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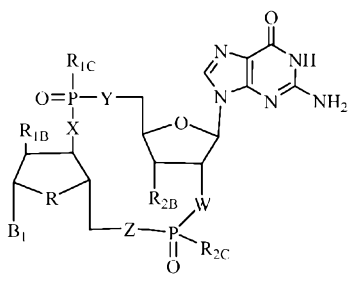
(54) Title: CYCLIC DINUCLEOTIDES AS STING AGONISTS

(57) Abstract: Disclosed are compounds, compositions and methods for treating of diseases, syndromes, or disorders that are affected by the modulation of STING. Such compounds are represented by Formula (I) as follows: wherein R, R_{1B}, R_{1C}, R_{2B}, R_{2C}, B_1, W, X, Y, Z are defined herein.



Formula (I)

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CYCLIC DINUCLEOTIDES AS STING AGONISTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This Application claims priority to United States Provisional Patent Application No. 62/451,301 filed January 27, 2017; United States Provisional Patent Application No. 62/454,135, filed February 3, 2017; and United States Provisional Patent Application No. 62/451,351, filed January 27, 2017; which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to novel compounds which are STING (Stimulator of Interferon Genes) agonists and are useful for the treatment of disorders that are affected by the modulation of the STING protein. The invention also relates to pharmaceutical compositions comprising one or more of such compounds, processes to prepare such compounds and compositions, and use of such compounds or pharmaceutical compositions for the treatment of various diseases, syndromes and disorders. The invention may be involved in the activation of the downstream signaling pathway, further resulting in the activation of second messengers and growth factors, and the production of interferon involved in the innate and adaptive immunity. More particularly, the present invention relates to the use of such compounds or pharmaceutical compositions for the treatment of various infections, diseases, syndromes and disorders including, but not limited to, melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and antiviral therapy.

BACKGROUND OF THE INVENTION

STING (stimulator of interferon genes), also known as TMEM173, MITA, MPYS, and ERIS, is a transmembrane receptor located inside the cell and a key sensor of cytosolic nucleic acids (Zhong B, et al. “The Adaptor Protein MITA Links Virus-Sensing Receptors to IRF3

Transcription Factor Activation". *Immunity*. 2008. vol. 29: 538-550). Recent studies have revealed the biology of STING and its role in mobilizing an innate immune response resulting in robust antitumor activity in mouse models. Activation of the STING pathway results in production of Type I interferons (mainly IFN-α and IFN-β) induced through the IRF3 (interferon regulatory factor 3) pathway. Activation of IRF3 is thought to be mediated by TBK1 that recruits and phosphorylates IRF3 thus forming an IRF3 homodimer capable of entering the nucleus to transcribe type I interferon and other genes (Liu S, et al. "Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation" *Science*. 2015: 2630-2637). TBK1 also activates the nuclear factor kappa-light-chain-enhancer of activated B cells pathway which leads to production of pro-inflammatory cytokines (IL-1α, IL-1β, IL-2, IL-6, TNF-α, etc.), via the oncogenic transcription factor NF-κB. In addition, STING activates STAT6 (signal transducer and activator of transcription 6) to induce (Th2-type), increase (IL-12) or decrease (IL-10) production of various cytokines, including the chemokines CCL2, CCL20, and CCL26 (Chen H, et al. "Activation of STAT6 by STING Is Critical for Antiviral Innate Immunity" *Cell.* 2011, vol.14: 433-446). Direct phosphorylation of STING on Ser366 upon activation has also been reported to occur through TBK1 or ULK1 (Corrales, L. et al "Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity" *Cell Reports*, 2015, vol.11: 1-13; Konno, H. et al. "Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling" *Cell*, 2013, vol. 155: 688-698).

The natural ligand that binds to and activates STING (2',3')cyclic guanosine monophosphate-adenosine monophosphate (2',3'-cGAMP) and the enzyme responsible for its synthesis (cGAS, also known as C6orf150 or MB21D1) have been elucidated providing an opportunity to modulate this pathway. cGAMP is a high affinity ligand for STING produced in mammalian cells that serves as an endogenous second messenger to activate the STING pathway. It is a cyclic dinucleotide with a unique 2',3' linkage produced by cGAS in the presence of exogenous double-stranded DNA (e.g. that released by invading bacteria, viruses or protozoa) or of self-DNA in mammals (Wu et al., 2013; Sun, L. et al. "Cyclic GMP-AMP

Synthase Is a Cytosolic DNA Sensor That Activates the Type I Interferon Pathway" *Science*, 2013, vol. 339: 786-791; Bhat N and Fitzgerald KA. "Recognition of Cytosolic DNA by cGAS and other STING-dependent sensors". *Eur J Immunol.* 2014 Mar; 44(3):634-40). STING activation can also occur through binding of exogenous (3',3) cyclic dinucleotides (c-di-GMP, c-di-AMP and 3'3'-cGAMP) that are released by invading bacteria (Zhang X, et al. "Cyclic GMP-AMP Containing Mixed Phosphodiester Linkages Is An Endogenous High-Affinity Ligand for STING" *Molecular Cell*, 2013, vol. 51: 226-235; Danilchanka, O and Mekalanos, JJ. "Cyclic Dinucleotides and the Innate Immune Response" *Cell.* 2013. vol. 154: 962-970).

Activation of the STING pathway triggers an immune response that results in generation of specific killer T-cells that can shrink tumors and provide long lasting immunity so they do not recur. The striking antitumor activity obtained with STING agonists in preclinical models has generated a high level of excitement for this target and small molecule compounds that can modulate the STING pathway have potential to treat both cancer and reduce autoimmune diseases.

Activation of the STING pathway also contributes to an antiviral response. Loss-of-functional response, either at the cellular or organism level, demonstrates an inability to control viral load in the absence of STING. Activation of the STING pathway triggers an immune response that results in antiviral and proinflammatory cytokines that combat the virus and mobilize the innate and adaptive arms of the immune system. Ultimately, long-lasting immunity is developed against the pathogenic virus. The striking antiviral activity obtained with STING agonists in preclinical models has generated a high level of excitement for this target and small molecule compounds that can modulate the STING pathway have potential to treat chronic viral infections, such as hepatitis B.

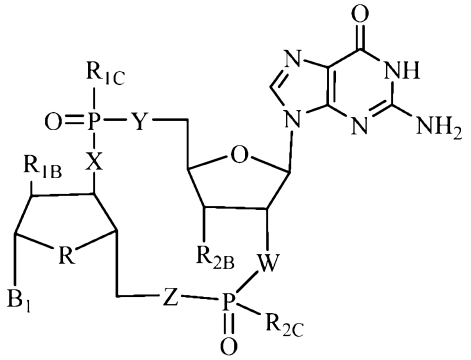
Chronic hepatitis B virus (HBV) infection is a significant global health problem, affecting over 5% of the world population (over 350 million people worldwide and 1.25 million individuals in the U.S.). Despite the availability of certain HBV vaccines and therapies, the burden of chronic HBV infection continues to be a significant unmet worldwide medical problem due to suboptimal treatment options and sustained rates of new infections in most parts

of the developing world. Current treatments are limited to only two classes of agents: interferon alpha and nucleoside analogues acting as inhibitors of the viral polymerase. Yet none of these therapies offer a cure to the disease, and drug resistance, low efficacy, and tolerability issues limit their impact. The low cure rates of HBV are attributed at least in part to the fact that complete suppression of virus production is difficult to achieve with a single antiviral agent. However, persistent suppression of HBV DNA slows liver disease progression and helps to prevent hepatocellular carcinoma. Current therapy goals for HBV-infected patients are directed to reducing serum HBV DNA to low or undetectable levels, and to ultimately reducing or preventing the development of cirrhosis and hepatocellular carcinoma. There is, therefore, a need in the art for therapeutic agents that can increase the suppression of virus production and that can treat, ameliorate, or prevent HBV infection. Administration of such therapeutic agents to an HBV infected patient, either as monotherapy or in combination with other HBV treatments or ancillary treatments, may lead to significantly reduced virus burden, improved prognosis, diminished progression of the disease and enhanced seroconversion rates.

The potential therapeutic benefits of enhancing both innate and adaptive immunity make STING an attractive therapeutic target that demonstrates impressive activity by itself and can also be combined with other immunotherapies.

SUMMARY OF THE INVENTION

The present invention is directed to compounds of Formula (I)



Formula (I)

wherein

R is CH_2 or O;

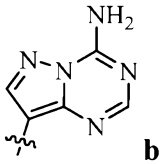
R_{1B} is hydrogen, hydroxy, or fluoro;

R_{1C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

R_{2B} is hydrogen, hydroxy, methoxy, or fluoro;

R_{2C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, **b7** and **b8**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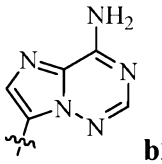
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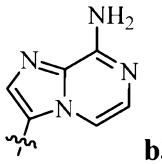
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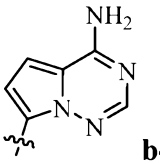
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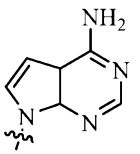
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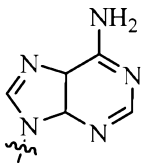
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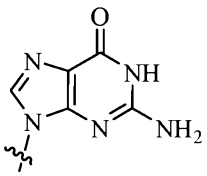
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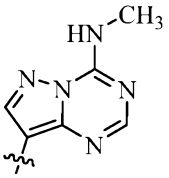

b8;

;

W is -O- or -NH-;

X is -O- or -NH-;

Y is $-CH_2-$, -O- or -NH-;

Z is $-CH_2-$, -O- or -NH-;

such that only one of X and Y is NH, and only one of W and Z is NH, in any instance;

and, such that when R is CH_2 and B_1 is selected from **b6** or **b7**, then R_{2B} is other than fluoro or hydroxy;

furthermore, provided that a compound of Formula (I) is other than a compound wherein R, W, X, Y, and Z, are each O; R_{1B} and R_{2B} are each hydroxy; B_1 is **b1**; and R_{1B} and R_{2B} are each hydroxy;

or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

The present invention also provides a pharmaceutical composition comprising, consisting of and/or consisting essentially of a pharmaceutically acceptable carrier, a pharmaceutically acceptable excipient, and/or a pharmaceutically acceptable diluent and a compound of Formula (I), or a pharmaceutically acceptable salt form thereof.

Also provided are processes for making a pharmaceutical composition comprising, consisting of, and/or consisting essentially of admixing a compound of Formula (I), and a pharmaceutically acceptable carrier, a pharmaceutically acceptable excipient, and/or a pharmaceutically acceptable diluent.

The present invention further provides methods for treating or ameliorating a viral infection, disease, syndrome, or condition in a subject, including a mammal and/or human in which the viral infection, disease, syndrome, or condition is affected by the agonism of STING, using a compound of Formula (I).

The present invention further provides methods for treating or ameliorating a viral infection, disease, syndrome, or condition in a subject, including a mammal and/or human, using a compound of Formula (I).

The present invention further provides methods for treating or ameliorating a viral infection, disease, syndrome, or condition in a subject, including a mammal and/or human in

which the viral infection, disease, syndrome, or condition is affected by the agonism of STING, selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B, using a compound of Formula (I).

The present invention further provides methods for treating or ameliorating a viral infection, disease, syndrome, or condition in a subject, including a mammal and/or human, selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B, using a compound of Formula (I).

The present invention is also directed to the use of any of the compounds described herein in the preparation of a medicament wherein the medicament is prepared for treating a viral infection, disease, syndrome, or condition that is affected by the agonism of STING, selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B, in a subject in need thereof.

The present invention is also directed to the use of any of the compounds described herein in the preparation of a medicament wherein the medicament is prepared for treating a viral infection, disease, syndrome, or condition selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B, in a subject in need thereof.

The present invention is also directed to the preparation of substituted cyclic dinucleotide derivatives that act as selective agonists of STING.

Exemplifying the invention are methods of treating a viral infection, disease, syndrome, or condition modulated by STING selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B, comprising administering to a subject in need thereof a therapeutically effective amount of any of the compounds or pharmaceutical compositions described above.

Exemplifying the invention are methods of treating a viral infection, disease, syndrome, or condition selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B, comprising administering to a subject

in need thereof a therapeutically effective amount of any of the compounds or pharmaceutical compositions described above.

In another embodiment, the present invention is directed to a compound of Formula (I) for use in the treatment of a viral infection, disease, syndrome, or condition affected by the agonism of STING selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B.

In another embodiment, the present invention is directed to a composition comprising a compound of Formula (I) for the treatment of a viral infection, disease, syndrome, or condition selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B.

DETAILED DESCRIPTION OF THE INVENTION

With reference to substituents, the term "independently" refers to the situation where when more than one substituent is possible, the substituents may be the same or different from each other.

The term "alkyl" whether used alone or as part of a substituent group, refers to straight and branched carbon chains having 1 to 8 carbon atoms. Therefore, designated numbers of carbon atoms (e.g., C_{1-8}) refer independently to the number of carbon atoms in an alkyl moiety or to the alkyl portion of a larger alkyl-containing substituent. In substituent groups with multiple alkyl groups such as, $(C_{1-6}alkyl)_2amino$-, the $C_{1-6}alkyl$ groups of the dialkylamino may be the same or different.

The term "alkoxy" refers to an -O-alkyl group, wherein the term "alkyl" is as defined above.

The terms "alkenyl" and "alkynyl" refer to straight and branched carbon chains having 2 to 8 carbon atoms, wherein an alkenyl chain contains at least one double bond and an alkynyl chain contains at least one triple bond.

The term "cycloalkyl" refers to saturated or partially saturated, monocyclic or

polycyclic hydrocarbon rings of 3 to 14 carbon atoms. Examples of such rings include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and adamantyl.

The term "heterocyclyl" refers to a nonaromatic monocyclic or bicyclic ring system having 3 to 10 ring members that include at least 1 carbon atom and from 1 to 4 heteroatoms independently selected from N, O, and S. Included within the term heterocyclyl is a nonaromatic cyclic ring of 5 to 7 members in which 1 to 2 members are N, or a nonaromatic cyclic ring of 5 to 7 members in which 0, 1 or 2 members are N and up to 2 members are O or S and at least one member must be either N, O, or S; wherein, optionally, the ring contains 0 to 1 unsaturated bonds, and, optionally, when the ring is of 6 or 7 members, it contains up to 2 unsaturated bonds. The carbon atom ring members that form a heterocycle ring may be fully saturated or partially saturated. The term "heterocyclyl" also includes two 5 membered monocyclic heterocycloalkyl groups bridged to form a bicyclic ring. Such groups are not considered to be fully aromatic and are not referred to as heteroaryl groups. When a heterocycle is bicyclic, both rings of the heterocycle are non-aromatic and at least one of the rings contains a heteroatom ring member. Examples of heterocycle groups include, and are not limited to, pyrrolinyl (including 2*H*-pyrrole, 2-pyrrolinyl or 3-pyrrolinyl), pyrrolidinyl, imidazolinyl, imidazolidinyl, pyrazolinyl, pyrazolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, and piperazinyl. Unless otherwise noted, the heterocycle is attached to its pendant group at any heteroatom or carbon atom that results in a stable structure.

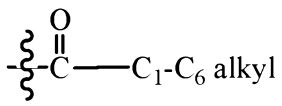
The term "aryl" refers to an unsaturated, aromatic monocyclic or bicyclic ring of 6 to 10 carbon members. Examples of aryl rings include phenyl and naphthalenyl. The term "heteroaryl" refers to an aromatic monocyclic or bicyclic aromatic ring system having 5 to 10 ring members and which contains carbon atoms and from 1 to 4 heteroatoms independently selected from the group consisting of N, O, and S. Included within the term heteroaryl are aromatic rings of 5 or 6 members wherein the ring consists of carbon atoms and has at least one heteroatom member. Suitable heteroatoms include nitrogen, oxygen, and sulfur. In the case of 5 membered rings, the heteroaryl ring preferably contains one member of nitrogen, oxygen or sulfur and, in addition, up to 3 additional nitrogens. In the

case of 6 membered rings, the heteroaryl ring preferably contains from 1 to 3 nitrogen atoms. For the case wherein the 6 membered ring has 3 nitrogens, at most 2 nitrogen atoms are adjacent. Examples of heteroaryl groups include furyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolyl, isoindolyl, benzofuryl, benzothienyl, indazolyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, benzisoxazolyl, benzothiadiazolyl, benzotriazolyl, quinolinyl, isoquinolinyl and quinazolinyl. Unless otherwise noted, the heteroaryl is attached to its pendant group at any heteroatom or carbon atom that results in a stable structure.

The term "halogen" or "halo" refers to fluorine, chlorine, bromine and iodine atoms.

Whenever the term "alkyl" or "aryl" or either of their prefix roots appear in a name of a substituent (e.g., arylalkyl, alkylamino) the name is to be interpreted as including those limitations given above for "alkyl" and "aryl." Designated numbers of carbon atoms (e.g., C_1-C_6) refer independently to the number of carbon atoms in an alkyl moiety, an aryl moiety, or in the alkyl portion of a larger substituent in which alkyl appears as its prefix root. For alkyl and alkoxy substituents, the designated number of carbon atoms includes all of the independent members included within a given range specified. For example C_{1-6} alkyl would include methyl, ethyl, propyl, butyl, pentyl and hexyl individually as well as sub-combinations thereof (e.g., C_{1-2}, C_{1-3}, C_{1-4}, C_{1-5}, C_{2-6}, C_{3-6}, C_{4-6}, C_{5-6}, C_{2-5}, etc.).

In general, under standard nomenclature rules used throughout this disclosure, the terminal portion of the designated side chain is described first followed by the adjacent functionality toward the point of attachment. Thus, for example, a "C_1-C_6 alkylcarbonyl" substituent refers to a group of the formula:



The term "R" at a stereocenter designates that the stereocenter is purely of the *R*-configuration as defined in the art; likewise, the term "S" means that the stereocenter is

purely of the *S*-configuration. As used herein, the terms "*R" or "*S" at a stereocenter are used to designate that the stereocenter is of pure but unknown configuration. As used herein, the term "RS" refers to a stereocenter that exists as a mixture of the *R*- and *S*-configurations. Similarly, the terms "*RS" or "*SR" refer to a stereocenter that exists as a mixture of the *R*- and *S*-configurations and is of unknown configuration relative to another stereocenter within the molecule.

Compounds containing one stereocenter drawn without a stereo bond designation are a mixture of two enantiomers. Compounds containing two stereocenters both drawn without stereo bond designations are a mixture of four diastereomers. Compounds with two stereocenters both labeled "RS" and drawn with stereo bond designations are a two-component mixture with relative stereochemistry as drawn. Compounds with two stereocenters both labeled "*RS" and drawn with stereo bond designations are a two-component mixture with relative stereochemistry unknown. Unlabeled stereocenters drawn without stereo bond designations are a mixture of the *R*- and *S*-configurations. For unlabeled stereocenters drawn with stereo bond designations, the absolute stereochemistry is as depicted.

Unless otherwise noted, it is intended that the definition of any substituent or variable at a particular location in a molecule be independent of its definitions elsewhere in that molecule. It is understood that substituents and substitution patterns on the compounds of the present invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art as well as those methods set forth herein.

The term "subject" refers to an animal, preferably a mammal, most preferably a human, who has been the object of treatment, observation or experiment.

The term "therapeutically effective amount" refers to an amount of an active compound or pharmaceutical agent, including a compound of the present invention, which elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation or partial alleviation of the symptoms of the disease, syndrome,

condition, or disorder being treated.

