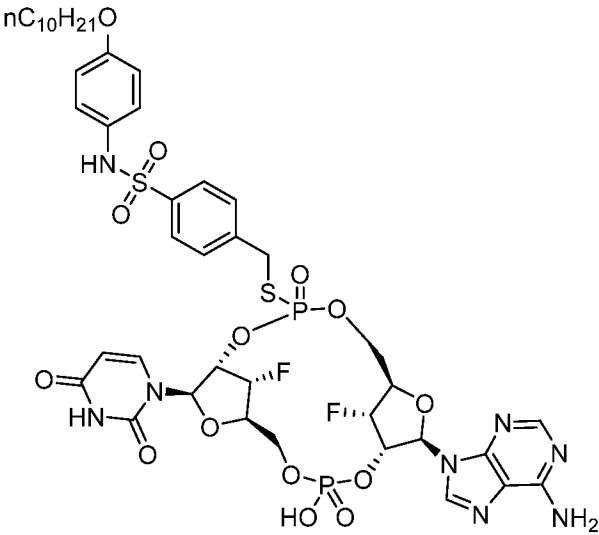
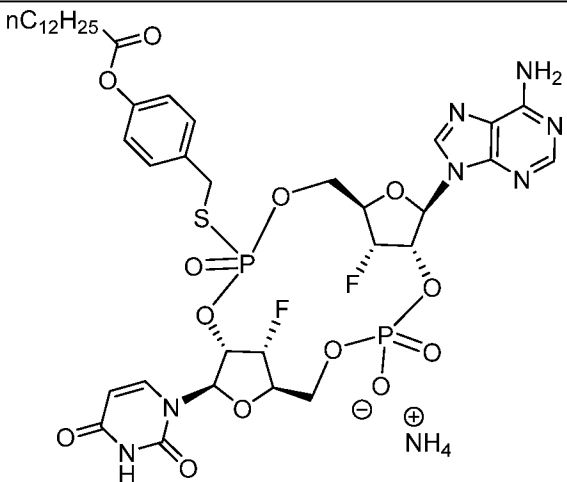
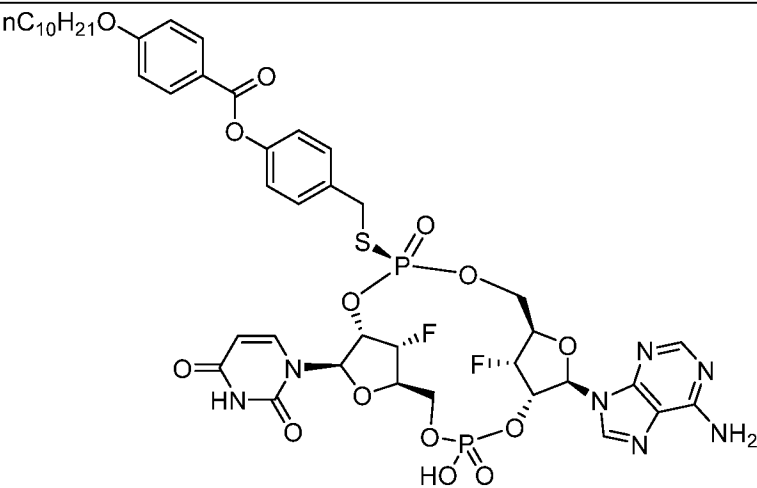
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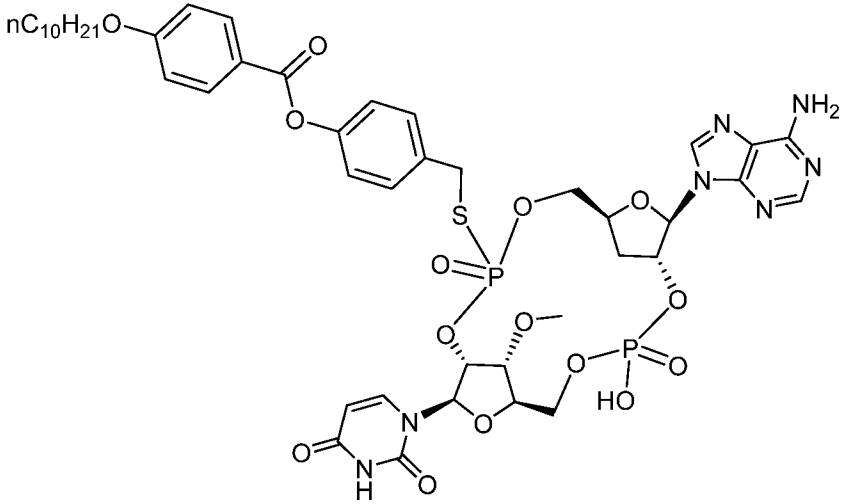
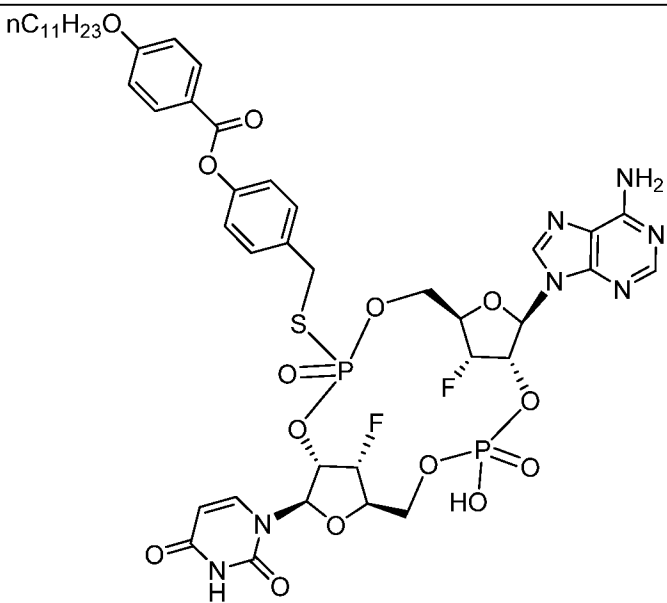
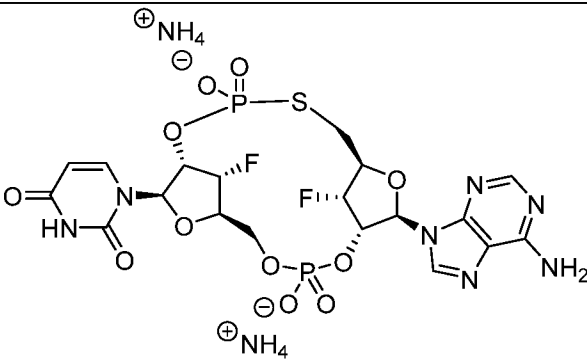
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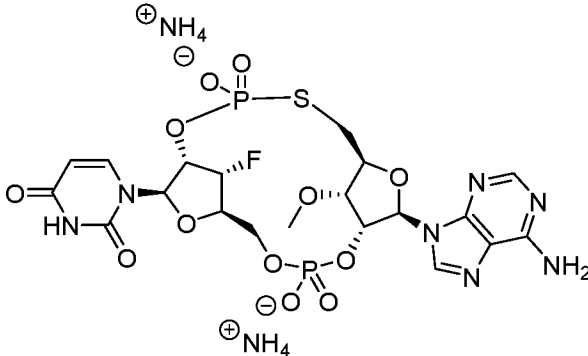
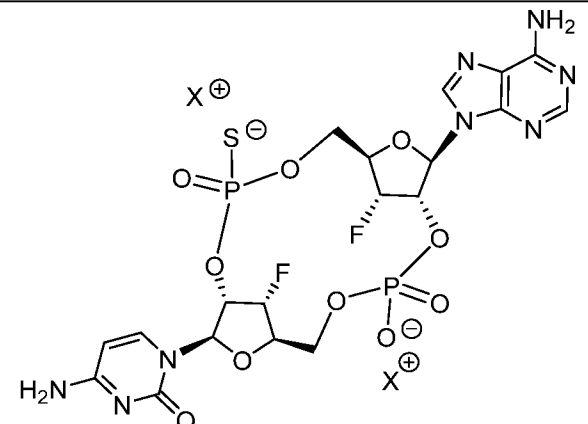
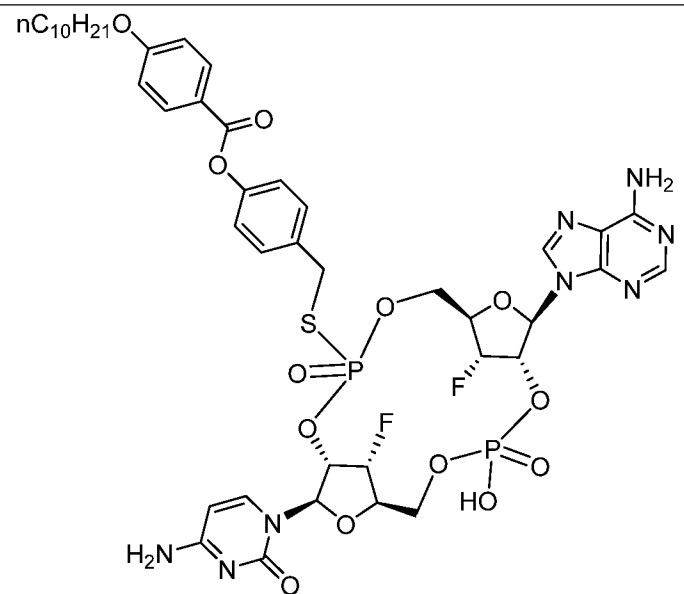
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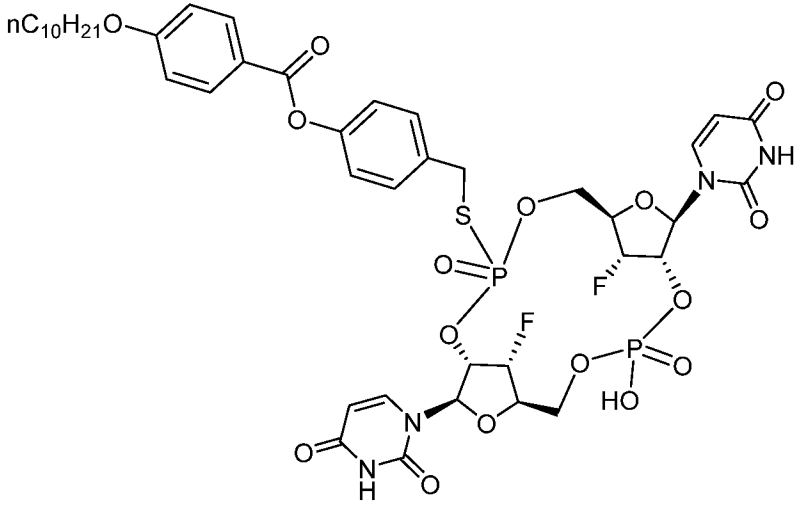
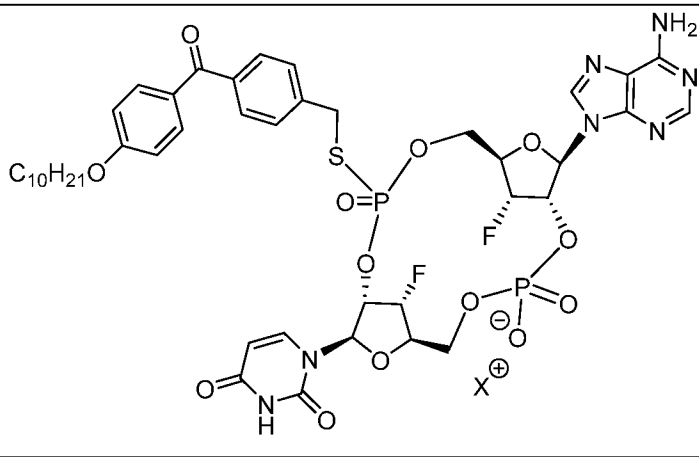