The term "composition" refers to a product that includes the specified ingredients in therapeutically effective amounts, as well as any product that results, directly, or indirectly, from combinations of the specified ingredients in the specified amounts.

The term "STING agonist" is intended to encompass a compound that interacts with STING by binding to it and inducing downstream signal transduction characterized by activation of the molecules associated with STING function. This includes direct phosphorylation of STING, IRF3 and/or NF-κB and could also include STAT6. STING pathway activation results in increased production of type I interferons (mainly IFN-α and IFN-β) and expression of interferon-stimulated genes (Chen H, et al. "Activation of STAT6 by STING Is Critical for Antiviral Innate Immunity". *Cell.* 2011, vol.14: 433-446; and Liu S-Y, et al. "Systematic identification of type I and type II interferon-induced antiviral factors". *Proc. Natl. Acad. Sci.* 2012:vol.109 4239-4244).

The term "STING-modulated" is used to refer to a condition affected by STING directly or via the STING pathway, including but not limited to, viral infections, diseases or conditions such as melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B infection.

As used herein, unless otherwise noted, the term "disorder modulated by STING" shall mean any viral infection, disease, disorder or condition characterized in that at least one of its characteristic symptoms is alleviated or eliminated upon treatment with a STING agonist. Suitable examples include, but are not limited to melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B.

As used herein, unless otherwise noted, the term "affect" or "affected" (when referring to a viral infection, disease, syndrome, condition or disorder that is affected by agonism of STING) includes a reduction in the frequency and / or severity of one or more symptoms or manifestations of said viral infection, disease, syndrome, condition or disorder; and / or include the prevention of the development of one or more symptoms or manifestations of said viral infection, disease, syndrome, condition or disorder or the development of the viral infection, disease, condition, syndrome or disorder.

The compounds of the instant invention are useful in methods for treating or ameliorating a viral infection, disease, a syndrome, a condition or a disorder that is affected by the agonism of STING. Such methods comprise, consist of and/or consist essentially of administering to a subject, including an animal, a mammal, and a human in need of such treatment, amelioration and / or prevention, a therapeutically effective amount of a compound of Formula (I), or an enantiomer, diastereomer, solvate or pharmaceutically acceptable salt thereof.

In particular, the compounds of Formula (I), or an enantiomer, diastereomer, solvate or pharmaceutically acceptable salt form thereof are useful for treating or ameliorating diseases, syndromes, conditions, or disorders such as melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B.

More particularly, the compounds of Formula (I), or an enantiomer, diastereomer, solvate or pharmaceutically acceptable salt form thereof are useful for treating or ameliorating melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula (I), or an enantiomer, diastereomer, solvate or pharmaceutically acceptable salt form thereof as herein defined.

Some embodiments disclosed herein relate to methods of ameliorating and/or treating a viral infection including infections caused by *Hepadnaviridae* such as hepatitis B virus or HBV. The methods can include administering to a subject identified as suffering from a viral infection an effective amount of one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, or a pharmaceutical composition that includes one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof.

Other embodiments disclosed herein relate to a method of ameliorating and/or treating a viral infection that can include contacting a cell infected with the virus with an effective amount of one or more compounds described herein (for example, a compound of Formula (I), or a pharmaceutically acceptable salt form thereof), or a pharmaceutical composition that includes one or more compounds described herein, or a pharmaceutically acceptable salt thereof. Still other embodiments described herein relate to using one or more compounds of Formula (I), or a

pharmaceutically acceptable salt form thereof, in the manufacture of a medicament for ameliorating and/or treating a viral infection.

Yet still other embodiments described herein relate to one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, or a pharmaceutical composition that includes one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, that can be used for ameliorating and/or treating a viral infection. Some embodiments disclosed herein relate to a method of inhibiting replication of a virus that can include contacting a cell infected with the virus with an effective amount of one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, or a pharmaceutical composition that includes one or more compounds described herein, or a pharmaceutically acceptable salt form thereof.

Other embodiments described herein relate to using one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof) in the manufacture of a medicament for inhibiting replication of a virus. Still other embodiments described herein relate to one or more compounds described herein (for example, a compound of Formula (I), or a pharmaceutically acceptable salt form thereof), or a pharmaceutical composition that includes one or more compounds described herein, or a pharmaceutically acceptable salt form thereof, that can be used for inhibiting replication of a virus.

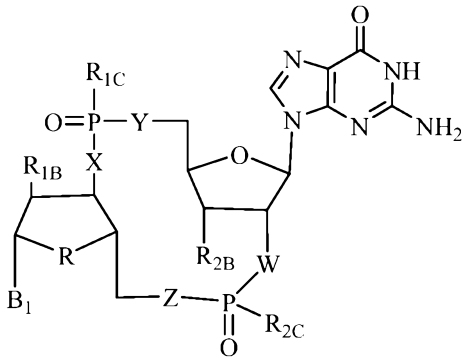
In some embodiments, the viral infection can be a hepatitis B viral infection. The methods can include administering to a subject identified as suffering from HBV an effective amount of one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, or a pharmaceutical composition that includes one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof.

Other embodiments disclosed herein relate to a method of ameliorating and/or treating a viral infection that can include contacting a cell infected with HBV with an effective amount of one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, or a pharmaceutical composition that includes one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof. Still other embodiments described herein relate to using one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, in the manufacture of a medicament for ameliorating and/or treating HBV.

Yet still other embodiments described herein relate to one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, or a pharmaceutical composition that includes one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, that can be used for ameliorating and/or treating HBV. Some embodiments disclosed herein relate to a method of inhibiting replication of HBV that can include contacting a cell infected with the virus with an effective amount of one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, or a pharmaceutical composition that includes one or more compounds of Formula (I), or a pharmaceutically acceptable salt thereof.

Other embodiments described herein relate to using one or more compounds of Formula (I), or a pharmaceutically acceptable salt thereof) in the manufacture of a medicament for inhibiting replication of HBV. Still other embodiments described herein relate to one or more compounds of Formula (I), or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition that includes one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, that can be used for inhibiting replication of HBV.

An embodiment of the present invention is directed to a compound of Formula (I)



Formula (I)

wherein

R is CH_2 or O;

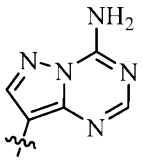
R_{1B} is hydrogen, hydroxy, or fluoro;

R_{1C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

R_{2B} is hydrogen, hydroxy, methoxy, or fluoro;

R_{2C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, and **b8**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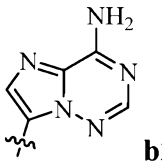
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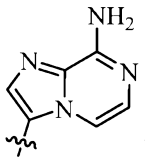
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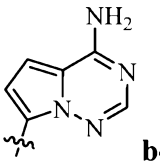
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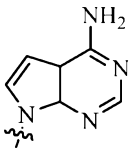
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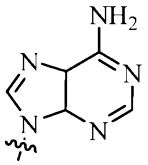
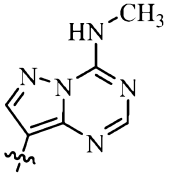

b6, and

b8;

W is -O- or -NH-;

X is -O- or -NH-;

Y is $-CH_2-$, -O-, or -NH-;

Z is $-CH_2-$, -O-, or -NH-;

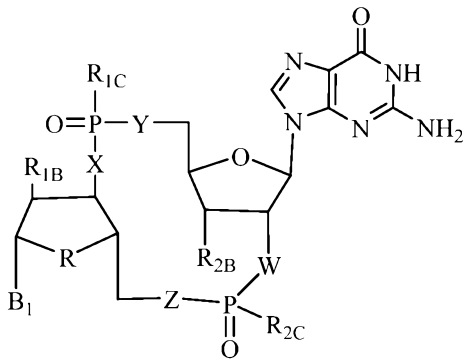
such that only one of X and Y is NH, and only one of W and Z is NH, in any instance;

and, such that when R is CH_2 and B_1 is **b6**, then R_{2B} is other than fluoro or hydroxy;

furthermore, provided that a compound of Formula (I) is other than a compound wherein R, W, X, Y, and Z, are each O; R_{1B} and R_{2B} are each hydroxy; B_1 is **b1**; and R_{1B} and R_{2B} are each hydroxy;

or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

An embodiment of the present invention is directed to a compound of Formula (I)



Formula (I)

wherein

R is CH_2 or O;

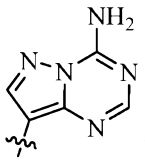
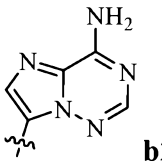
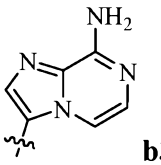
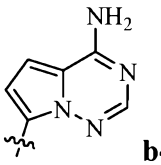
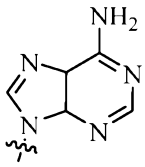
R_{1B} is hydrogen, hydroxy, or fluoro;

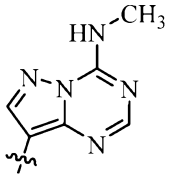
R_{1C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

R_{2B} is hydrogen, hydroxy, methoxy, or fluoro;

R_{2C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b6**, and **b8**

 **b1**,  **b2**,  **b3**,  **b4**,  **b6**,

and  **b8** ;

W is -O-;

X is -O- or -NH-;

Y is -CH_2-, -O-, or -NH-;

Z is -CH_2-, -O-, or -NH-;

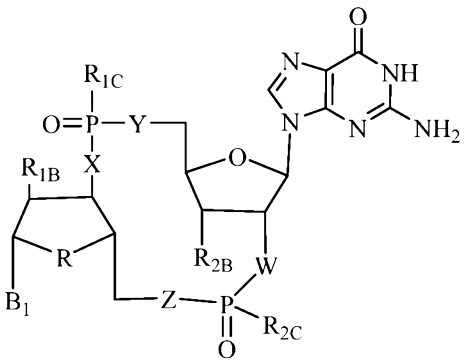
such that only one of X and Y is NH in any instance;

and, such that when R is CH_2 and B_1 is **b6**, then R_{2B} is other than fluoro or hydroxy;

furthermore, provided that a compound of Formula (I) is other than a compound wherein R, X, Y, and Z, are each O; R_{1B} and R_{2B} are each hydroxy; B_1 is **b1**; and R_{1B} and R_{2B} are each hydroxy;

or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

An embodiment of the present invention is directed to a compound of Formula (I)



Formula (I)

wherein

R is CH_2 or O;

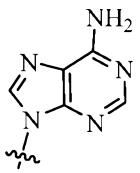
R_{1B} is hydrogen, hydroxy, or fluoro;

R_{1C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

R_{2B} is hydrogen, hydroxy, methoxy, or fluoro;

R_{2C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

B_1 is **b6**



b6 ;

W is -O-;

X is -O- or -NH-;

Y is $-CH_2-$, -O-, or -NH-;

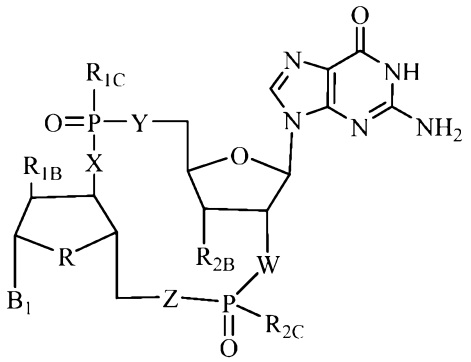
Z is $-CH_2-$, -O-, or -NH-;

such that only one of X and Y is NH in any instance;

and, such that when R is CH_2 and B_1 is **b6**, then R_{2B} is other than fluoro or hydroxy;

or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

An embodiment of the present invention is directed to a compound of Formula (I)



Formula (I)

wherein

R is CH_2 or O;

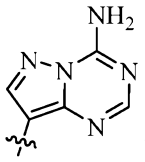
R_{1B} is hydrogen, hydroxy, or fluoro;

R_{1C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

R_{2B} is hydrogen, hydroxy, methoxy, or fluoro;

R_{2C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

B_1 is selected from the group consisting of rings **b1, b2, b3, b4, b6,** and **b8**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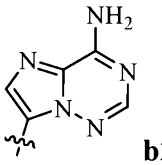
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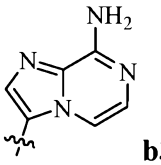
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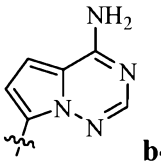
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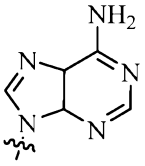
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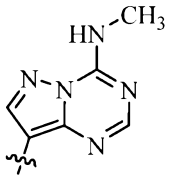
and

b8 ;

W is -NH-;

X is -O- or -NH-;

Y is $-CH_2-$, -O-, or -NH-;

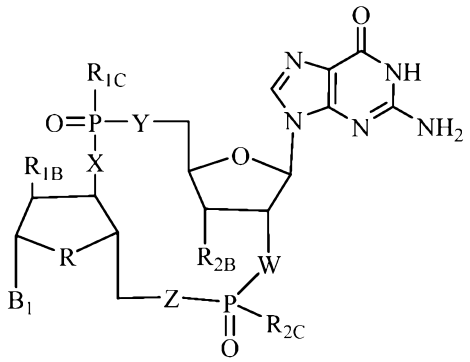
Z is $-CH_2-$, -O-, or -NH-;

such that only one of X and Y is NH in any instance;

and, such that when R is CH_2 and B_1 is **b6**, then R_{2B} is other than fluoro or hydroxy;

or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

An embodiment of the present invention is directed to a compound of Formula (I)



Formula (I)

wherein

R is CH_2 or O;

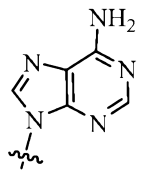
R_{1B} is hydrogen, hydroxy, or fluoro;

R_{1C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

R_{2B} is hydrogen, hydroxy, methoxy, or fluoro;

R_{2C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

B_1 is **b6**



b6 ;

W is -NH-;

X is -O- or -NH-;

Y is -CH_2-, -O-, or -NH-;

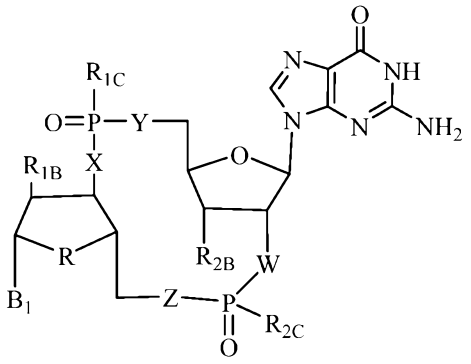
Z is -CH_2-, -O-, or -NH-;

such that only one of X and Y is NH in any instance;

and, such that when R is CH_2 and B_1 is **b6**, then R_{2B} is other than fluoro or hydroxy;

or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

An embodiment of the present invention is directed to a compound of Formula (I)



Formula (I)

wherein

R is CH_2;

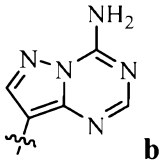
R_{1B} is hydrogen, hydroxy, or fluoro;

R_{1C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

R_{2B} is hydrogen, hydroxy, methoxy, or fluoro;

R_{2C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, and **b8**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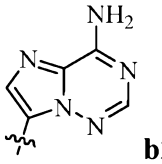
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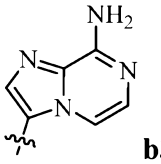
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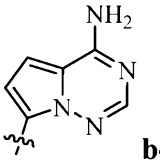
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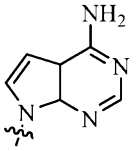
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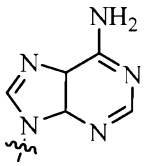
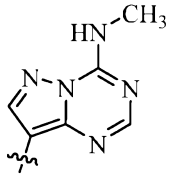

b6, and

b8;

W is -O- or -NH-;

X is -O- or -NH-;

Y is $-CH_2-$, -O-, or -NH-;

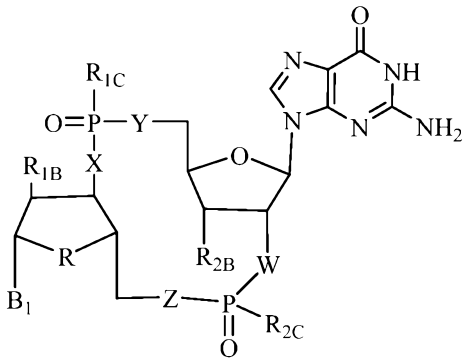
Z is $-CH_2-$, -O-, or -NH-;

such that only one of X and Y is NH, and only one of W and Z is NH, in any instance;

and, such that when B_1 is **b6**, then R_{2B} is other than fluoro or hydroxy;

or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

An embodiment of the present invention is directed to a compound of Formula (I)



Formula (I)

wherein

R is CH_2;

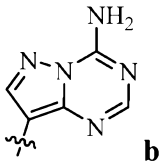
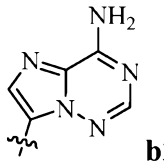
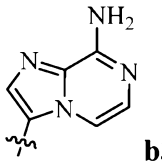
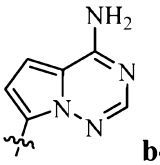
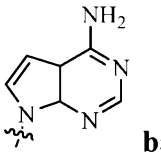
R_{1B} is hydrogen, hydroxy, or fluoro;

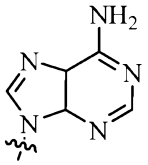
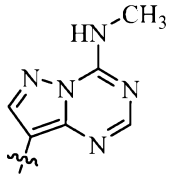
R_{1C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

R_{2B} is hydrogen, hydroxy, methoxy, or fluoro;

R_{2C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, and **b8**

 **b1,**  **b2,**  **b3,**  **b4,**  **b5,**

 **b6,** and  **b8**;

W is -O- ;

X is -O- ;

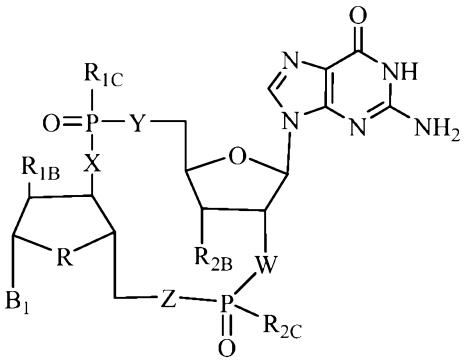
Y is -CH_2- or -O- ;

Z is -CH_2- or -O-;

such that when B_1 is or **b6**, then R_{2B} is other than fluoro or hydroxy;

or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

An embodiment of the present invention is directed to a compound of Formula (I)



Formula (I)

wherein

R is O;

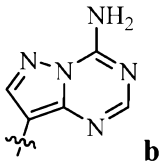
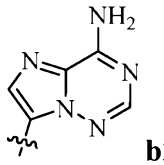
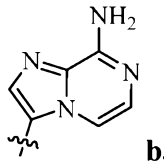
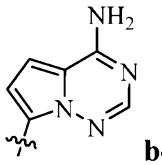
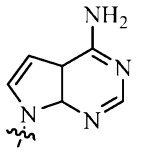
R_{1B} is hydrogen, hydroxy, or fluoro;

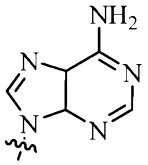
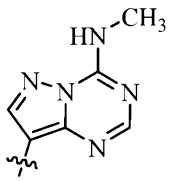
R_{1C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

R_{2B} is hydrogen, hydroxy, methoxy, or fluoro;

R_{2C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, and **b8**

 **b1,**  **b2,**  **b3,**  **b4,**  **b5,**

 **b6,** and  **b8**;

W is -O- or -NH-;

X is -O- or -NH-;

Y is -CH_2-, -O-, or -NH-;

Z is -CH_2-, -O-, or -NH-;

such that only one of X and Y is NH, and only one of W and Z is NH, in any instance;

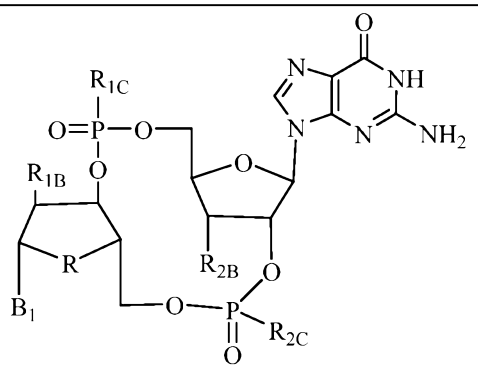
furthermore, provided that a compound of Formula (I) is other than a compound wherein R, W, X, Y, and Z, are each O; R_{1B} and R_{2B} are each hydroxy; B_1 is **b1**; and R_{1B} and R_{2B} are each hydroxy;

or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

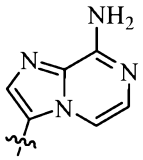
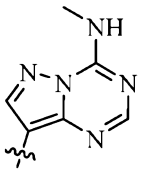
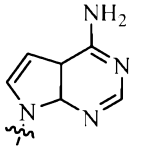
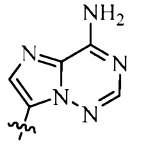
Embodiments of the present invention further include a compound of Formula (Ia), wherein W, X, Y, and Z are each O, as herein defined, or an enantiomer, diastereomer, solvate, or a pharmaceutically acceptable salt form thereof, wherein the substituents

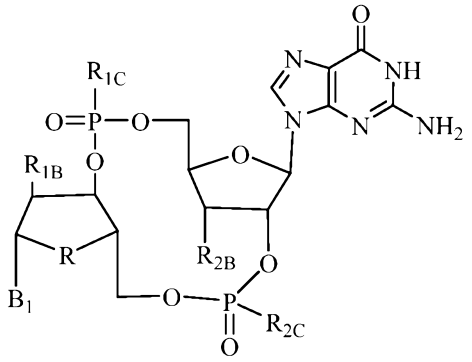
selected from one or more of the variables defined herein (e.g. R, R_{1B}, R_{1C},, R_{2B}, R_{2C}, and B_1) are independently selected to be any individual substituent or any subset of substituents from those exemplified in the listing in Table 1, below.

Table 1.

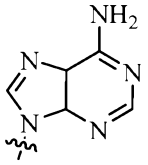
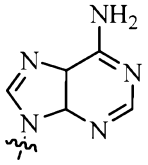
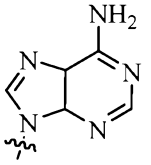
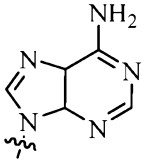
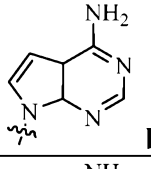
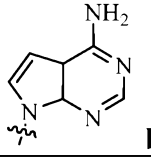


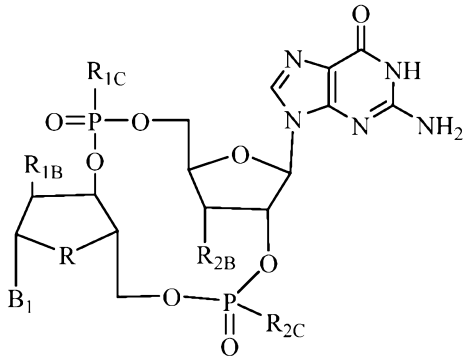
Formula (Ia)

Cpd No.	R	R_{1B}	R_{1C}	R_{2C}	R_{2B}	B_1
1	-O-	OH	OH	OH	OH	 b3
2	-O-	OH	OH	OH	OH	 b8
3	$-CH_2-$	OH	OH	OH	OH	 b5
4	-O-	OH	OH	OH	OH	 b2

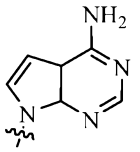
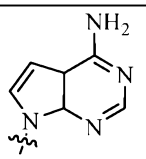
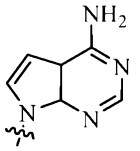


Formula (Ia)

Cpd No.	R	R_{1B}	R_{1C}	R_{2C}	R_{2B}	B_1
5	-CH_2-	OH	(*R) SH	(*R) SH	OCH_3	 b6
6	-CH_2-	OH	(*S) SH	(*R) SH	OCH_3	 b6
7	-CH_2-	OH	(*S) SH	(*S) SH	OCH_3	 b6
8	-CH_2-	OH	(*R) SH	(*S) SH	OCH_3	 b6
9	-CH_2-	OH	OH	OH	OCH_3	 b5
10	-CH_2-	OH	(*S) SH	(*S) SH	OCH_3	 b5

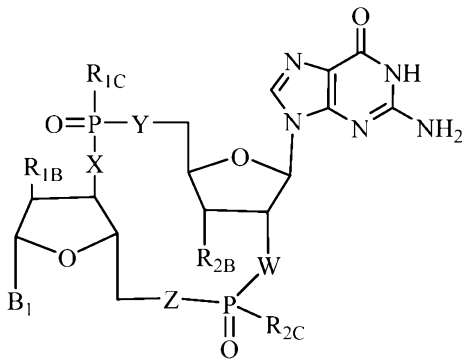


Formula (Ia)

Cpd No.	R	R_{1B}	R_{1C}	R_{2C}	R_{2B}	B_1
11	-CH_2-	OH	(*S) SH	(*R) SH	OCH_3	 b5
12	-CH_2-	OH	(*R) SH	(*S) SH	OCH_3	 b5
13	-CH_2-	OH	(*R) SH	(*R) SH	OCH_3	 b5

Embodiments of the present invention further include a compound of Formula (Ib), wherein R is O, as herein defined, or an enantiomer, diastereomer, solvate, or a pharmaceutically acceptable salt form thereof, wherein the substituents selected from one or more of the variables defined herein (e.g. R_{1B}, R_{1C},, R_{2B}, R_{2C}, W, X, Y, Z, and B_1) are independently selected to be any individual substituent or any subset of substituents from those exemplified in the listing in Table 2, below.

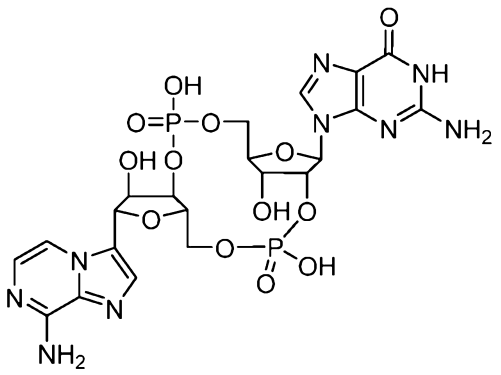
Table 2.



Formula (Ib)

Cpd No.	R_{1B}	R_{1C}	W	X	Y	Z	R_{2B}	R_{2C}	B_1
14	F	OH	-O-	-O-	-O-	CH_2	OH	OH	b6
15	OH	OH	-O-	-O-	-O-	CH_2	F	OH	b6
16	OH	-O-	-O-	-O-	CH_2	CH_2	OH	OH	b6
17	H	OH	-O-	NH	-O-	-O-	OH	OH	b6
18	F	OH	-O-	NH	-O-	-O-	OH	OH	b6
19	F	OH	-O-	-O-	NH	-O-	OH	OH	b6
20	OH	OH	-O-	NH	-O-	-O-	OH	OH	b6
21	OH	OH	-O-	-O-	-O-	NH	F	OH	b6
22	OH	OH	-O-	-O-	NH	NH	OH	OH	b6
23	F	OH	-O-	-O-	-O-	NH	F	OH	b6
24	OH	OH	-O-	-O-	NH	-O-	F	OH	b6
25	F	OH	-O-	-O-	-O-	NH	OCH_3	OH	b6
26	F	OH	-O-	NH	-O-	-O-	OCH_3	(*R)-SH	b6
27	F	OH	-O-	NH	-O-	-O-	OCH_3	(*S)-SH	b6
28	F	(*R)-SH	-O-	NH	-O-	-O-	OCH_3	(*R)-SH	b6
29	F	(*R)-SH	-O-	NH	-O-	-O-	OCH_3	(*S)-SH	b6
30	F	(*S)-SH	-O-	NH	-O-	-O-	OCH_3	(*S)-SH	b6
31	F	(*S)-SH	-O-	NH	-O-	-O-	OCH_3	(*R)-SH	b6
32	F	(*RS)-SH	-O-	-O-	-O-	$-CH_2-$	OCH_3	OH	b6
33	F	OH	-NH-	-O-	-O-	-O-	OCH_3	OH	b6

A further embodiment of the present invention is directed to a compound of Formula (I), selected from compounds 1 to 33,



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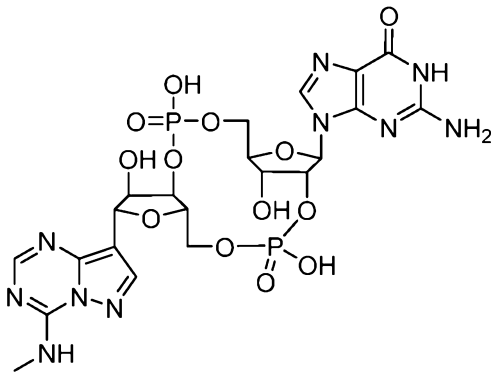

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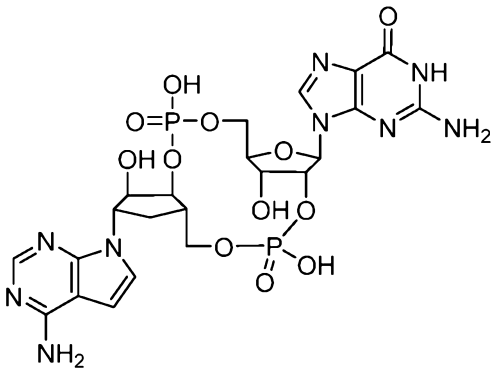

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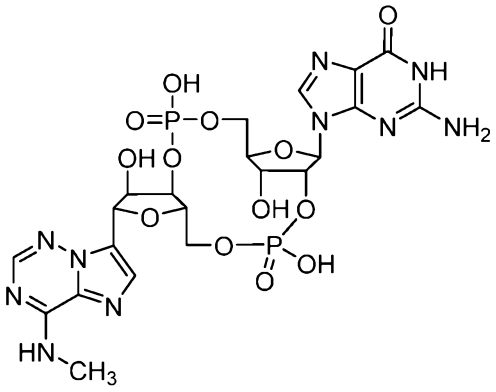

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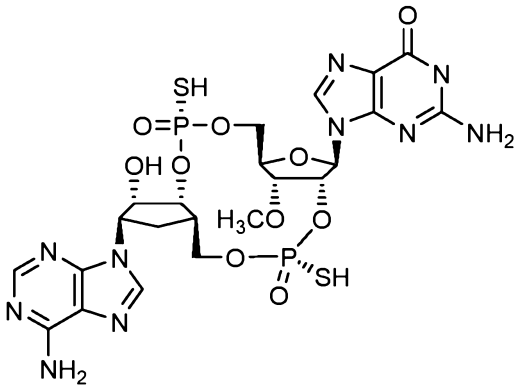

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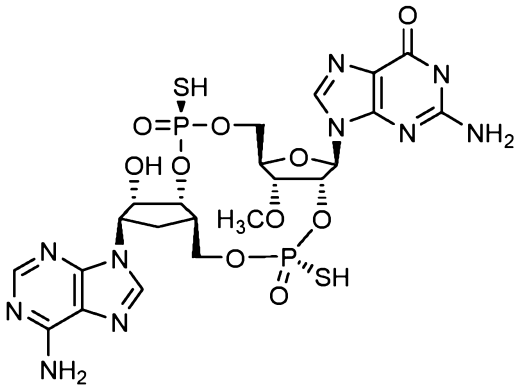

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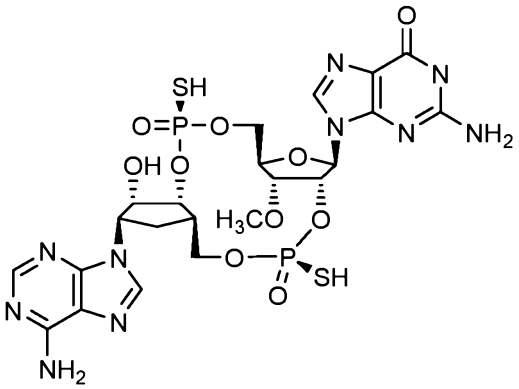

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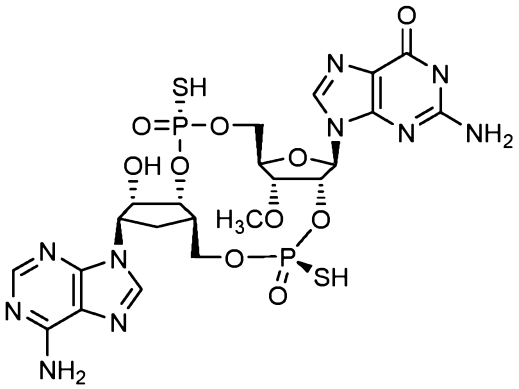

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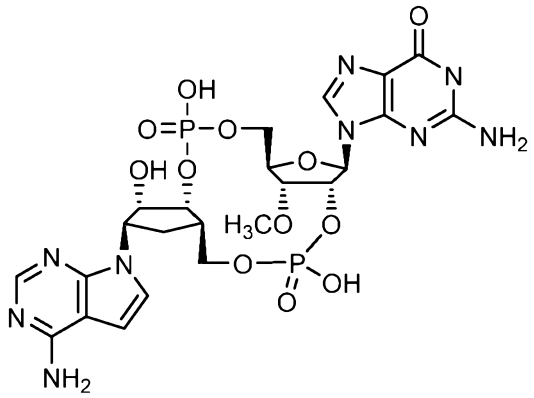

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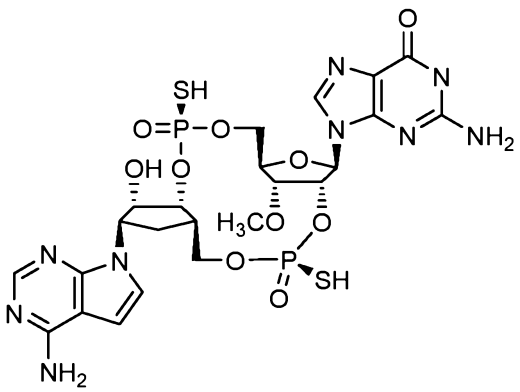

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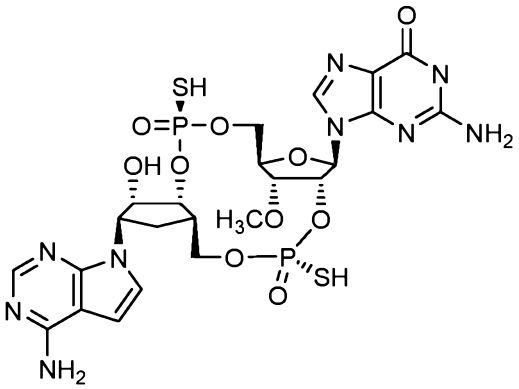

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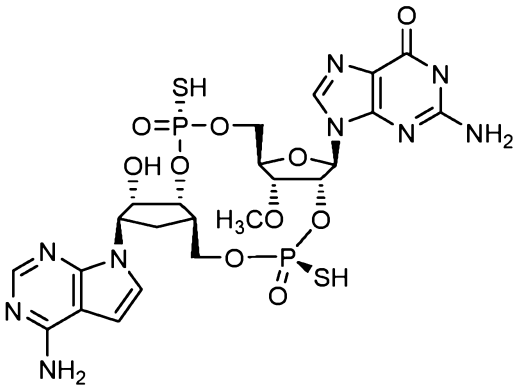

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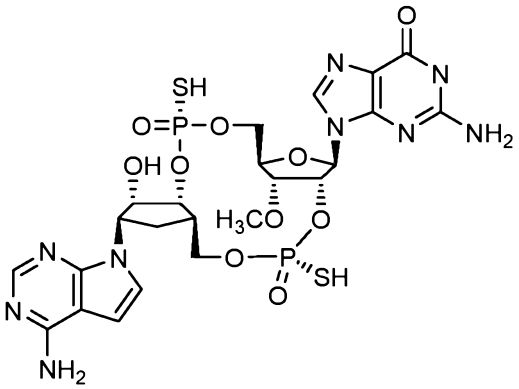

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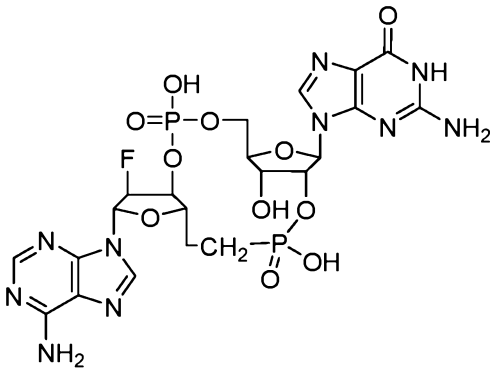

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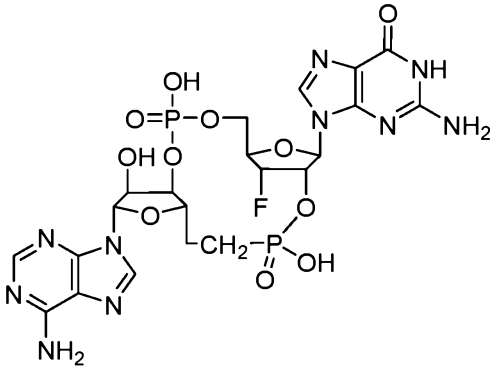

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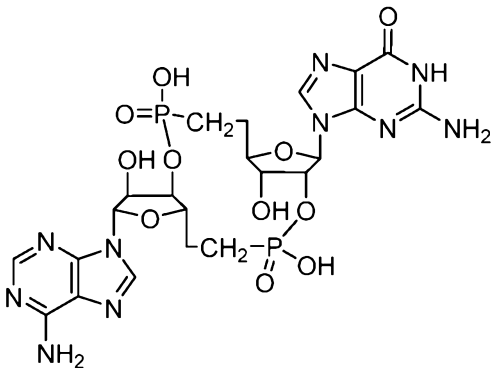

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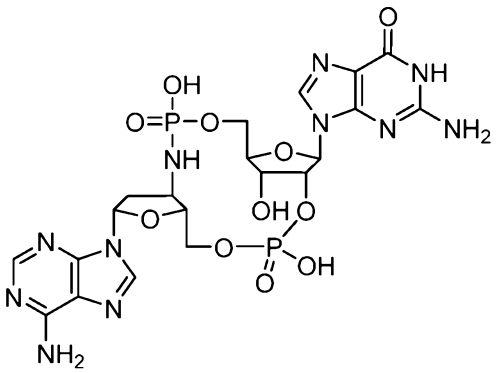