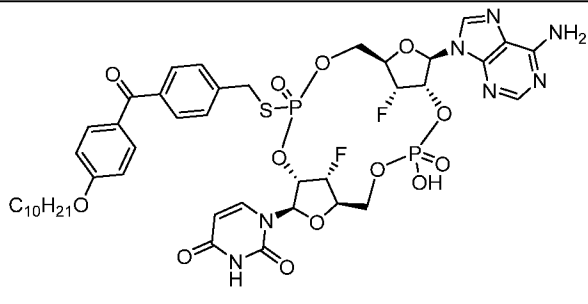
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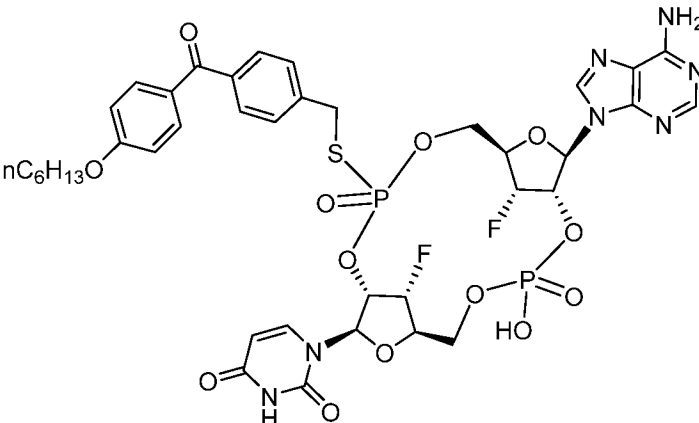
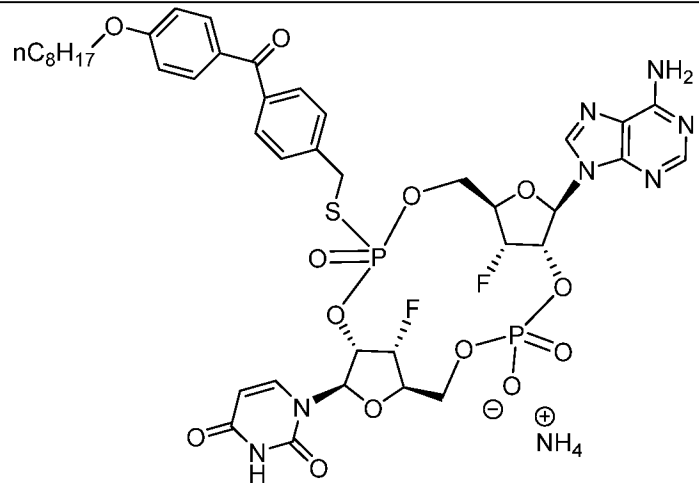
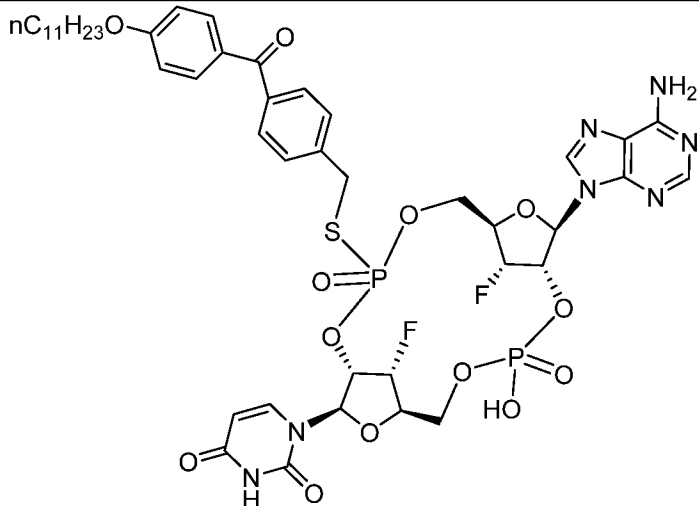
99	 Chemical structure 99: A complex molecule featuring a central sugar moiety (ribose) linked via phosphonate groups to a thymine base and an adenine base. The thymine base is further linked to a sulfonamide group, which is connected to a phenyl ring substituted with an $nC_{10}H_{21}O$ group.
100	 Chemical structure 100: A complex molecule featuring a central sugar moiety (ribose) linked via phosphonate groups to a thymine base and an adenine base. The thymine base is further linked to a sulfonamide group, which is connected to a phenyl ring substituted with an $nC_{12}H_{25}$ group. The molecule also includes a phosphate group and an ammonium ion (NH_4^+).
101	 Chemical structure 101: A complex molecule featuring a central sugar moiety (ribose) linked via phosphonate groups to a thymine base and an adenine base. The thymine base is further linked to a sulfonamide group, which is connected to a phenyl ring substituted with an $nC_{10}H_{21}O$ group.