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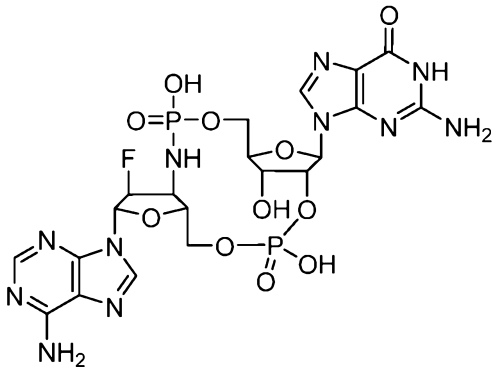

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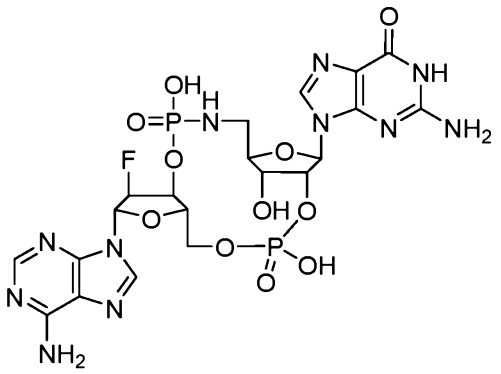

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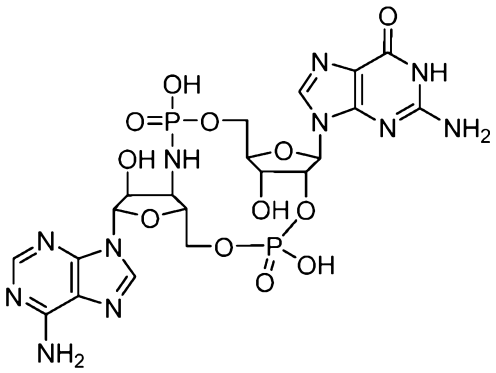

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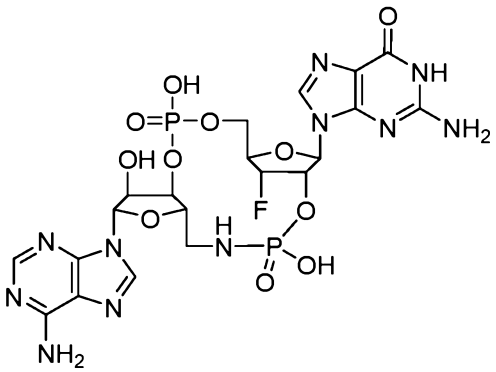

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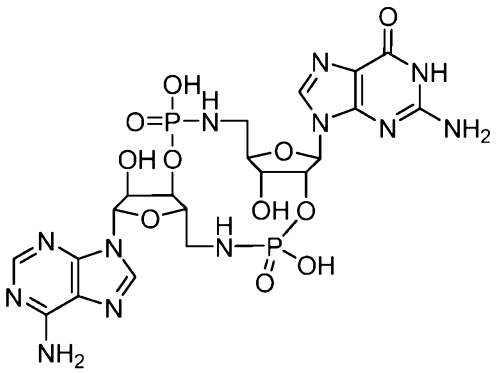

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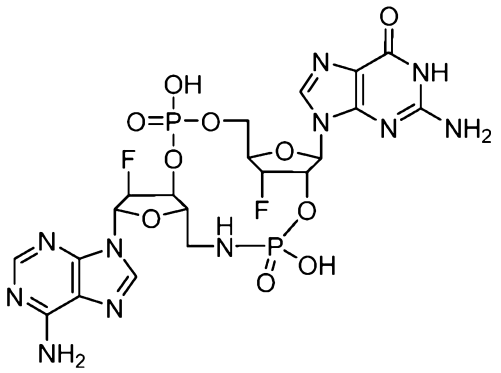

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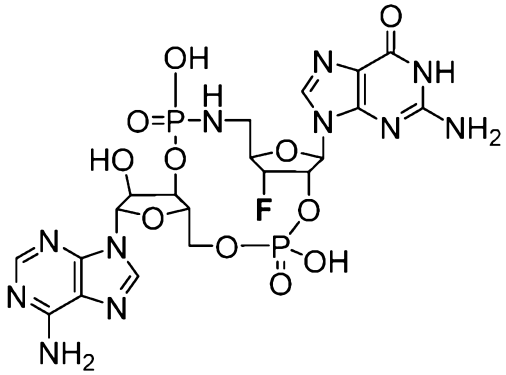

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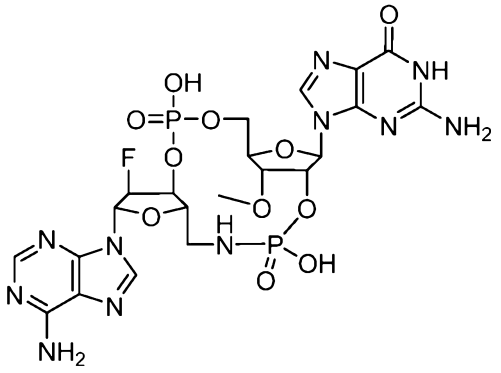

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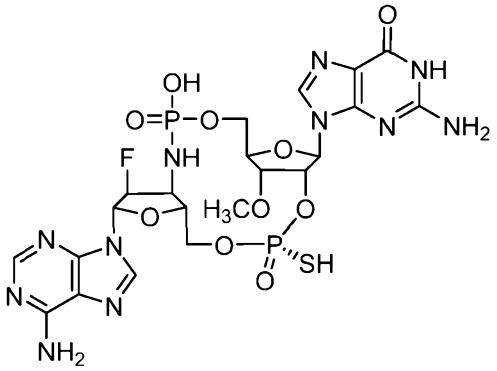

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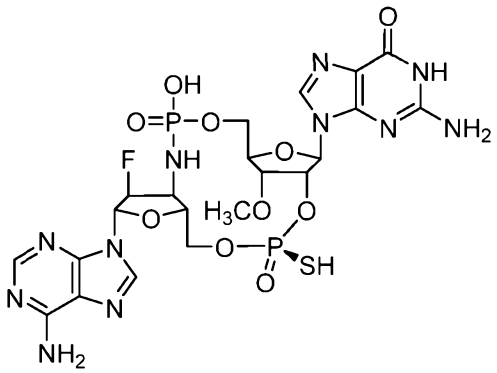

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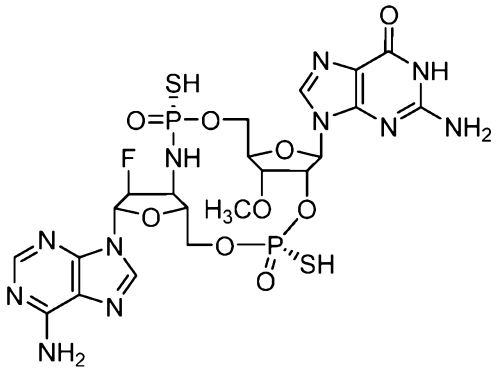

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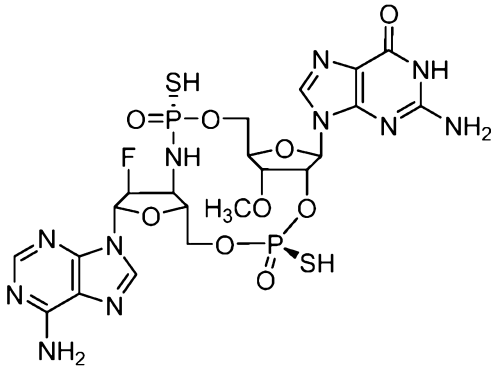

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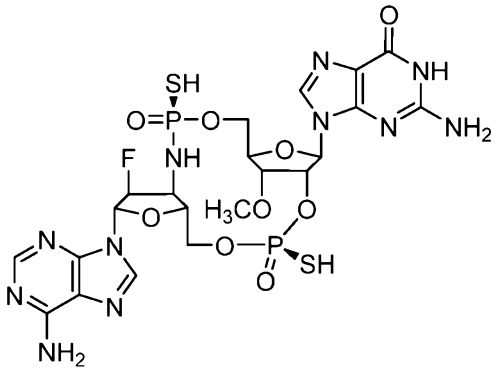

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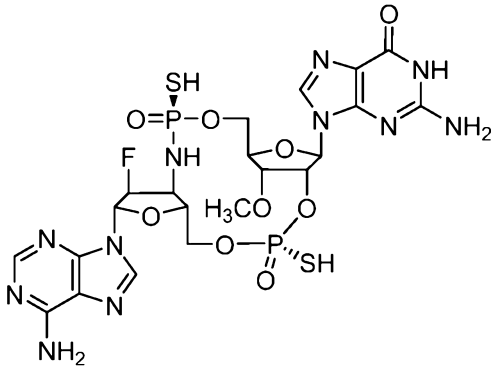

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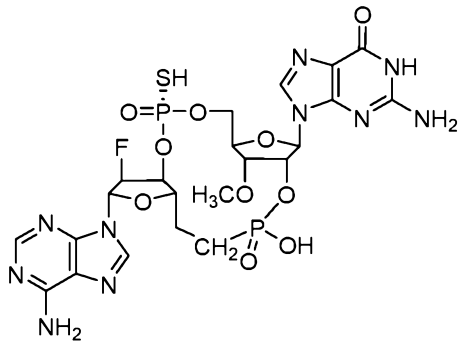

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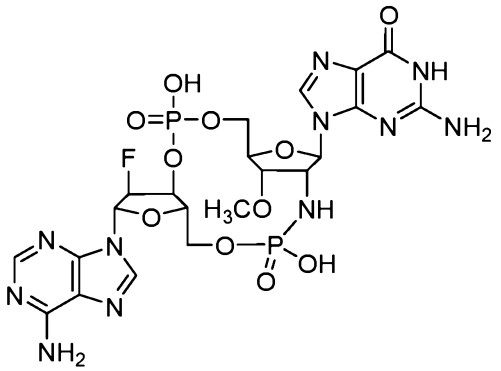


33;

or a pharmaceutically acceptable salt form thereof.

For use in medicine, salts of compounds of Formula (I) refer to non-toxic "pharmaceutically acceptable salts." Other salts may, however, be useful in the preparation of compounds of Formula (I) or of their pharmaceutically acceptable salt forms thereof. Suitable pharmaceutically acceptable salts of compounds of Formula (I) include acid addition salts that can, for example, be formed by mixing a solution of the compound with a solution of a pharmaceutically acceptable acid such as, hydrochloric acid, sulfuric acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid. Furthermore, where the compounds of Formula (I) carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may include alkali metal salts such as, sodium or potassium salts; alkaline earth metal salts such as, calcium or magnesium salts; and salts formed with suitable organic ligands such as, quaternary ammonium salts. Thus, representative pharmaceutically acceptable salts

include acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, oleate, pamoate (embonate), palmitate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate.

Representative acids and bases that may be used in the preparation of pharmaceutically acceptable salts include acids including acetic acid, 2,2-dichloroacetic acid, acylated amino acids, adipic acid, alginic acid, ascorbic acid, L-aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, (+)-camphoric acid, camphorsulfonic acid, (+)-(1S)-camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxy-ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, D-gluconic acid, D-glucoronic acid, L-glutamic acid, α-oxo-glutaric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, (+)-L-lactic acid, (±)-DL-lactic acid, lactobionic acid, maleic acid, (-)-L-malic acid, malonic acid, (±)-DL-mandelic acid, methanesulfonic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, nitric acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid, phosphoric acid, L-pyroglutamic acid, salicylic acid, 4-amino-salicylic acid, sebaic acid, stearic acid, succinic acid, sulfuric acid, tannic acid, (+)-L-tartaric acid, thiocyanic acid, p-toluenesulfonic acid and undecylenic acid; and bases including ammonia, L-arginine, benethamine, benzathine, calcium hydroxide, choline, deanol, diethanolamine, diethylamine, 2-(diethylamino)-ethanol, ethanolamine, ethylenediamine, N-methyl-glucamine, hydrabamine, 1H-imidazole, L-lysine, magnesium hydroxide, 4-(2-

hydroxyethyl)-morpholine, piperazine, potassium hydroxide, 1-(2-hydroxyethyl)-pyrrolidine, sodium hydroxide, triethanolamine, tromethamine, and zinc hydroxide.

Embodiments of the present invention include prodrugs of compounds of Formula (I). In general, such prodrugs will be functional derivatives of the compounds that are readily convertible *in vivo* into the required compound. Thus, in the methods of treating or preventing embodiments of the present invention, the term "administering" encompasses the treatment or prevention of the various diseases, conditions, syndromes and disorders described with the compound specifically disclosed or with a compound that may not be specifically disclosed, but which converts to the specified compound *in vivo* after administration to a patient. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs", ed. H. Bundgaard, Elsevier, 1985.

Where the compounds according to embodiments of this invention have at least one chiral center, they may accordingly exist as enantiomers. Where the compounds possess two or more chiral centers, they may additionally exist as diastereomers. It is to be understood that all such isomers and mixtures thereof are encompassed within the scope of the present invention. Furthermore, some of the crystalline forms for the compounds may exist as polymorphs and as such are intended to be included in the present invention. In addition, some of the compounds may form solvates with water (i.e., hydrates) or common organic solvents, and such solvates are also intended to be encompassed within the scope of this invention. The skilled artisan will understand that the term compound as used herein, is meant to include solvated compounds of Formula (I).

Where the processes for the preparation of the compounds according to certain embodiments of the invention give rise to mixture of stereoisomers, these isomers may be separated by conventional techniques such as, preparative chromatography. The compounds may be prepared in racemic form, or individual enantiomers may be prepared either by enantiospecific synthesis or by resolution. The compounds may, for example, be resolved into their component enantiomers by standard techniques such as, the formation of diastereomeric pairs by salt formation with an optically active acid such as,

(-)-di-p-toluoyl-d-tartaric acid and/or (+)-di-p-toluoyl-l-tartaric acid followed by fractional crystallization and regeneration of the free base. The compounds may also be resolved by formation of diastereomeric esters or amides, followed by chromatographic separation and removal of the chiral auxiliary. Alternatively, the compounds may be resolved using a chiral HPLC column.

One embodiment of the present invention is directed to a composition, including a pharmaceutical composition, comprising, consisting of, and/or consisting essentially of the (+)-enantiomer of a compound of Formula (I) wherein said composition is substantially free from the (-)-isomer of said compound. In the present context, substantially free means less than about 25 %, preferably less than about 10 %, more preferably less than about 5 %, even more preferably less than about 2 % and even more preferably less than about 1 % of the (-)-isomer calculated as

$$\%(+)\text{-enantiomer} = \frac{(mass\,(+)\text{-enantiomer})}{(mass\,(+)\text{-enantiomer}) + (mass(-)\text{-enantiomer})} \times 100$$

Another embodiment of the present invention is a composition, including a pharmaceutical composition, comprising, consisting of, and consisting essentially of the (-)-enantiomer of a compound of Formula (I) wherein said composition is substantially free from the (+)-isomer of said compound. In the present context, substantially free from means less than about 25 %, preferably less than about 10 %, more preferably less than about 5 %, even more preferably less than about 2 % and even more preferably less than about 1 % of the (+)-isomer calculated as

$$\%(-)\text{-enantiomer} = \frac{(mass\,(-)\text{-enantiomer})}{(mass\,(+)\text{-enantiomer}) + (mass(-)\text{-enantiomer})} \times 100$$

During any of the processes for preparation of the compounds of the various embodiments of the present invention, it may be necessary and/or desirable to protect

sensitive or reactive groups on any of the molecules concerned. This may be achieved by means of conventional protecting groups such as those described in *Protective Groups in Organic Chemistry*, *Second Edition*, J.F.W. McOmie, Plenum Press, 1973; T.W. Greene & P.G.M. Wuts, *Protective Groups in Organic Synthesis*, John Wiley & Sons, 1991; and T.W. Greene & P.G.M. Wuts, *Protective Groups in Organic Synthesis*, *Third Edition,* John Wiley & Sons, 1999. The protecting groups may be removed at a convenient subsequent stage using methods known from the art.

Even though the compounds of embodiments of the present invention (including their pharmaceutically acceptable salts and pharmaceutically acceptable solvates) can be administered alone, they will generally be administered in admixture with a pharmaceutically acceptable carrier, a pharmaceutically acceptable excipient and/or a pharmaceutically acceptable diluent selected with regard to the intended route of administration and standard pharmaceutical or veterinary practice. Thus, particular embodiments of the present invention are directed to pharmaceutical and veterinary compositions comprising compounds of Formula (I) and at least one pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, and/or pharmaceutically acceptable diluent.

By way of example, in the pharmaceutical compositions of embodiments of the present invention, the compounds of Formula (I) may be admixed with any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilizing agent(s), and combinations thereof.

Solid oral dosage forms such as, tablets or capsules, containing the compounds of the present invention may be administered in at least one dosage form at a time, as appropriate. It is also possible to administer the compounds in sustained release formulations.

Additional oral forms in which the present inventive compounds may be administered include elixirs, solutions, syrups, and suspensions; each optionally containing flavoring agents and coloring agents.

Alternatively, compounds of Formula (I) can be administered by inhalation

(intratracheal or intranasal) or in the form of a suppository or pessary, or they may be applied topically in the form of a lotion, solution, cream, ointment or dusting powder. For example, they can be incorporated into a cream comprising, consisting of, and/or consisting essentially of an aqueous emulsion of polyethylene glycols or liquid paraffin. They can also be incorporated, at a concentration of between about 1 % and about 10 % by weight of the cream, into an ointment comprising, consisting of, and/or consisting essentially of a wax or soft paraffin base together with any stabilizers and preservatives as may be required. An alternative means of administration includes transdermal administration by using a skin or transdermal patch.

The pharmaceutical compositions of the present invention (as well as the compounds of the present invention alone) can also be injected parenterally, for example, intracavernosally, intravenously, intramuscularly, subcutaneously, intradermally, or intrathecally. In this case, the compositions will also include at least one of a suitable carrier, a suitable excipient, and a suitable diluent.

For parenteral administration, the pharmaceutical compositions of the present invention are best used in the form of a sterile aqueous solution that may contain other substances, for example, enough salts and monosaccharides to make the solution isotonic with blood.

In addition to the above described routes of administration for the treatment of cancer, the pharmaceutical compositions may be adapted for administration by intratumoral or peritumoral injection. The activation of the immune system in this manner to kill tumors at a remote site is commonly known as the abscopal effect and has been demonstrated in animals with multiple therapueutic modalities, (van der Jeught, et al., *Oncotarget*, 2015, 6(3), 1359-1381). A further advantage of local or intratumoral or peritumoral administration is the ability to achieve equivalent efficacy at much lower doses, thus minimizing or eliminating adverse events that may be observed at much higher doses (Marabelle, A., et al., *Clinical Cancer Research*, 2014, 20(7), 1747-1756).

For buccal or sublingual administration, the pharmaceutical compositions of the present invention may be administered in the form of tablets or lozenges, which can be

formulated in a conventional manner.