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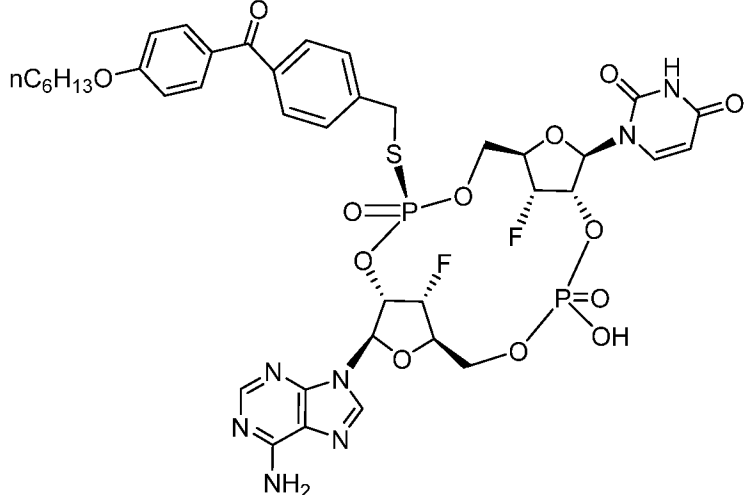
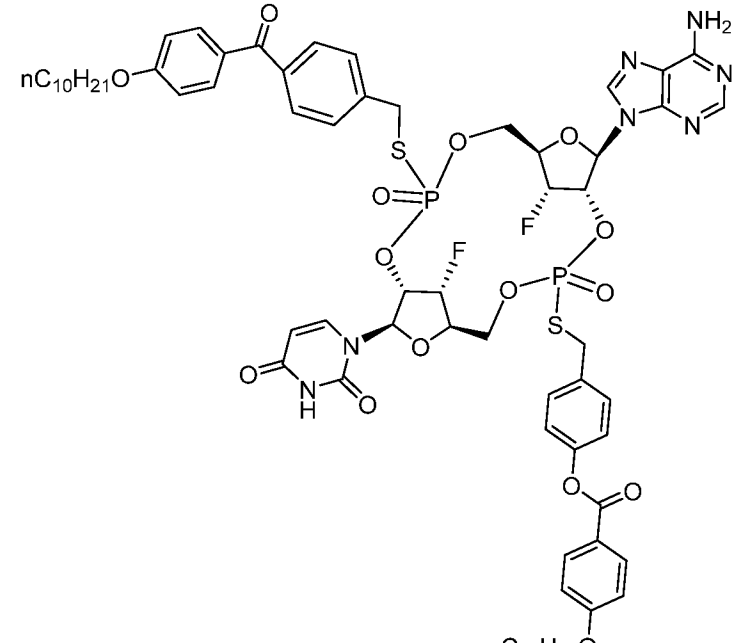
105	 Chemical structure 105: A complex molecule featuring a long alkyl chain ($nC_{10}H_{21}O$) attached to a benzene ring, which is linked via an ester to another benzene ring. This second benzene ring is connected to a sulfur atom, which is part of a cyclic phosphonate structure. The structure includes a pyrimidine ring, a ribose sugar, and a phosphate group.
106	 Chemical structure 106: Similar to structure 105, but with a different alkyl chain ($nC_{11}H_{23}O$) and a fluorine atom on the ribose sugar.
107	 Chemical structure 107: A complex molecule featuring a long alkyl chain ($nC_{10}H_{21}O$) attached to a benzene ring, which is linked via an ester to another benzene ring. This second benzene ring is connected to a sulfur atom, which is part of a cyclic phosphonate structure. The structure includes a pyrimidine ring, a ribose sugar, and a phosphate group.