By way of further example, pharmaceutical compositions containing at least one of the compounds of Formula (I) as the active ingredient can be prepared by mixing the compound(s) with a pharmaceutically acceptable carrier, a pharmaceutically acceptable diluent, and/or a pharmaceutically acceptable excipient according to conventional pharmaceutical compounding techniques. The carrier, excipient, and diluent may take a wide variety of forms depending upon the desired route of administration (e.g., oral, parenteral, etc.). Thus, for liquid oral preparations such as, suspensions, syrups, elixirs and solutions, suitable carriers, excipients and diluents include water, glycols, oils, alcohols, flavoring agents, preservatives, stabilizers, coloring agents and the like; for solid oral preparations such as, powders, capsules, and tablets, suitable carriers, excipients and diluents include starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like. Solid oral preparations also may be optionally coated with substances such as, sugars, or be enterically coated so as to modulate the major site of absorption and disintegration. For parenteral administration, the carrier, excipient and diluent will usually include sterile water, and other ingredients may be added to increase solubility and preservation of the composition. Injectable suspensions or solutions may also be prepared utilizing aqueous carriers along with appropriate additives such as, solubilizers and preservatives.

A therapeutically effective amount of a compound of Formula (I) or a pharmaceutical composition thereof includes a dose range from about 0.01 mg to about 3000 mg, or any particular amount or range therein, in particular from about 0.05 mg to about 1000 mg, or any particular amount or range therein, or, more particularly, from about 0.05 mg to about 250 mg, or any particular amount or range therein, of active ingredient in a regimen of about 1 to about 4 times per day for an average (70 kg) human; although, it is apparent to one skilled in the art that the therapeutically effective amount for a compound of Formula (I) will vary as will the diseases, syndromes, conditions, and disorders being treated.

For oral administration, a pharmaceutical composition is preferably provided in the form of tablets containing about 1.0, about 10, about 50, about 100, about 150, about 200, about 250, and about 500 milligrams of a compound of Formula (I).

Advantageously, a compound of Formula (I) may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three and four times daily.

Optimal dosages of a compound of Formula (I) to be administered may be readily determined and will vary with the particular compound used, the mode of administration, the strength of the preparation and the advancement of the disease, syndrome, condition or disorder. In addition, factors associated with the particular subject being treated, including subject gender, age, weight, diet and time of administration, will result in the need to adjust the dose to achieve an appropriate therapeutic level and desired therapeutic effect. The above dosages are thus exemplary of the average case. There can be, of course, individual instances wherein higher or lower dosage ranges are merited, and such are within the scope of this invention.

Compounds of Formula (I) may be administered in any of the foregoing compositions and dosage regimens or by means of those compositions and dosage regimens established in the art whenever use of a compound of Formula (I) is required for a subject in need thereof.

As STING protein agonists, the compounds of Formula (I) are useful in methods for treating or preventing a viral infection, disease, a syndrome, a condition or a disorder in a subject, including an animal, a mammal and a human in which the viral infection, disease, the syndrome, the condition or the disorder is affected by the modulation, including agonism, of the STING protein. Such methods comprise, consist of and/or consist essentially of administering to a subject, including an animal, a mammal, and a human, in need of such treatment or prevention, a therapeutically effective amount of a compound, salt or solvate of Formula (I).

In one embodiment, the present invention is directed to a compound of Formula (I), or a pharmaceutically acceptable salt form thereof, for the use in the treatment of cancer, and cancer diseases and conditions, or a viral infection.

Examples of cancer diseases and conditions for which compounds of Formula (I), or pharmaceutically acceptable salts or solvates thereof, may have potentially beneficial antitumor effects include, but are not limited to, cancers of the lung, bone, pancreas, skin, head, neck,

uterus, ovaries, stomach, colon, breast, esophagus, small intestine, bowel, endocrine system, thyroid gland, parathyroid gland, adrenal gland, urethra, prostate, penis, testes, ureter, bladder, kidney or liver; rectal cancer; cancer of the anal region; carcinomas of the fallopian tubes, endometrium, cervix, vagina, vulva, renal pelvis, renal cell; sarcoma of soft tissue; myxoma; rhabdomyoma; fibroma; lipoma; teratoma; cholangiocarcinoma; hepatoblastoma; angiosarcoma; hemagioma; hepatoma; fibrosarcoma; chondrosarcoma; myeloma; chronic or acute leukemia; lymphocytic lymphomas; primary CNS lymphoma; neoplasms of the CNS; spinal axis tumors; squamous cell carcinomas; synovial sarcoma; malignant pleural mesotheliomas; brain stem glioma; pituitary adenoma; bronchial adenoma; chondromatous hanlartoma; inesothelioma; Hodgkin's Disease or a combination of one or more of the foregoing cancers. Suitably the present invention relates to a method for treating or lessening the severity of cancers selected from the group consisting of brain (gliomas), glioblastomas, astrocytomas, glioblastoma multiforme, Bannayan-Zonana syndrome, Cowden disease, Lhermitte-Duclos disease, Wilm's tumor, Ewing's sarcoma, Rhabdomyosarcoma, ependymoma, medulloblastoma, head and neck, kidney, liver, melanoma, ovarian, pancreatic, adenocarcinoma, ductal madenocarcinoma, adenosquamous carcinoma, acinar cell carcinoma, glucagonoma, insulinoma, prostate, sarcoma, osteosarcoma, giant cell tumor of bone, thyroid, lymphoblastic T cell leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, hairy-cell leukemia, acute lymphoblastic leukemia, acute myelogenous leukemia, chronic neutrophilic leukemia, acute lymphoblastic T cell leukemia, plasmacytoma, Immunoblastic large cell leukemia, mantle cell leukemia, multiple myeloma, megakaryoblastic leukemia, multiple myeloma, acute megakaryocytic leukemia, pro myelocytic leukemia, erythroleukemia, malignant lymphoma, hodgkins lymphoma, non-hodgkins lymphoma, lymphoblastic T cell lymphoma, Burkitt's lymphoma, follicular lymphoma, neuroblastoma, bladder cancer, urothelial cancer, vulval cancer, cervical cancer, endometrial cancer, renal cancer, mesothelioma, esophageal cancer, salivary gland cancer, hepatocellular cancer, gastric cancer, nasopharangeal cancer, buccal cancer, cancer of the mouth, GIST (gastrointestinal stromal tumor) and testicular cancer.

In another embodiment, the present invention is directed to a compound of Formula (I), or a pharmaceutically acceptable salt form thereof, for use in the treatment of a disorder affected

by the agonism of STING selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, fibrosarcoma, and hepatitis B.

The disclosed compounds of Formula (I) may be useful in combination with one or more additional compounds useful for treating HBV infection. These additional compounds may comprise other disclosed compounds and/or compounds known to treat, prevent, or reduce the symptoms or effects of HBV infection. Such compounds include, but are not limited to, HBV polymerase inhibitors, interferons, viral entry inhibitors, viral maturation inhibitors, literature-described capsid assembly modulators, reverse transcriptase inhibitors, immunomodulatory agents, TLR-agonists, and other agents with distinct or unknown mechanisms that affect the HBV life cycle or that affect the consequences of HBV infection.

In non-limiting examples, the disclosed compounds may be used in combination with one or more drugs (or a salt thereof) selected from the group comprising:

HBV reverse transcriptase inhibitors, and DNA and RNA polymerase inhibitors including, but not limited to, lamivudine (3TC, Zeffix, Heptovir, Epivir, and Epivir-HBV), entecavir (Baraclude, Entavir), adefovir dipivoxil (Hepsara, Preveon, bis-POM PMEA), tenofovir disoproxil fumarate (Viread, TDF or PMPA);

interferons including, but not limited to, interferon alpha (IFN-α), interferon beta (IFN-β), interferon lambda (IFN-λ), and interferon gamma (IFN-γ);

viral entry inhibitors;

viral maturation inhibitors;

capsid assembly modulators, such as, but not limited to, BAY 41-4109;

reverse transcriptase inhibitors;

immunomodulatory agents such as TLR-agonists; and

agents of distinct or unknown mechanisms, such as, but not limited to, AT-61 ((E)-N-(1-chloro-3-oxo-1-phenyl-3-(piperidin-1-yl)prop-1-en-2-yl)benzamide), AT-130 ((E)-N-(1-bromo-1-(2-methoxyphenyl)-3-oxo-3-(piperidin-1-yl)prop-1-en-2-yl)-4-nitrobenzamide), and analogs thereof.

In one embodiment, the additional therapeutic agent is an interferon. The term "interferon" or "IFN" refers to any member of the family of highly homologous species-specific

proteins that inhibit viral replication and cellular proliferation and modulate immune response. For example, human interferons are grouped into three classes: Type I, which includes interferon-alpha (IFN-α), interferon-beta (IFN-β), and interferon-omega (IFN-ω), Type II, which includes interferon-gamma (IFN-γ), and Type III, which includes interferon-lambda (IFN-λ). Recombinant forms of interferons that have been developed and are commercially available are encompassed by the term "interferon" as used herein. Subtypes of interferons, such as chemically modified or mutated interferons, are also encompassed by the term "interferon" as used herein. Chemically modified interferons may include pegylated interferons and glycosylated interferons. Examples of interferons also include, but are not limited to, interferon-alpha-2a, interferon-alpha-2b, interferon-alpha-n1, interferon-beta-1a, interferon-beta-1b, interferon-lamda-1, interferon-lamda-2, and interferon-lamda-3. Examples of pegylated interferons include pegylated interferon-alpha-2a and pegylated interferon alpha-2b.

Accordingly, in one embodiment, the compounds of Formula (I) can be administered in combination with an interferon selected from the group consisting of interferon alpha (IFN-α), interferon beta (IFN-β), interferon lambda (IFN-λ), and interferon gamma (IFN-γ). In one specific embodiment, the interferon is interferon-alpha-2a, interferon-alpha-2b, or interferon-alpha-n1. In another specific embodiment, the interferon-alpha-2a or interferon-alpha-2b is pegylated. In a preferred embodiment, the interferon-alpha-2a is pegylated interferon-alpha-2a (PEGASYS). In another embodiment, the additional therapeutic agent is selected from immune modulator or immune stimulator therapies, which includes biological agents belonging to the interferon class.

Further, the additional therapeutic agent may be an agent that disrupts the function of other essential viral protein(s) or host proteins required for HBV replication or persistence.

In another embodiment, the additional therapeutic agent is an antiviral agent that blocks viral entry or maturation or targets the HBV polymerase such as nucleoside or nucleotide or non-nucleos(t)ide polymerase inhibitors. In a further embodiment of the combination therapy, the reverse transcriptase inhibitor or DNA or RNA polymerase inhibitor is Zidovudine, Didanosine, Zalcitabine, ddA, Stavudine, Lamivudine, Abacavir, Emtricitabine, Entecavir, Apricitabine,

Atevirapine, ribavirin, acyclovir, famciclovir, valacyclovir, ganciclovir, valganciclovir, Tenofovir, Adefovir, PMPA, cidofovir, Efavirenz, Nevirapine, Delavirdine, or Etravirine.

In an embodiment, the additional therapeutic agent is an immunomodulatory agent that induces a natural, limited immune response leading to induction of immune responses against unrelated viruses. In other words, the immunomodulatory agent can effect maturation of antigen presenting cells, proliferation of T-cells and cytokine release (e.g., IL-12, IL-18, IFN-alpha, -beta, and -gamma and TNF-alpha among others),

In a further embodiment, the additional therapeutic agent is a TLR modulator or a TLR agonist, such as a TLR-7 agonist or TLR-9 agonist. In further embodiment of the combination therapy, the TLR-7 agonist is selected from the group consisting of SM360320 (9-benzyl-8-hydroxy-2-(2-methoxy-ethoxy)adenine) and AZD 8848 (methyl [3-({[3-(6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)propyl][3-(4-morpholinyl)propyl]amino}methyl)phenyl]acetate).

In any of the methods provided herein, the method may further comprise administering to the individual at least one HBV vaccine, a nucleoside HBV inhibitor, an interferon or any combination thereof. In an embodiment, the HBV vaccine is at least one of RECOMBIVAX HB, ENGERIX-B, ELOVAC B, GENEVAC-B, or SHANVAC B.

In one embodiment, the methods described herein further comprise administering at least one additional therapeutic agent selected from the group consisting of nucleotide/nucleoside analogs, entry inhibitors, fusion inhibitors, and any combination of these or other antiviral mechanisms.

In another aspect, provided herein is method of treating an HBV infection in an individual in need thereof, comprising reducing the HBV viral load by administering to the individual a therapeutically effective amount of a disclosed compound alone or in combination with a reverse transcriptase inhibitor; and further administering to the individual a therapeutically effective amount of HBV vaccine. The reverse transcriptase inhibitor may be at least one of Zidovudine, Didanosine, Zalcitabine, ddA, Stavudine, Lamivudine, Abacavir, Emtricitabine, Entecavir, Apricitabine, Atevirapine, ribavirin, acyclovir, famciclovir, valacyclovir, ganciclovir, valganciclovir, Tenofovir, Adefovir, PMPA, cidofovir, Efavirenz, Nevirapine, Delavirdine, or Etravirine.

In another aspect, provided herein is a method of treating an HBV infection in an individual in need thereof, comprising reducing the HBV viral load by administering to the individual a therapeutically effective amount of a disclosed compound alone or in combination with an antisense oligonucleotide or RNA interference agent that targets HBV nucleic acids; and further administering to the individual a therapeutically effective amount of HBV vaccine. The antisense oligonucleotide or RNA interference agent possesses sufficient complementarity to the target HBV nucleic acids to inhibit replication of the viral genome, transcription of viral RNAs, or translation of viral proteins.

In another embodiment, the disclosed compound and the at least one additional therapeutic agent are co-formulated. In yet another embodiment, the disclosed compound and the at least one additional therapeutic agent are co-administered. For any combination therapy described herein, synergistic effect may be calculated, for example, using suitable methods such as the Sigmoid-E_{max} equation (Holford & Scheiner, 19981, Clin. Pharmacokinet. 6: 429-453), the equation of Loewe additivity (Loewe & Muischnek, 1926, Arch. Exp. Pathol Pharmacol. 114: 313-326) and the median-effect equation (Chou & Talalay, 1984, Adv. Enzyme Regul. 22: 27-55). Each equation referred to above may be applied to experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

In an embodiment of any of the methods of administering combination therapies provided herein, the method can further comprise monitoring or detecting the HBV viral load of the subject, wherein the method is carried out for a period of time including until such time that the HBV virus is undetectable.

Abbreviations used in the instant specification, particularly the schemes and examples, are as follows:

ACN	acetonitrile

AcOH	glacial acetic acid
ADDP	azodicarboxylic dipiperidide
aq.	aqueous
Bn or Bzl	benzyl
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
Boc	tert-butyloxycarbonyl
conc.	concentrated
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-dicyclohexyl-carbodiimide
DCE	1,2-dichloroethane
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIBAL	diisobutylaluminum hydride
DIPEA or DIEA	diisopropyl-ethyl amine
DMA	dimethylaniline
DMAP	4-dimethylaminopyridine
DME	dimethoxyethane
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DMT	4,4'-dimethoxytrityl
DPPA	diphenylphosphoryl azide
dppf	1,1′-bis(diphenylphosphino)ferrocene
EA	ethyl acetate
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ESI	electrospray ionization
EtOAc or EA	ethyl acetate
EtOH	ethanol
GCMS	gas chromatography-mass spectrometry

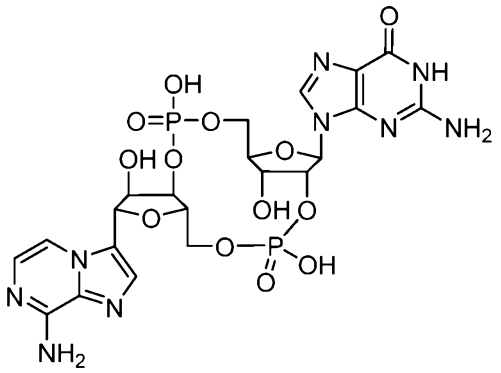
h or hr(s)	hour or hours
HEK	human embryonic kidney
HPLC	high performance liquid chromatography
LAH	lithium aluminum hydride
LDA	lithium diisopropylamide
LHMDS	lithium bis(trimethylsilyl)amide
MEK	methyl ethyl ketone
MeOH	methanol
MHz	megahertz
min	minute or minutes
MS	mass spectrometry
Ms	methanesulfonyl
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMM	N-methylmorpholine
NMP	*N*-methylpyrrolidone
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
PE	petrolum ether
RP	reverse-phase
rt or RT	room temperature
R_t	retention time
Sec	second or seconds
SEM-Cl	2-(trimethylsilyl)ethoxymethyl chloride
TBAF	tetrabutylammonium fluoride
TBDMS	t-butyldimethylsilyl
TBP	tributyl phosphate
TEA or Et_3N	triethylamine
TFA	trifluoroacetic acid

THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	tetramethylsilane
Ts	4-toluenesulfonyl

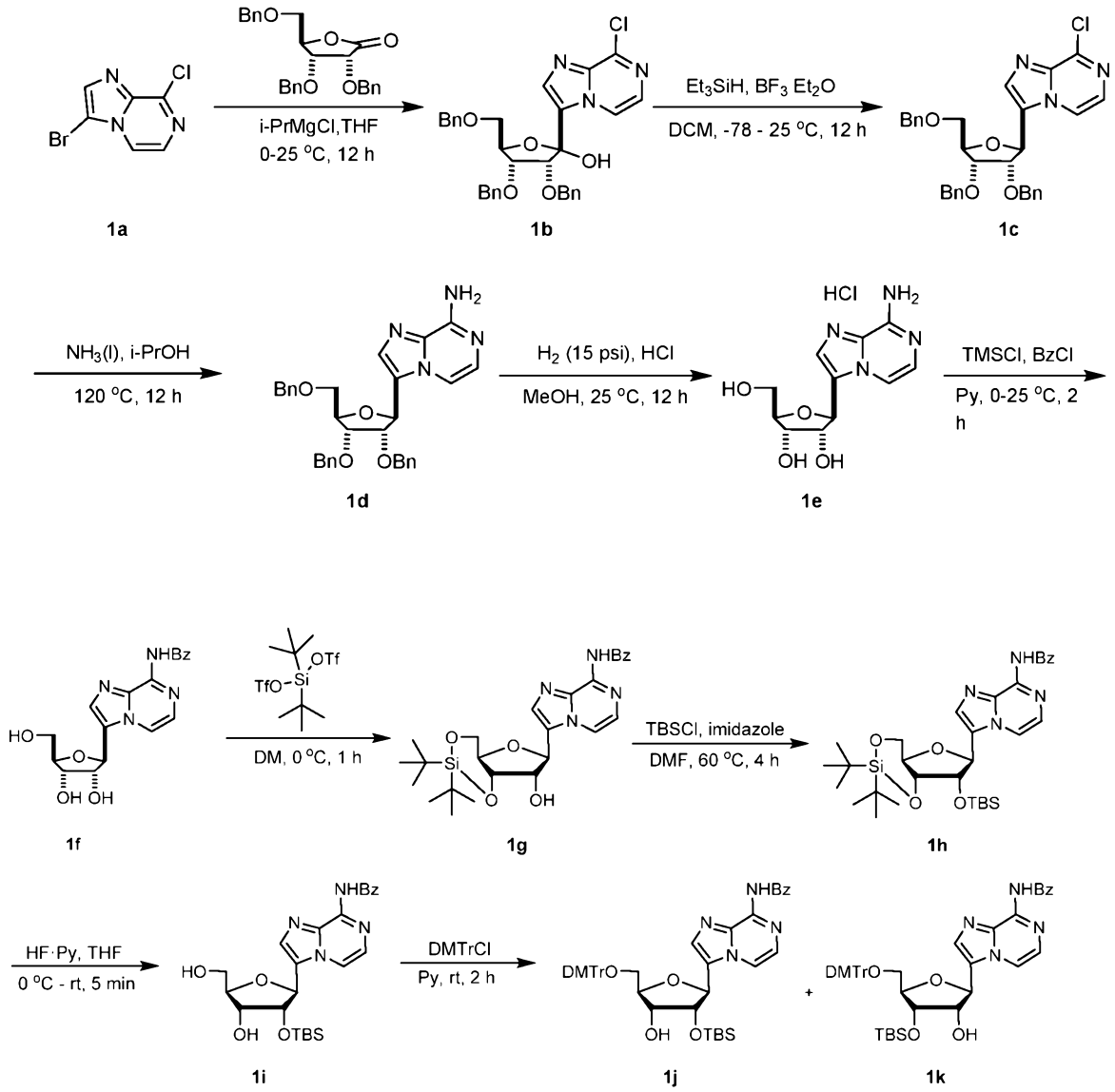
Specific Examples

The reaction scheme illustrated in Example 1 describes one possible route to the preparation of compound **1**, and pharmaceutically acceptable salt forms thereof, of the present invention.