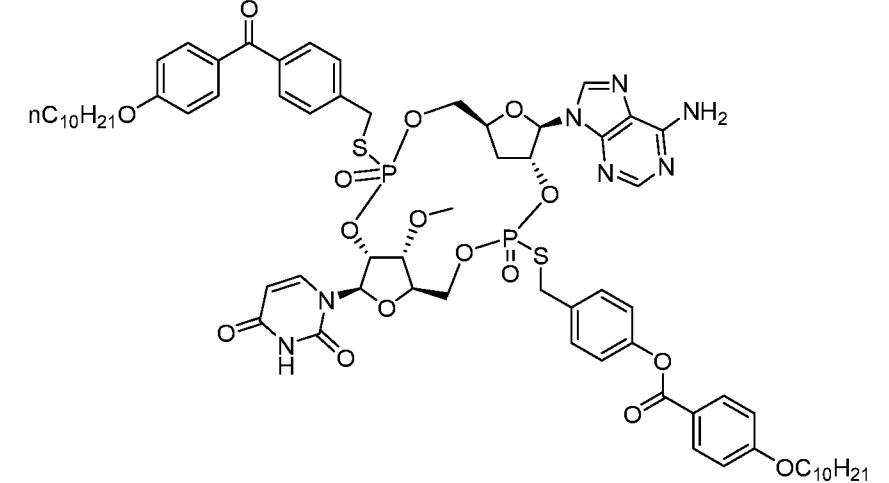
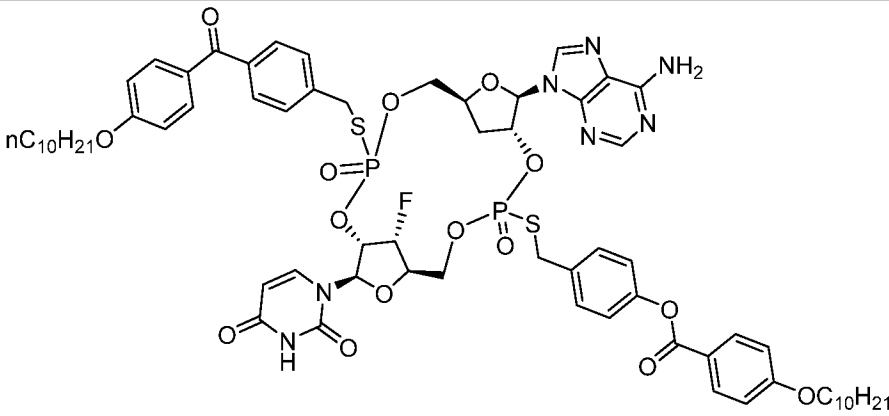
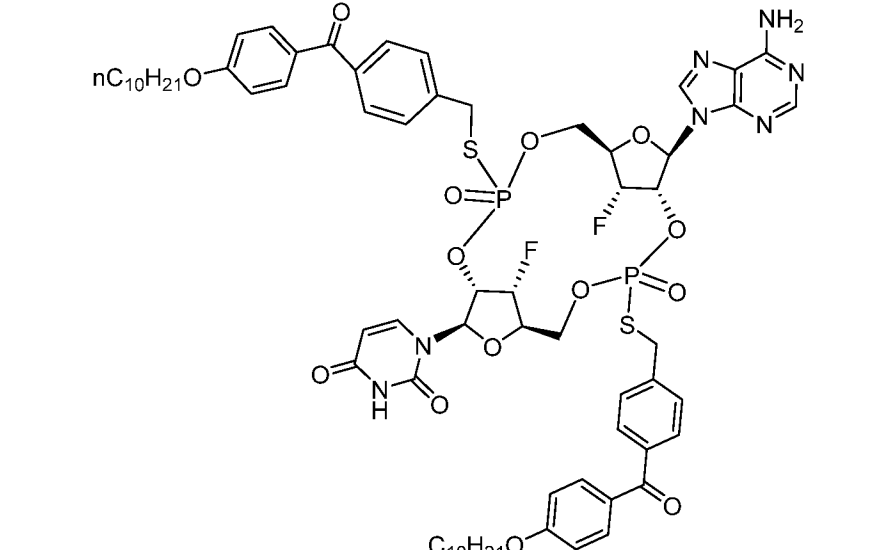
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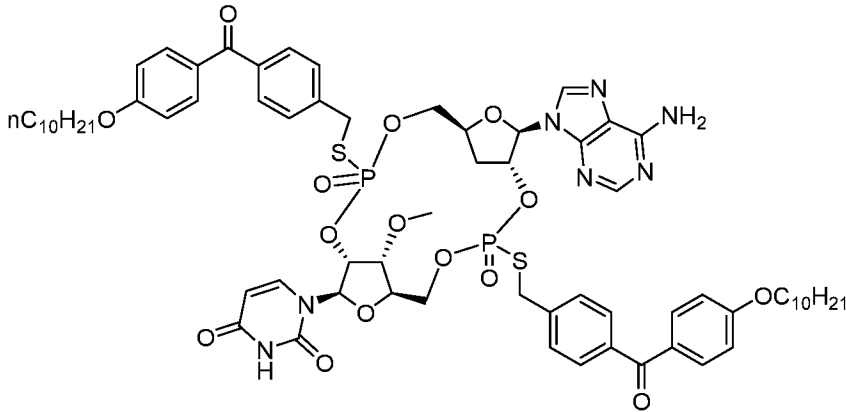
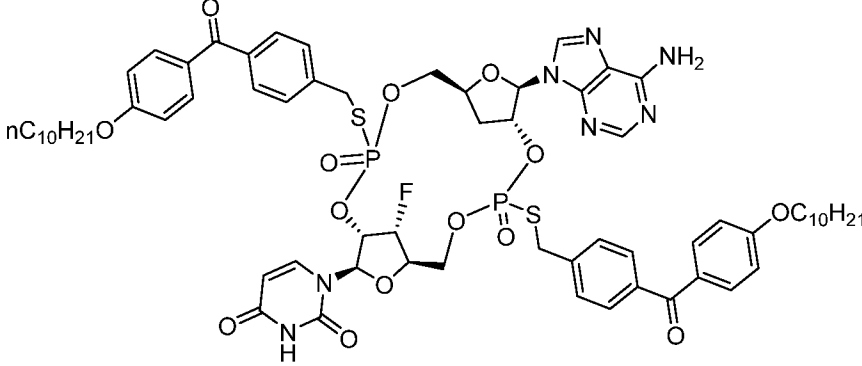
111	 Chemical structure 111: A complex molecule featuring a central sugar moiety (ribose) linked via a phosphonate group to a long alkyl chain (nC₁₀H₂₁O) and a thiazine ring system.
112	 Chemical structure 112: A complex molecule featuring a central sugar moiety (ribose) linked via a phosphonate group to a long alkyl chain (C₁₀H₂₁O) and a thiazine ring system, with a counterion X⁺.
113	 Chemical structure 113: A complex molecule featuring a central sugar moiety (ribose) linked via a phosphonate group to a long alkyl chain (C₁₀H₂₁O) and a thiazine ring system, with a counterion X⁺.

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120	 Chemical structure 120: A complex molecule featuring a central dinucleotide core. The left sugar is substituted with an adenine base (NH₂ group). The right sugar is substituted with a thymine base (methyl group). The phosphate group is linked to a long alkyl chain (nC₆H₁₃O) via a sulfonate group. The molecule also contains a fluorine atom and a hydroxyl group.
121	 Chemical structure 121: A complex molecule featuring a central dinucleotide core. The left sugar is substituted with an adenine base (NH₂ group). The right sugar is substituted with a thymine base (methyl group). The phosphate group is linked to a long alkyl chain (nC₁₀H₂₁O) via a sulfonate group. The molecule also contains a fluorine atom and a hydroxyl group.

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or a pharmaceutically acceptable salt thereof.

32. A method of treating cancer in a subject, comprising administering to the subject a therapeutically effective amount of a compound of any one of the preceding claims.

33. The method of claim 32, wherein the cancer is a cancer of the breast, bone, brain, cervix, colon, gastrointestinal tract, eye, gall bladder, lymph nodes, blood, lung, liver, skin, mouth, prostate, ovary, penis, pancreas, uterus, testicles, stomach, thymus, thyroid, or other part of the body.

34. The method of claim 33, wherein the cancer is a cancer of the liver.

35. The method of any one of claims 32-34, wherein the method comprises intratumoral administration.

36. The method of any one of claims 32-35, wherein the method comprises oral administration.
37. The method of any one of claims 32-35, wherein the method comprises parenteral administration (e.g., intravenous, subcutaneous, intraperitoneal, or intramuscular administration).
38. The method of claim 37, wherein the parenteral administration comprises intraperitoneal administration.
39. The method of any one of claims 32-38, further comprising administration of an additional agent (e.g., an anticancer agent).
40. The method of claim 39, wherein the additional agent comprises methotrexate, 5-fluorouracil, doxorubicin, vincristine, bleomycin, vinblastine, dacarbazine, topotecan, cisplatin, epirubicin, or sorafenib tosylate.
41. A method of inducing the expression of a pattern recognition receptors (PRRs) for immune-modulation in a subject, comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-31.
42. A method of inducing the expression of a pattern recognition receptors for immunomodulation and inducing a therapeutic response in a subject having cancer, comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-31.
43. A method of inducing an immune response in a subject, comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-31.
44. The method of claim 43, wherein the immune response comprises antitumoral immunity.
45. The method of claim 43 or 44, wherein the immune response comprises induction of a PRR (e.g., STING, RIG-I, MDA5).

46. A method of treating a microbial infection in a subject, comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-31.

47. A method of inducing the expression of a pattern recognition receptor in a subject suffering from a microbial infection, comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-31.

48. A method of treating a viral infection in a subject, comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-31.

49. The method of claim 48, wherein the viral infection is Hepatitis C virus, Norovirus, Junin virus, Respiratory syncytial virus, or Dengue virus.