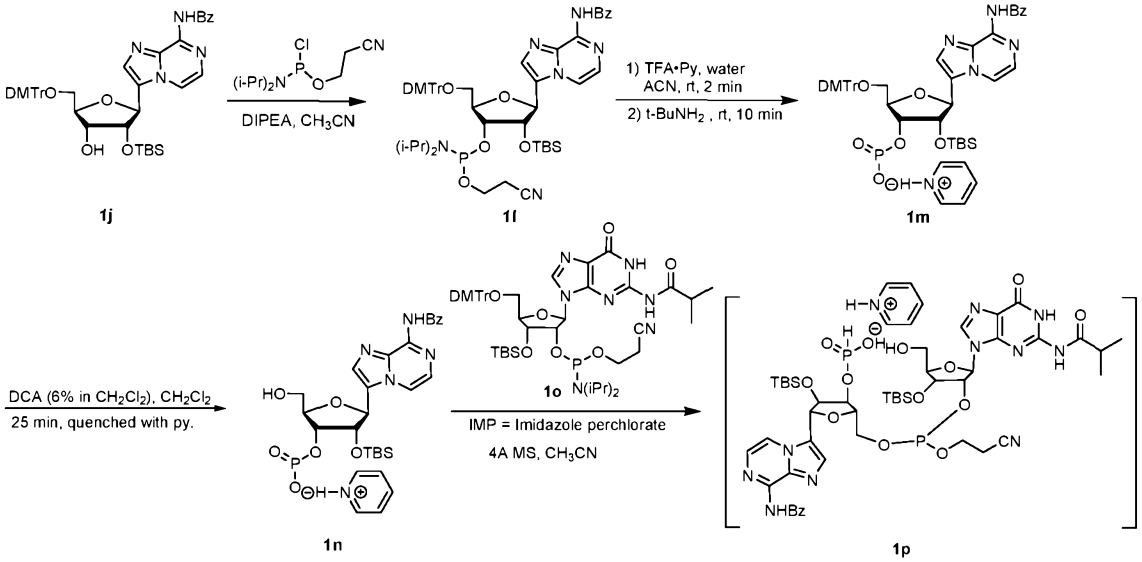
Example 1

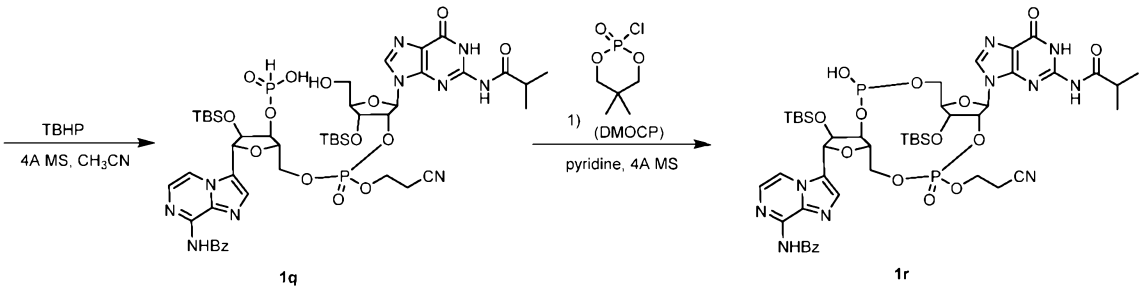


Compound 1

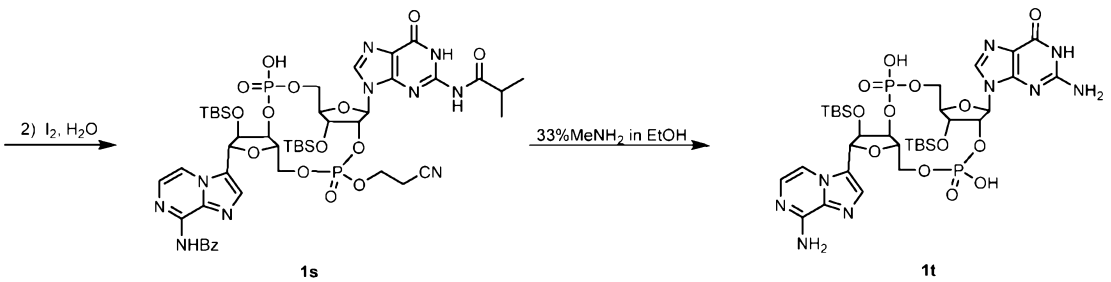

i-PrMgCl,THF
0-25 °C, 12 h
Et3SiH, BF3 Et2O
DCM, -78 - 25 °C, 12 h
NH3(l), i-PrOH
120 °C, 12 h
H2 (15 psi), HCl
MeOH, 25 °C, 12 h
TMSCl, BzCl
Py, 0-25 °C, 2 h
DM, 0 °C, 1 h
TBSCl, imidazole
DMF, 60 °C, 4 h
HF·Py, THF
0 °C - rt, 5 min
DMTrCl
Py, rt, 2 h
1a
1b
1c
1d
1e
1f
1g
1h
1i
1j
1k

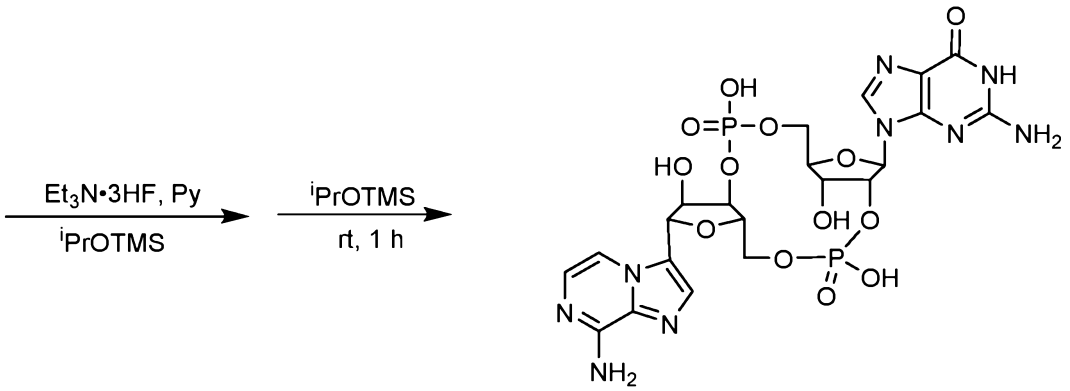
5


DMTrO
NHBz
OH
OTBS
(i-Pr)2N
Cl
CN
DIPEA, CH3CN
1) TFA•Py, water
ACN, rt, 2 min
2) t-BuNH2 , rt, 10 min
1j
1l
1m
DCA (6% in CH2Cl2), CH2Cl2
25 min, quenched with py.
1n
1o
N(iPr)2
IMP = Imidazole perchlorate
4A MS, CH3CN
1p

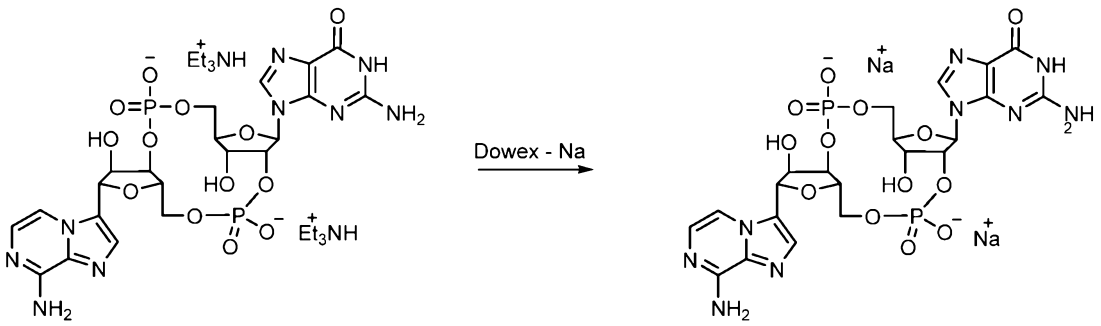

TBHP
4A MS, CH3CN
1q
1)
(DMOCP)
pyridine, 4A MS
1r

5


2) I2, H2O
1s
33%MeNH2 in EtOH
1t



Compound 1 triethylamine salt



Compound 1 triethylamine salt **Compound 1** Sodium salt

Step 1: Preparation of **compound 1f**

To a solution of compound **1e** (Journal of Heterocyclic Chemistry **1993**, *30*: 1213-1220) (5 g, 14.7 mmol) in pyridine (80.0 mL) was added TMSCl (9.61 g, 88.4 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 0.5 h. Benzoyl chloride (2.49 g, 17.7 mmol) was added dropwise at 0 °C. After 5 min, the mixture was warmed up to rt and stirred at 25 °C for 1.5 h. The mixture was diluted with water (5 mL) at 0 °C and $NH_3.H_2O$ (25%, 7.5 mL) was added dropwise. The reaction mixture was then concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography on silica gel (DCM: MeOH = 50:1 to 5:1) to afford compound **1f** (1.05 g, 1.08 mmol) as a white solid.

^{1}H NMR (400 MHz, DMSO d6) δ 10.86 (s, 1H), 8.59 (d, *J* = 4.8 Hz, 1H), 8.04 (d, *J* = 7.6 Hz, 2H), 7.76-7.37 (m, 2H), 7.65-7.61 (m, 1H), 7.56-7.53 (m, 2H), 5.28 (d, *J* = 6.8 Hz, 1H), 5.12 (d, *J* = 4.8 Hz, 1H), 5.07-5.02 (m, 2H), 4.30-4.25 (m, 1H), 4.06-4.05 (m, 1H), 3.94-3.93 (m, 1H), 3.39 (bar, 2H); ^{13}C NMR (400 MHz, DMSO d6) δ 165.7, 144.0, 136.4, 133.7, 132.9, 132.1, 128.5, 128.0, 126.9, 125.8, 117.6, 86.0, 74.8, 73.2, 71.2, 61.6; ESI-MS *m/z* 371 $(M+1)^+$.

Step 2: preparation of **compound 1g**

To a solution of compound **1f** (550 mg, 1.485 mmol) in DMF (10.0 mL) was added di-tert-butylsilanediyl bis(trifluoromethanesulfonate) (1.31 g, 2.97 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. 1H-Imidazole (505.49 mg, 7.42 mmol) was added in one portion at 0 °C. After 5 min, the mixture was stirred at 25 °C for 30 min. The mixture was diluted with DCM (30 mL) and washed with water/brine = 1/1 (20 mL x 2) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography on silica gel (Petroleum ether /EtOAc = 10/1 to 1/1) to afford compound **1g** (670 mg, 1.281 mmol) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) 9.49 (s, 1H), 8.09-7.96 (m, 2H), 7.90-7.84 (m, 1H), 7.84 - 7.78 (m, 1H), 7.67-7.47 (m, 4H), 5.34-5.23 (m, 1H), 4.57 (d, *J* =5.2 Hz, 1H), 4.49 (dd, *J* =5.2, 9.2 Hz, 1H), 4.16-4.06 (m, 1H), 4.03-3.92 (m, 2H), 1.06 (d, *J* =0.8 Hz, 18H); ESI-MS *m/z* 511.2 $(M+1)^+$.

Step 3: preparation of **compound 1h**

To a solution of compound **1g** (2.53 g, 4.95 mmol) in DMF (50 mL) was added imidazole (2.024 g, 29.72 mmol) and tert-butylchlorodimethylsilane (2.24 g, 14.86 mmol) at 0 °C. The mixture was stirred at 60 °C for 12 h. The mixture was diluted with EtOAc (100 mL) and washed with $NaHCO_3$ (50 mL), water (50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography on

silica gel (Petroleum ether /EtOAc = 10/1 to 3/1) to afford compound **3** (2.86 g, 4.577 mmol) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) 9.47 (s, 1H), 8.02 (d, *J* = 7.5 Hz, 2H), 7.93-7.75 (m, 2H), 7.68-7.45 (m, 4H), 5.17 (s, 1H), 4.66 (d, *J* = 5.0 Hz, 1H), 4.45 (dd, *J* = 5.3, 9.3 Hz, 1H), 4.21-4.15 (m, 1H), 3.92 (dd, J=5.3, 9.8 Hz, 1H), 3.85 (t, *J* = 9.8 Hz, 1H), 1.05 (d, J = 2.5 Hz, 18H), 0.92 (s, 9H), 0.15 (d, J = 7.5 Hz, 6H); ESI-MS *m/z* 625.7 $(M+1)^+$.

Step 4: preparation of **compound 1i**

Pyridine hydrofluoride (1.65 mL, 18.306 mmol) was carefully diluted with pyridine (12 mL) and then added dropwise to a solution of compound **1h** (2.86 g, 4.57 mmol) in THF (45 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 5 min. The reaction mixture was quenched by the addition of pyridine (12 mL), and diluted with CH_2Cl_2 (50 mL), washed with brine (50 mL x 2), dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography on silica gel (Petroleum ether /EtOAc = 10/1 to 0/1) to give compound **1i** (2.04 g, 4.21 mmol) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) 9.52 (s, 1H), 8.29 (d, *J* = 4.5 Hz, 1H), 8.04 (d, *J* = 7.0 Hz, 2H), 7.77 (d, J = 4.5 Hz, 1H), 7.64-7.58 (m, 2H), 7.57-7.49 (m, 2H), 5.09 (d, *J* =8.0 Hz, 1H), 4.51 (dd, *J* =5.5, 8.0 Hz, 1H), 4.30-4.16 (m, 2H), 4.04-3.85 (m, 2H), 3.49 (s, 1H), 2.82 (d, *J* = 2.5 Hz, 1H), 2.19 (br, s, 1H), 0.83 (s, 9H), -0.12 (s, 3H), -0.27 (s, 3H). ESI-MS *m/z* 485.1 $(M+1)^+$.

Step 5: preparation of **compound 1j**

To a solution of compound **1i** (1.84 g, 3.797 mmol) in pyridine (20 mL) was added DMTrCl (1.93 g, 5.695 mmol) at 25 °C. The reaction mixture was stirred at 25 °C for 12 h. The reaction mixture was quenched by the addition of MeOH (2.0 mL), diluted with EA (100 mL) and washed with brine (50 mL x 3), dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by flash

column chromatography on silica gel (Petroleum ether /EtOAc =10/1 to 1/1) to give compound **1j** (1.35 g, 1.715 mmol) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) 9.47 (s, 1H), 8.35 (d, J = 4.4 Hz, 1H), 8.03 (d, J = 7.0 Hz, 2H), 7.65-7.57 (m, 2H), 7.56-7.49 (m, 2H), 7.41 (d, J = 7.0 Hz, 2H), 7.34-7.20 (m, 9H), 7.04 (d, J = 5.0 Hz, 1H), 6.85-6.78 (m, 4H), 5.09 (d, J = 8.5 Hz, 1H), 4.76 (dd, J = 5.5, 8.5 Hz, 1H), 4.44 (d, J = 5.0 Hz, 1H), 4.27 (d, J = 1.5 Hz, 1H), 3.78 (d, J = 2.0 Hz, 6H), 3.50 (d, J = 2.0 Hz, 2H), 2.88 (d, J = 1.5 Hz, 1H), 0.84 (s, 9H), -0.14 (s, 3H), -0.31 (s, 3H); ESI-MS m/z 787.3 $(M+1)^+$.

Step 6: preparation of **compound 1l**

To a solution of compound **1j** (600 mg, 0.762 mmol) in THF (6.0 mL) was added N,N-diisopropylethylamine (591.2 mg, 4.57 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (541.333 mg, 2.28 mmol) at 0 °C. Then the reaction mixture was stirred at 25 °C for 2 h.

To a solution of compound **1j** (930 mg, 1.18 mmol) in THF (10.0 mL) was added N,N-Diisopropylethylamine (916.37 mg, 7.09 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (839.06 mg, 3.54 mmol) at 0 °C. Then the reaction mixture was stirred at 25 °C for 2 h. The reaction mixture was combined with the first reaction, and the combined mixture was quenched by addition of MeOH (5 mL) and diluted with EA (50 mL). The organic layer was washed with $NaHCO_3$ (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography on silica gel (Petroleum ether /EtOAc = 10/1 to 3/1) to afford compound **1l** (1.76 g, 1.78 mmol) as a yellow oil.

^{31}P NMR (162MHz, $CDCl_3$) 151.467 (s, 1P), 148.337 (s, 1P); ESI-MS m/z 904.4 $(M-(N(iPr)_2+OH))^+$.

Step 7: preparation of **compound 1m**

To a solution of compound **1l** (1.76 g, 1.783 mmol) in CH_3CN (10.0 mL) and water (64.236 uL, 3.56 mmol) was added pyridinium trifluoroacetate (413.16 mg, 2.14 mmol). After 5 min, tert-butylamine (10.0 mL) was added and the reaction mixture was stirred for 15 min at room temperature. The mixture was concentrated under reduced pressure to afford a white foam. The residue was dissolved in CH_3CN (10.0 mL) and concentrated to afford a foam, and this process was repeated one more time to give compound **1m** (1.82 g, crude) as a white foam, which was used for the next step without further purification.

Step 8: preparation of **compound 1n**

To a solution of compound **1m** (1.82 g, crude) in CH_2Cl_2 (24 mL) was added water (385.3 mg, 21.387 mmol), followed by 6% dichloroacetic acid in CH_2Cl_2 (24 mL). The reaction mixture was stirred at 25 °C for 30 min. Pyridine (10 mL) was added to quench the reaction, and the reaction mixture was stirred at 25 °C for 30 min. At that time, the reaction mixture was concentrated under reduced pressure to give a residue, which was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH = 100/1 to 5/1) to afford compound **1n** (910 mg, 1.66 mmol) as a white foam.

^1H NMR (400MHz, CD_3OD) 8.85 (d, J = 4.6 Hz, 1H), 8.11 (d, J = 7.3 Hz, 2H), 7.83 (s, 1H), 7.73-7.61 (m, 2H), 7.61-7.51 (m, 2H), 6.19 (s, 1H), 6.00 (s, 1H), 5.26 (d, J = 9.0 Hz, 1H), 4.74 (dd, J = 5.0, 10.5 Hz, 1H), 4.61 (dd, J = 5.2, 8.7 Hz, 1H), 4.33 (s, 1H), 3.96-3.88 (m, 1H), 3.84-3.75 (m, 1H), 0.76 (s, 9H), -0.09 (s, 3H), -0.39 (s, 3H); ESI-MS m/z 549.1 $(M+1)^+$.

Step 9: preparation of **compound 1p**

A solution of compound **1n** (910 mg, 1.659 mmol) and 4Å molecular sieves in dry acetonitrile (30 mL) was stirred at room temperature under nitrogen for 3 min. 1H-Imidazole perchlorate (5.14 g, 30.52 mmol) was added. After 10 min, (2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert- butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1H-purin-9(6H)-yl)tetrahydrofuran-3-yl (2-cyanoethyl)

diisopropylphosphoramidite, **1o** (1.77 g, 1.825 mmol) in MeCN (15 mL) was added at 25 °C. The mixture was stirred at 25 °C for 50 min. The reaction mixture (0.03687 M in MeCN, 45 mL) was used for the next step without further purification.

Step 10: preparation of **compound 1q**

To a solution of compound **1p** (0.03687 M in MeCN, 45 mL) was added tert-butyl hydroperoxide (1.510 mL, 8.303 mmol) at 25 °C. The reaction mixture was stirred at 25 °C for 1 h. The reaction mixture was filtered, and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by reverse phase preparative HPLC (neutral, column: Waters Xbridge 150x25 5μM; mobile phase: water (10 mM NH_4HCO_3)-ACN, B%: 33%-53%, 25mL/min, Gradient Time: 8 min) to afford compound **1q** (602 mg, 0.532 mmol) as a white solid.

^{31}P NMR (162 MHz, CD_3OD) 4.05 (s, 1P), 3.92 (s, 1P), -2.20 (s, 1P), -2.98 (s, 1P); ESI-MS *m/z* 1131.1 $(M+1)^+$.

Step 11: preparation of **compound 1r**

To a solution of compound **1q** (600 mg, 0.53 mmol) and 4Å molecular sieves in pyridine (150 mL) was added DMOCP (293.669 mg, 1.591 mmol) at 25 °C. The mixture was stirred at 25 °C for 1 h. The reaction mixture (0.00353 M in Py, 150 mL) was used for the next step without further purification.

Step 12: preparation of **compound 1s**

A solution of compound **1r** (0.00353 M in Py, 150 mL) was added water (95.48 mg, 5.30 mmol) and I_2 (672.594 mg, 2.65 mmol, 5.0 eq) at 25 °C. The mixture was stirred at 25 °C for 1 h. The reaction was quenched with aqueous Na_2SO_3 (50 mL). The mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse phase preparative HPLC (column: Agela Durashell C18 150x25 5μM ; mobile phase: water (10mM NH_4HCO_3)-CH_3CN from 35% to 55%, flow rate: 25 mL/min) to afford compound **1s** (285 mg, 0.25 mmol) as a white solid.

ESI-MS *m/z* 1130.1 $(M+1)^+$.

Step 13: preparation of **compound 1t**

Compound **1s** (120 mg, 0.106 mmol) in $MeNH_2$ (33% in EtOH, 10 mL) was stirred at 25 °C for 16 h. The reaction mixture was concentrated under reduced pressure to give crude compound **1t** (125 mg) as a yellow solid, which was used for the next step without further purification.

Step 14: preparation of **compound 1, triethylammonium salt**

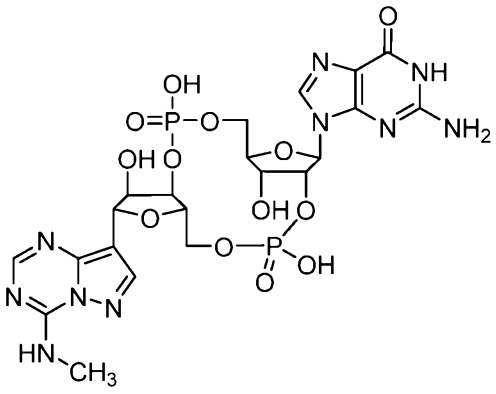
To a solution of compound **1t** (125 mg, crude) in Py (5 mL) was added Et_3N (1.402 g, 13.86 mmol) and triethylamine trihydrofluoride (1.117 g, 6.929 mmol, 50.0 eq) at 25 °C. The reaction mixture was stirred at 50 °C for 12 h. The mixture was dissolved in THF (3 mL) and isopropoxytrimethylsilane (3.66 g, 27.71 mmol) was added at 25 °C and stirred for 12 h. The mixture was concentrated under reduced pressure to give a residue (108 mg, crude) as a brown solid, collected as batch 1.

To a solution of compound **1t** (183 mg, crude) in Py (6 mL) was added triethylamine (2.05 g, 20.29 mmol) and triethylamine trihydrofluoride (1.63 g, 10.145 mmol) at 25 °C. The reaction mixture was stirred at 50 °C for 12 h. The mixture was dissolved in THF (5 mL) and isopropoxytrimethylsilane was (5.36 g, 40.58 mmol) added at 25 °C and stirred for 12 h. The mixture was concentrated under reduced pressure to give a residue as batch 2. The batch 2 was combined with batch 1 and purified by reverse phase preparative HPLC (column: Agela Durashell C18 150x25 5μM; mobile phase: water (0.05% ammonia hydroxide v/v)-ACN from 0% to 15 %, flow rate: 35 ml/min) to afford compound **1, triethylammonium salt** (125 mg, 0.186 mmol) as a white solid. ^1H NMR (400 MHz, D_2O) 7.77 (s, 1H), 7.63 (s, 1H), 7.50-7.16 (m, 2H), 5.86 (d, J =7.5 Hz, 1H), 5.74 (s, 1H), 5.13 (s, 1H), 4.90 (s, 1H), 4.54-4.42 (m, 2H), 4.32 (br s, 1H), 4.21 (br, d, J =8.5 Hz, 4H), 3.87 (d, J =11.5 Hz, 1H); ESI-MS *m/z* 674.0 $(M+1)^+$.

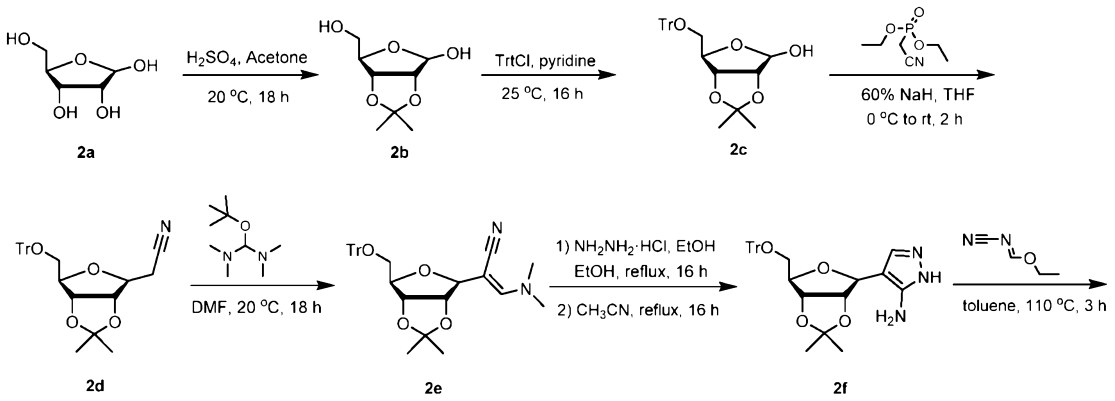
Step 15: preparation of **compound 1, sodium salt**

Compound **1, triethylammonium salt** was dried under high vacuum to give a white solid (125 mg). Dowex 50W x 8, 200-400 (H form, 10 mL) was added to a beaker (for 125 mg of compound **1, triethylammonium salt**) and washed with de-ionized water (2x). Then to the resin was added 15% H_2SO_4 in de-ionized H_2O (50 mL) and the mixture was stirred for 15 min and decanted (1x). The resin was transferred to a column with 15% H_2SO_4 in de-ionized H_2O and washed with 15% H_2SO_4 (at least 4 CV), and then with de-ionized H_2O until it was neutral. The resin was transferred back into the beaker, and 15% NaOH in de-ionized H_2O solution (50 mL) was added and the mixture was stirred for 15 min and decanted (1x). The resin was transferred to the column and washed with 15% NaOH in de-ionized H_2O (at least 4 CV), and then with de-ionized H_2O until it was neutral (at least 4 CV). Compound **1, triethylammonium salt** was dissolved in de-ionized H_2O (125 mg in 10 mL), added to the top of the column, and eluted with de-ionized H_2O. The converted sodium salt was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to give **compound 1, sodium salt** (85 mg, 0.117 mmol) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) 8.01 (s, 1H), 7.74 (d, J = 5.0 Hz, 2H), 7.26 (s, 1H), 6.02 (br d, J = 8.5 Hz, 1H), 5.28 (s, 1H), 5.10 (s, 1H), 4.62 (t, J = 4.3 Hz, 1H), 4.56 (d, J = 3.5 Hz, 1H), 4.45 - 3.99 (m, 6H), 3.86 (d, J = 11.5 Hz, 1H); ^{31}P NMR (162 MHz, CD_3OD) 0.63 (s, 1P), -2.91 (s, 1P); ESI-MS m/z 673.9 $(M+1)^+$.

Example 2



Compound 2



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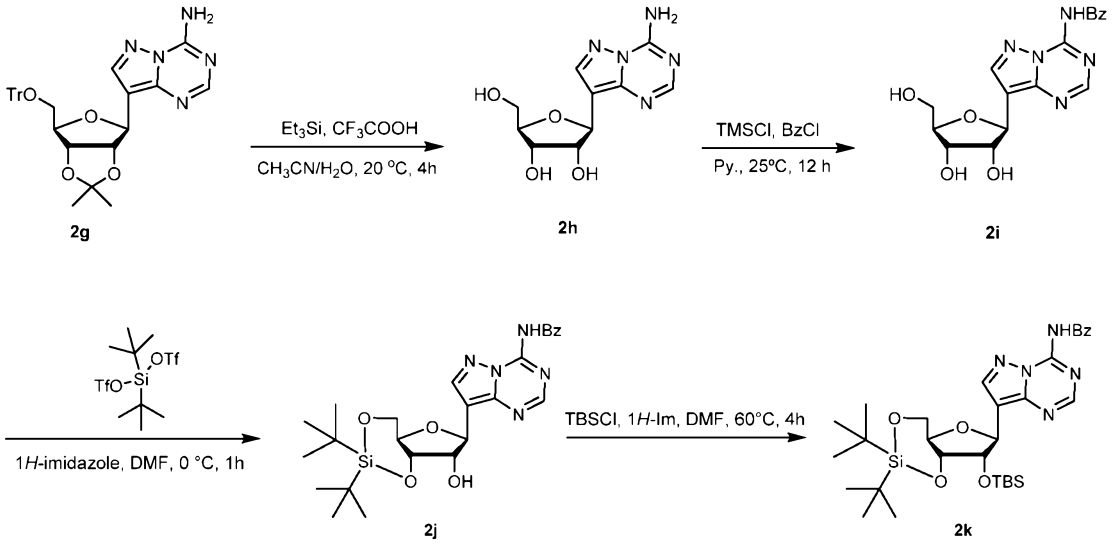
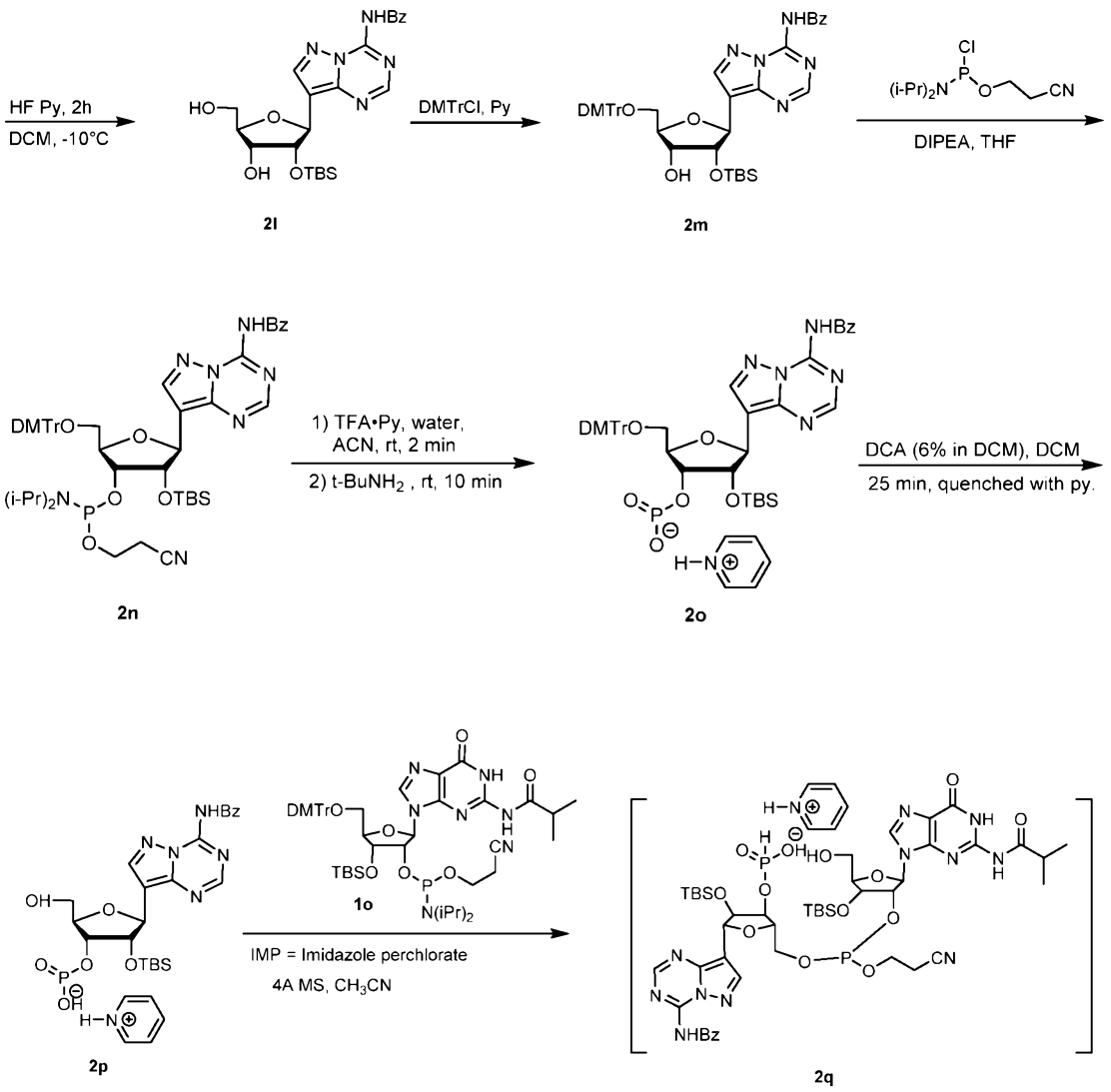
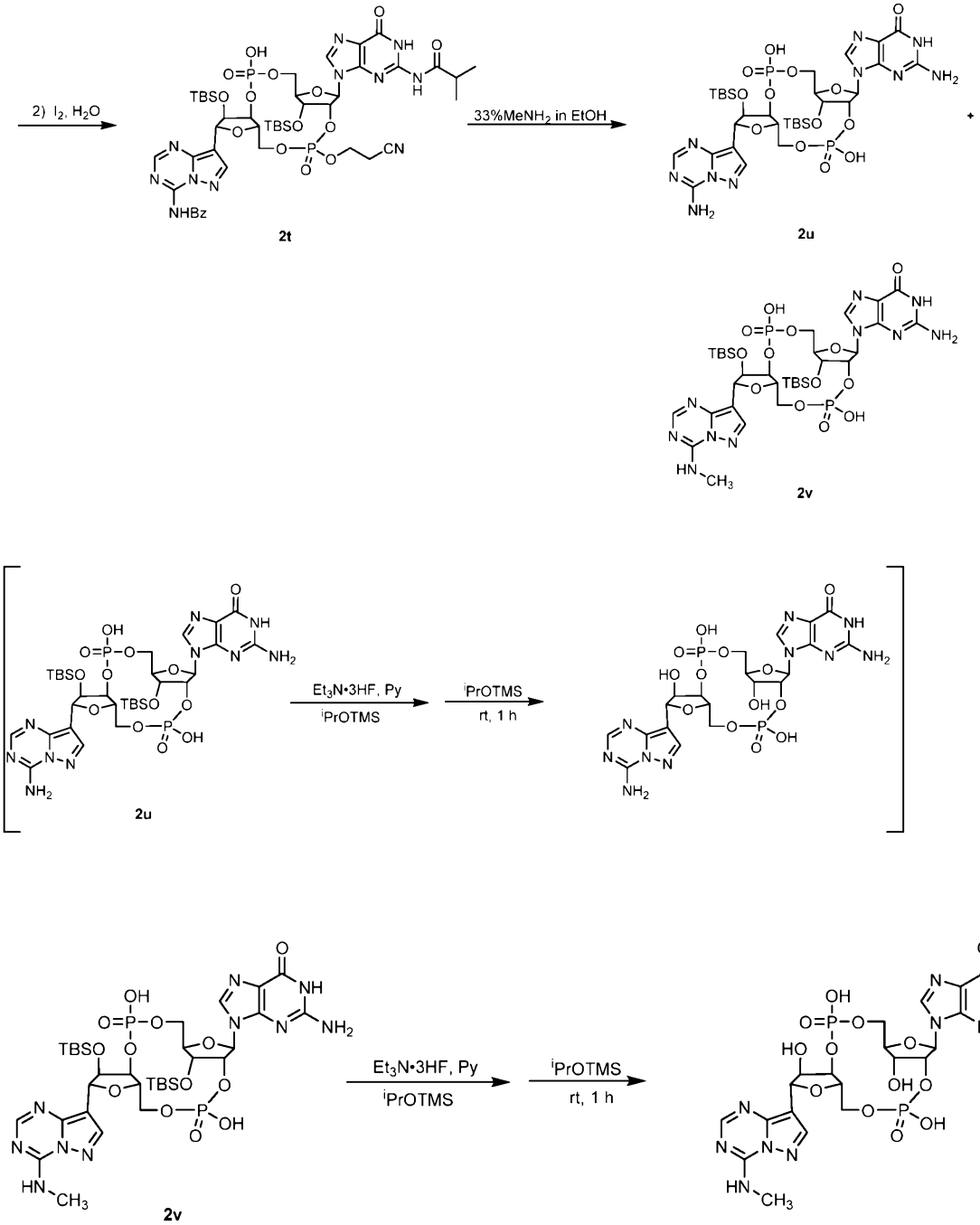
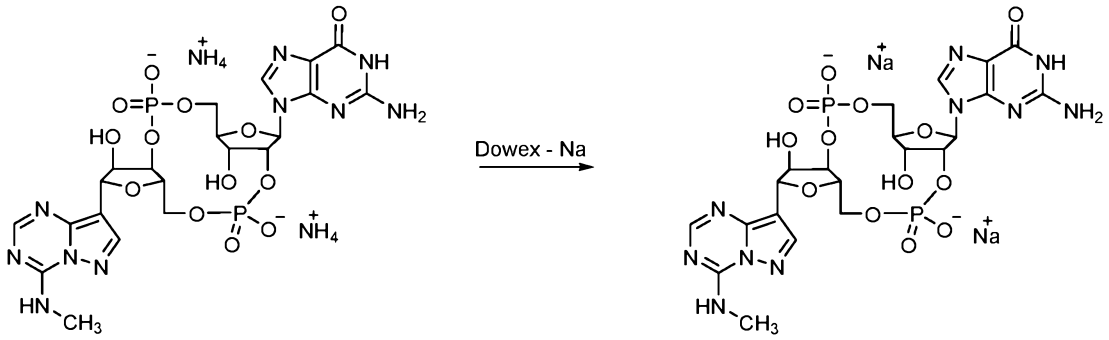



HF Py, 2h
DCM, -10°C
DMTrCl, Py
DIPEA, THF
1) TFA•Py, water, ACN, rt, 2 min
2) t-BuNH2 , rt, 10 min
DCA (6% in DCM), DCM
25 min, quenched with py.
IMP = Imidazole perchlorate
4A MS, CH3CN
TBHP
4A MS, CH3CN
1)
(DMOCP)
pyridine, 4A MS
2l
2m
2n
2o
2p
1o
2q
2r
2s



Compound 2, ammonium salt

5



Compound 2, Ammonium salt

Compound 2, Sodium salt

Step 1: preparation of **compound 2j**

To a solution of **2i** (WO2013179289) (2 g, 5.39 mmol) in DMF (20 mL) was added dropwise di-tert-butylsilanediyl bis(trifluoromethanesulfonate) (2.61 g, 5.92 mmol) at 0 °C under N_2. After 1 h, imidazole (458.32 mg, 6.73 mmol) was added in one portion at 0 °C. After 5 min, the mixture was stirred at room temperature for 25 min. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with water. The organic layer was dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The residue was recrystallized from CH_3CN and CH_2Cl_2 to give compound **2j** (2.1 g, 4.10 mmol, crude) as a white solid.