Figure 1A

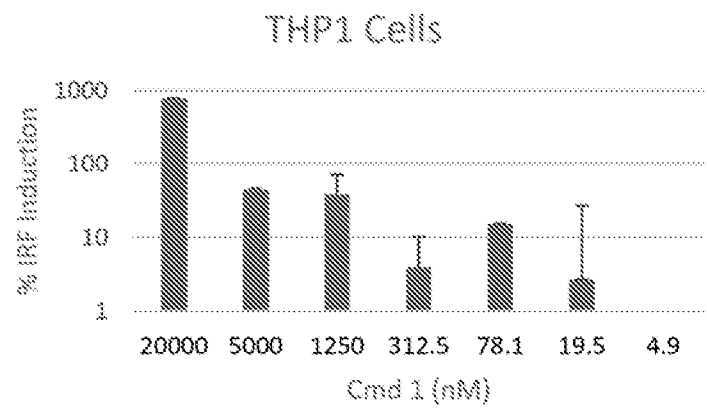


Figure 1B

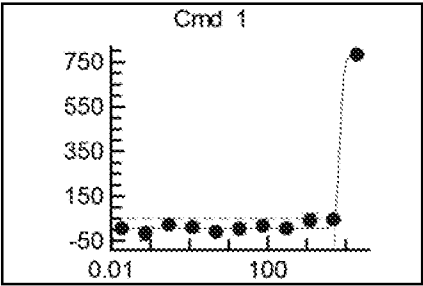


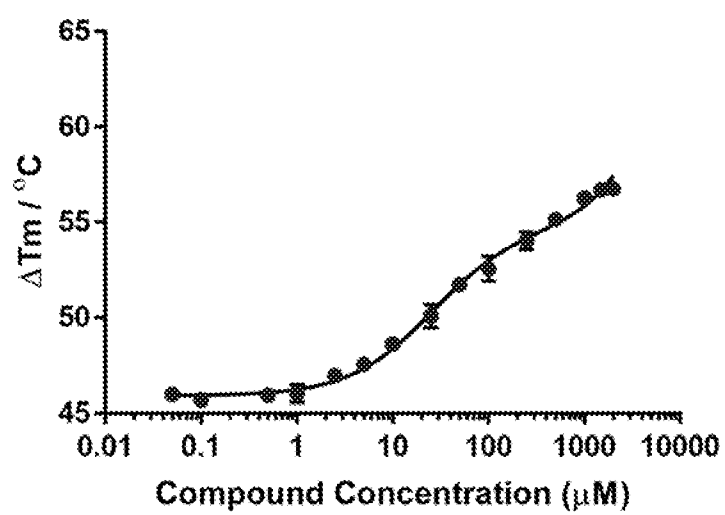
Figure 2

Figure 3A

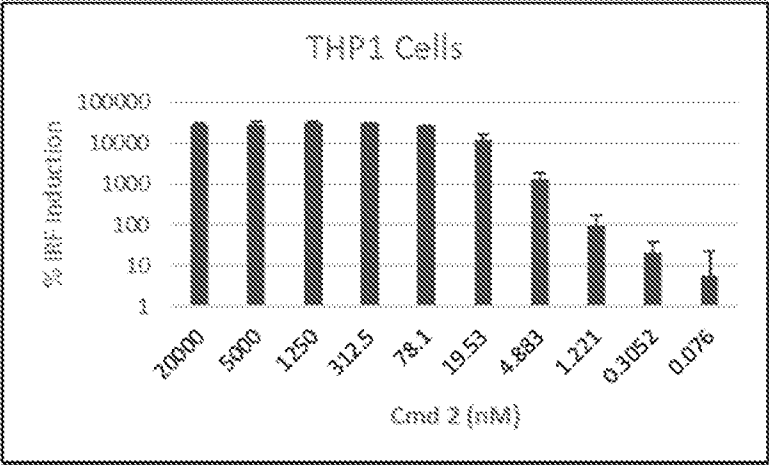


Figure 3B

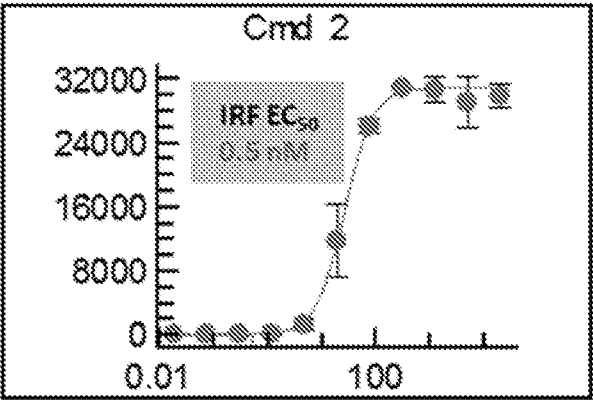


Figure 3C

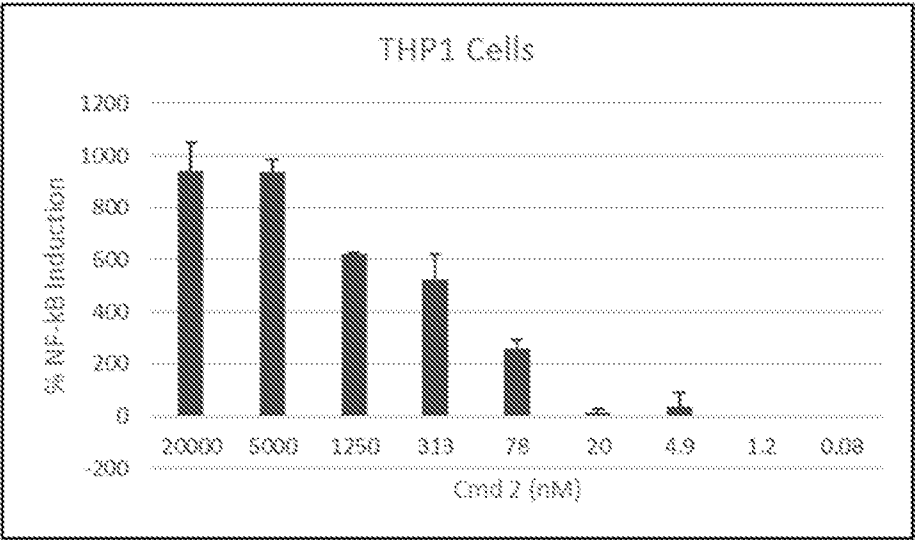


Figure 3D

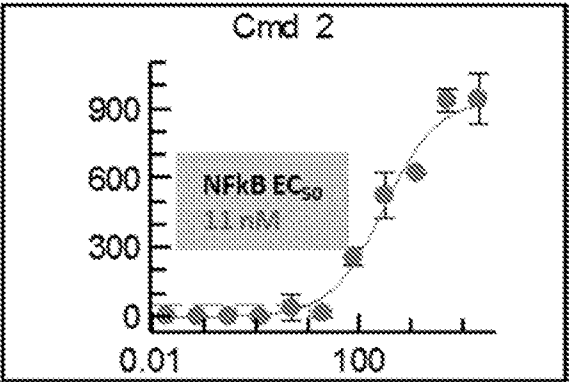


Figure 4A

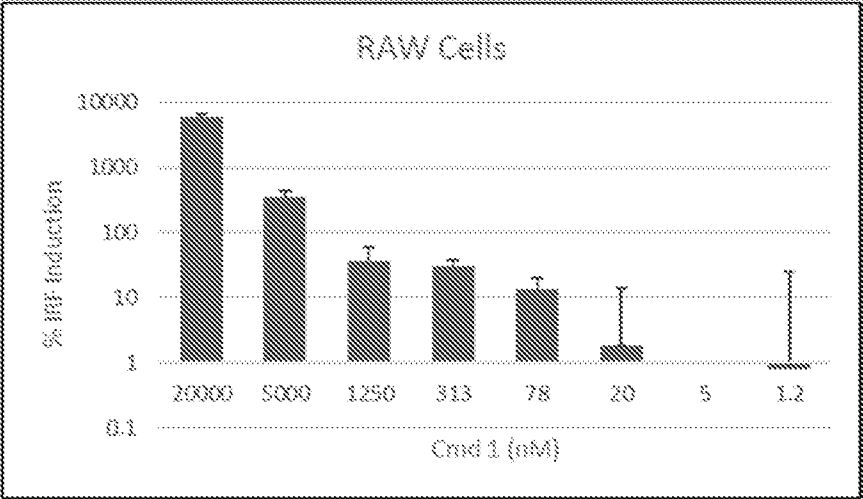


Figure 4B

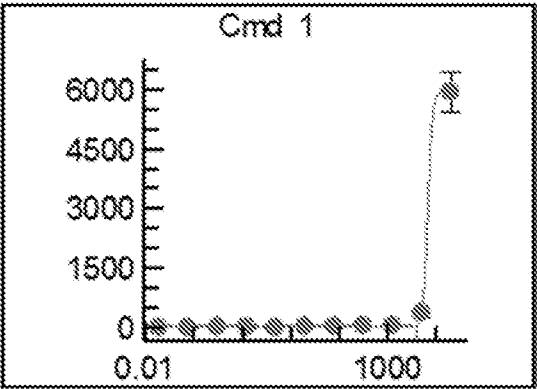


Figure 5A

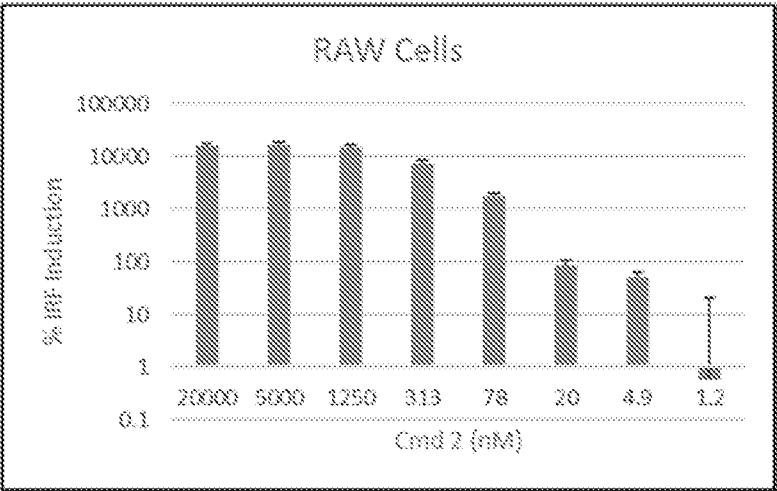
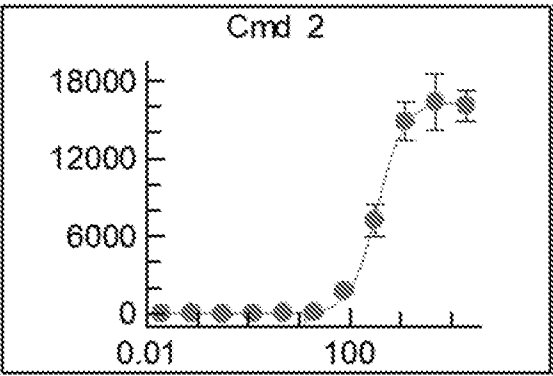


Figure 5B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/40811

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 31/522, 31/7084, 47/55 (2019.01)

CPC - A61K 31/522, 31/7084, 47/55

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.
X	US 2018/0118777 A1 (MEMORIAL SLOAN KETTERING CANCER CENTER, et al.) 03 May 2018; paragraphs [0030], [0156]-[0157], [0159]-[0165], [0167]	1-2, 4/1-2
Y	WO 2018/009648 A1 (SPEROVIE BIOSCIENCES, INC.) 11 January 2018; page 2, last paragraph; page 3, first paragraph; page 5, second paragraph; page 45, fourth structure	1-2, 3/1-2, 4/1-2, 31
Y	US 2017/0340658 A1 (INVIVOGEN) 30 November 2017; paragraph [0069]	1-2, 3/1-2, 4/1-2, 31

☐ 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

15 August 2019 (15.08.2019)

Date of mailing of the international search report

24 OCT 2019

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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/40811

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.: 5-30, 32-49
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:

----Continued Within the Next Supplemental Box----

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.:

1-2, 3/1-2, 4/1-2, 31
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/40811

-Continued from Box No. III Observations where unity of invention is lacking -

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 examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-4, 31; and a compound of Formula (I), wherein Z is S; each of B1 and B2 is independently a purinyl nucleobase; each of X1 and X2 is independently O; each of Y1 and Y2 is independently O; each of L1 and L2 is independently absent; each of R1 and R2 is independently hydrogen; and each of R3 and R4 is independently hydrogen (compound structure) are directed toward compounds.

The compounds will be searched to the extent the compound encompasses a compound of Formula (I), wherein Z is S; each of B1 and B2 is independently a purinyl nucleobase; each of X1 and X2 is independently O; each of Y1 and Y2 is independently O; each of L1 and L2 is independently absent; each of R1 and R2 is independently hydrogen; and each of R3 and R4 is independently hydrogen (first exemplary compound structure). Applicant is invited to elect additional compound(s), with fully specified structure (e.g. no optional or variable atoms, bonds, or substituents) for each, where available as an option in at least one searchable claim, to be searched. Additional compound(s) will be searched upon the payment of additional fees. It is believed that claims 1-2 (each in-part) and 4 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass a compound of Formula (I), wherein Z is S; each of B1 and B2 is independently a purinyl nucleobase; each of X1 and X2 is independently O; each of Y1 and Y2 is independently O; each of L1 and L2 is independently absent; each of R1 and R2 is independently hydrogen; and each of R3 and R4 is independently hydrogen (compound structure). Applicants must specify the searchable claims that encompass any additionally elected compound structure(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a compound of Formula (I), wherein Z is O; each of B1 and B2 is independently a purinyl nucleobase; each of X1 and X2 is independently O; each of Y1 and Y2 is independently O; each of L1 and L2 is independently absent; each of R1 and R2 is independently hydrogen; and each of R3 and R4 is independently hydrogen (compound structure).

Groups I+ share the technical features including: a compound of Formula (I).

However, these shared technical features are previously disclosed by WO 2018/009648 A1 (SPEROVIE BIOSCIENCES, INC.) (hereinafter 'Sperovie') in view of US 2017/0340658 A1 (INVIVOGEN) (hereinafter 'Invivogen').

Sperovie discloses a compound similar to a compound of Formula (I): as shown, wherein Z is S; each of B1 and B2 is independently a purinyl nucleobase; each of X1 and X2 is independently O; each of Y1 and Y2 is independently O; each of L1 and L2 is independently absent; each of R1 and R2 is independently hydrogen; and each of R3 and R4 is independently hydrogen (compound of formula I wherein X is S, B1 and B2 are purinyl nucleobases, X1 and X2 are O, Y1 and Y2 are O, L1 and L2 are absent, R1 and R2 are hydrogen, and R3 and R4 are hydrogen; page 2, last paragraph; page 3, first paragraph), but Sperovie does not disclose wherein the phosphate linkages are bound to the 2' positions of the ribose rings. However, Invivogen discloses a cyclic dinucleotide compound comprising phosphate linkages bound to the 2' positions of the ribose rings (cyclic dinucleotide of formula CL611 (ribose rings linked as the 2' positions); paragraph [0069]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the compound, as previously disclosed by Sperovie, in order to have provided a cyclic dinucleotide compound comprising phosphate linkages bound to the 2' positions of the ribose rings, as previously disclosed by Invivogen, for providing cyclic dinucleotide compounds useful as agonists of the STING receptor in the treatment of pancreatic cancer (Invivogen; abstract; Sperovie; abstract; page 62, second paragraph).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Sperovie and Invivogen references, unity of invention is lacking.