Step 2: preparation of **compound 2k**

A solution of **2j** (2.1 g, 4.10 mmol), imidazole (2.24 g, 32.84 mmol) and TBSCl (2.78 g, 18.47 mmol) in DMF (20 mL) was stirred at 60 °C for 3 h. The mixture was quenched with water and extracted with EtOAc. The organic layer was washed with water, dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2: MeOH = 20:1) to afford compound **2k** (2.22 g, 3.54 mmol) as a white solid.

Step 3: preparation of **compound 2l**

A solution of **2k** (2.52 g, 4.03 mmol) in CH_2Cl_2 (30 mL) was stirred at 0 °C. To the mixture was added pyridinium fluoride (1.64 mL, 18.15 mmol) and the reaction was

stirred at rt for 8 h. The mixture was quenched with water and extracted with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2: MeOH = 10:1) to afford compound **2l** (1.60 g, 3.3 mmol) as a white solid.

Step 4: preparation of **compound 2m**

A solution of **2l** (2.34 g, 4.81 mmol) in pyridine (23 mL) was stirred at room temparature, to which was added 4,4'-(chloro(phenyl)methylene)bis(methoxybenzene) (3.26 g, 9.62 mmol) and the reaction was stirred at room temperature for 2 h. The mixture was quenched with water and extracted with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2: MeOH = 10:1) to afford **2m** (3.59 g, 4.56 mmol) as a yellow solid.

Step 5: preparation of **compound 2n**

To a solution of **2m** (500 mg, 0.64 mmol) and DIPEA (246.0 mg, 1.90 mmol) in THF (1.56 mL) was added 3-((chloro(diisopropylamino)phosphino)oxy)propanenitrile (450.5 mg, 1.90 mmol) at 15 °C. The mixture was stirred at rt for 1 h. Water was added to the mixture and the mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine. The organic layer was dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (petroleum ether : EtOAc =1 : 2) to afford compound **2n** (498 mg, 0.55 mmol) as a yellow oil. ^{1}H NMR (400MHz, $CDCl_3$) 10.28 (s, 1H), 8.54 (s, 1H), 8.42 - 8.33 (m, 2H), 7.93 - 7.77 (m, 6H), 7.66 (t, J=8.4 Hz, 4H), 7.59 (s, 1H), 7.55 (s, 1H), 7.52 - 7.43 (m, 1H), 7.11 (d, J=8.4 Hz, 4H), 5.55 - 5.40 (m, 1H), 5.41 - 5.35 (m, 1H), 5.09 (s, 1H), 4.62 (s, 1H), 4.59 - 4.36 (m, 2H), 4.05 (s, 6H), 3.87 - 3.81 (m, 1H), 3.57 - 3.53 (m, 1H), 3.01 - 2.90 (m, 2H), 1.03 (s, 9H), 0.31 (s, 3H), 0.00 (s, 3H); ESI-MS *m/z* 927.8 $(M+Na)^+$.

Step 6: preparation of **compound 2o**

To a solution of **2n** (813 mg, 0.898 mmol) in water and acetonitrile was added pyridinium trifluoroacetate (208.18 mg, 1.078 mmol) at room temperature. t-Butylamine (3.44 mL) was added. The resulting mixture was stirred at room temperature for 20 min. The mixture was concentrated to afford the crude product **2o** (836.39 mg, 0.898 mmol, crude) as a yellow solid, which was co-evaporated with CH_2Cl_2 (3x) and used directly for the following step.

Step 7: preparation of **compound 2p**

To a solution of **2o** (836.39 mg, 0.898 mmol) in water and CH_2Cl_2 was added dichloroacetic acid (407.149 mg, 3.158 mmol) at room temperature over 0.5 h. Pyridine (0.145 mL, 1.80 mmol) was added. After 10 min, The mixture was concentrated and the resultant residue was purified by flash chromatography (silica gel, CH_2Cl_2 : MeOH=1 : 0 to 5:1) to afford compound **2p** (467.2 mg, 0.744 mmol) as a white solid.

Step 8: preparation of **compound 2r**

A solution of **2p** (467.2 mg, 0.744 mmol) and 4A molecular sieves (0.5 g) in CH_3CN (27 mL) was stirred at room temperature under an Argon atmosphere for 3 min. Imidazole perchlorate (2.51 g, 14.89 mmol) was added. After 10 min, (2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1H-purin-9(6H)-yl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite, **1o** (866.53 mg, 0.89 mmol) in CH_3CN was added. The mixture was stirred at 26 °C for 1 h. tert-Butyl hydroperoxide (0.74 mL, 3.72 mmol) was added. The final mixture was stirred at 26 °C for 1 h. The mixture was concentrated and the residue was purified by reverse phase preparative HPLC to afford 2 fractions of the desired product **2r** (62.7 mg, 0.054 mmol and 99.4 mg, 0.076 mmol) as a white solid. ESI-MS *m/z* 1132.7 $(M+H)^+$.

Step 9: preparation of **compound 2t**

To a solution of **2r** (99.4 mg, 0.088 mmol) and 4A molecular sieves in pyridine (43 mL) was added DMOCP (48.61 mg, 0.263 mmol) at room temperature under an Argon atmosphere. The mixture was stirred at 26 °C for 1 h. Water (15.81 mg, 0.87 mmol) and I_2 (111.41 mg, 0.44 mmol) were added. The reaction mixture was stirred at 26 °C for 1 h. The reaction was quenched with a solution of Na_2SO_3 (sat'd). The mixture was filtered, and the filtrate concentrated under reduced pressure to give a crude product which was purified by reverse phase preparative HPLC to give compound **2t** (50 mg, 0.044 mmol) as a white solid. ESI-MS *m/z* 1130.4 $(M+H)^+$.

Step 10: preparation of **compound 2u** and **compound 2v**

Compound **2t** (50 mg, 0.044 mmol) was treated with a solution of $MeNH_2$ in EtOH (33%, 20 mL) and was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure to give compounds **2u** and **2v** (40 mg, 0.044 mmol), which were used for the next step without purification. ESI-MS *m/z* compound **2u** 903.3; compound **2v** 917.2 $(M+H)^+$.

Step 11: preparation of **compound 2**

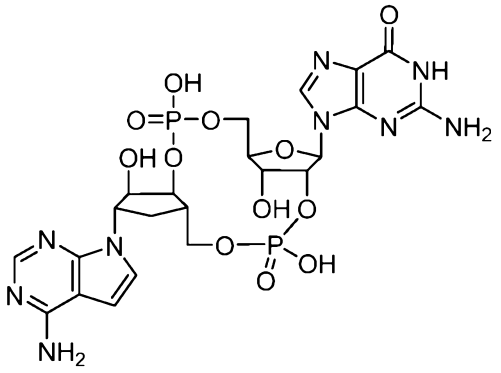
To a solution of compounds **2u** and **2v** (40 mg, 0.044 mmol) in pyridine (1.95 mL) was added triethlyamine (537.20 mg, 5.31 mmol) and triethlyammonium fluoride (427.92 mg, 2.65 mmol). The mixture was stirred at 50 °C for 10 h. Isopropoxytrimethylsilane (1.76 g, 13.27 mmol) was added and the reaction was stirred at room temperature for 2 h. The mixture was concentrated and the residue was purified reverse phase preparative HPLC to afford **compound 2 ammonium salt** (11.7 mg, 0.017 mmol), each as a white solid. **Compound 2**: ^1H NMR (400MHz, D_2O) 8.12 - 7.95 (m, 3H), 5.92 (d, J=8.4 Hz, 1H), 5.47 (d, J=3.6 Hz, 1H), 5.27 (d, J=5.2 Hz, 1H), 4.98 - 4.81 (m, 1H), 4.59 -4.44 (m, 2H), 4.37 (s, 1H), 4.31 - 4.15 (m, 3H), 4.14 - 4.07 (m, 1H), 4.02 (br d, J=10.4 Hz, 1H), 3.12 (d, J=4.4 Hz, 3H).

Step 12: preparation of **compound 2 sodium salt**

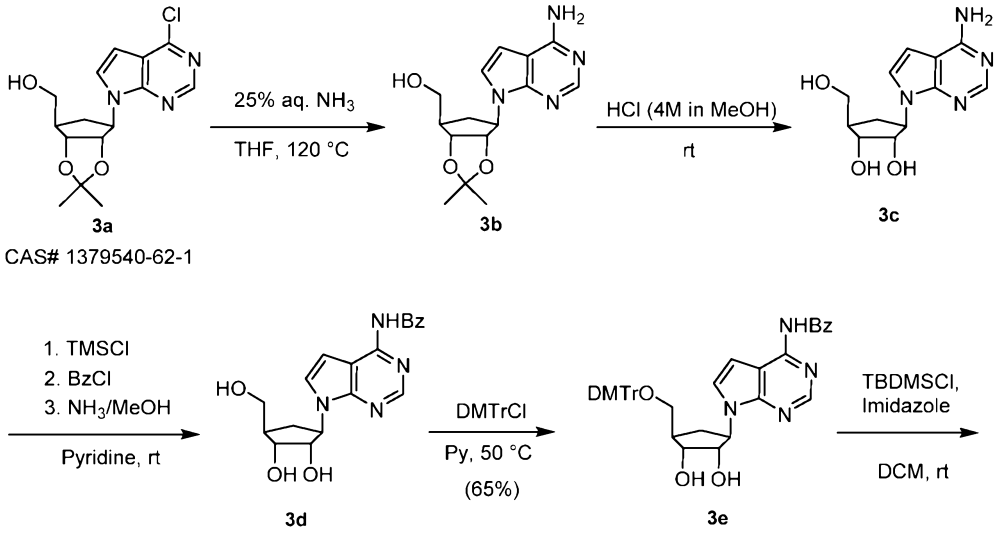
A 10 mL volume of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 11.7 mg of **compound 2 ammonium salt**) and washed with DI H_2O (2x). Then to the resin was added 15% H_2SO_4 in DI H_2O (50 mL), the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to a column with 15% H_2SO_4 in DI H_2O and washed with 15% H_2SO_4 (at least 4 CV), and then with DI H_2O until it was neutral. The resin was transferred back into the beaker, and 15% NaOH in DI H_2O solution (50 mL) was added, and the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to the column and washed with 15% NaOH in DI H_2O (at least 4 CV), and then with H_2O until it was neutral (at least 4 CV). A was dissolved in DI water (11.7 mg in 5 mL), added to the top of the column, and eluted with DI H_2O. Compound **7** was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to give **compound 2 sodium salt** (8.8 mg, 0.012 mmol) as a white solid. ^{1}H NMR (400 MHz, D_2O) 8.06 (s, 1H), 7.98 (s, 1H), 7.85 (s, 1H), 5.90 (d, J=8.4 Hz, 1H), 5.47 (d, J = 4.0 Hz, 1H), 5.27 (s, 1H), 4.96 (s, 1H), 4.58 - 4.50 (m, 1H), 4.36 (s, 1H), 4.29 - 4.22 (m, 2H), 4.29 - 4.22 (m, 1H), 4.17 (s, 1H), 4.14 - 4.08 (m, 1H), 4.03 (d, J=12.0 Hz, 1H), 3.10 (s, 3H); ^{31}P NMR (162 MHz, D_2O) -1.13, -1.75; ESI-MS *m/z* 688.8 $(M+H)^+$.

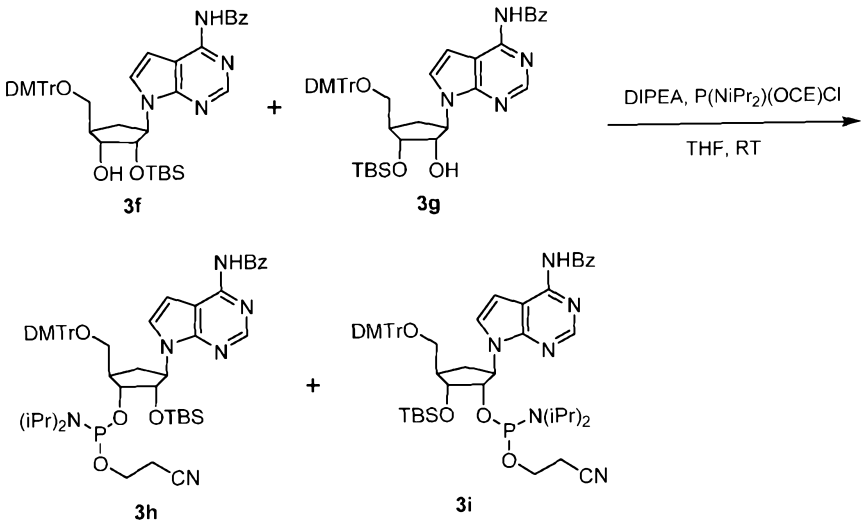
The reaction scheme illustrated in Example 3 describes one possible route to the preparation of compound **3**, and pharmaceutically acceptable salt forms thereof, of the present invention.

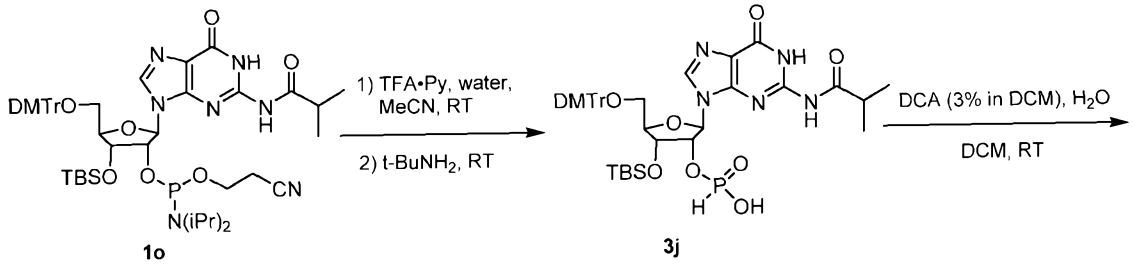
Example 3



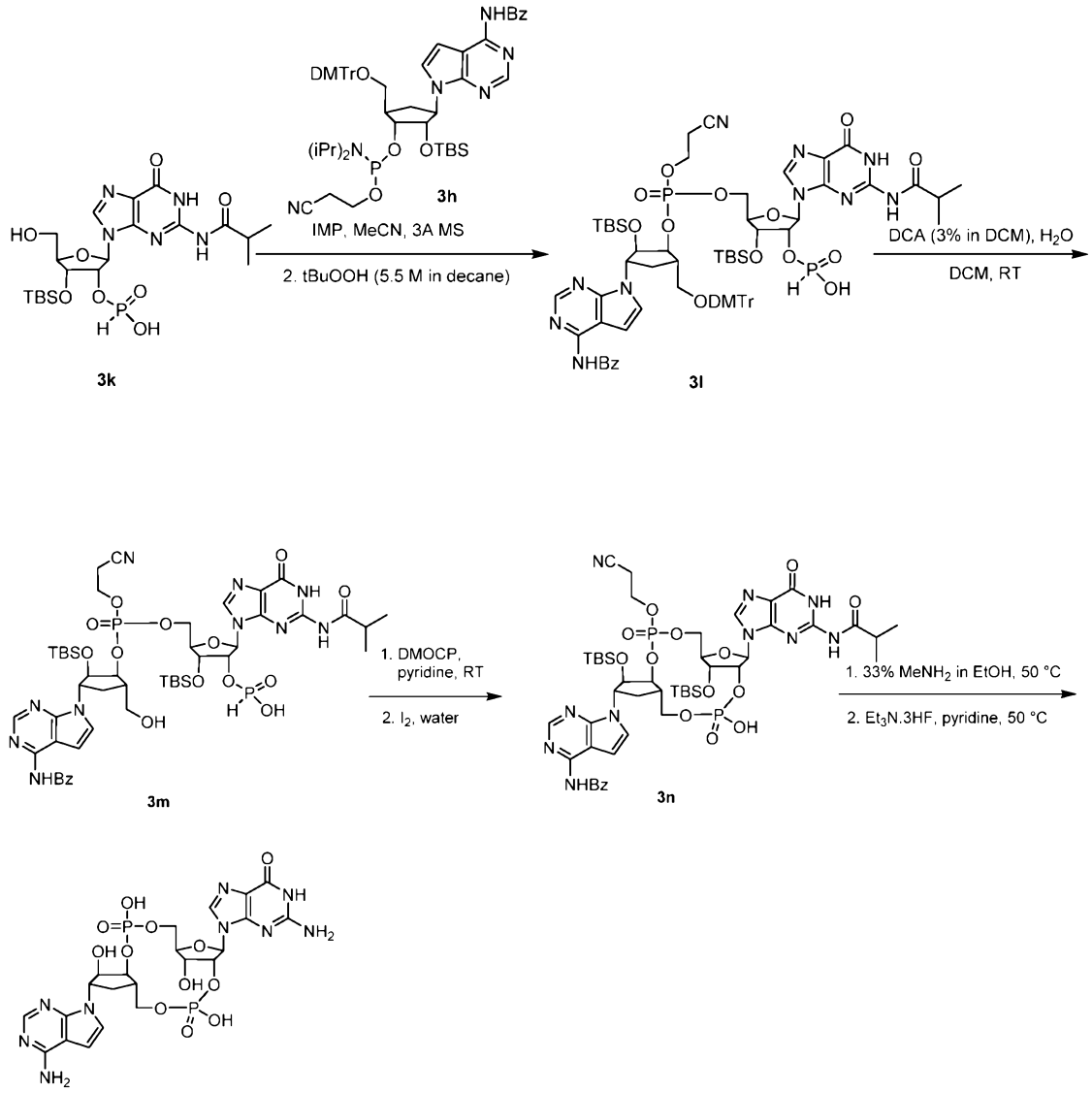
Compound 3



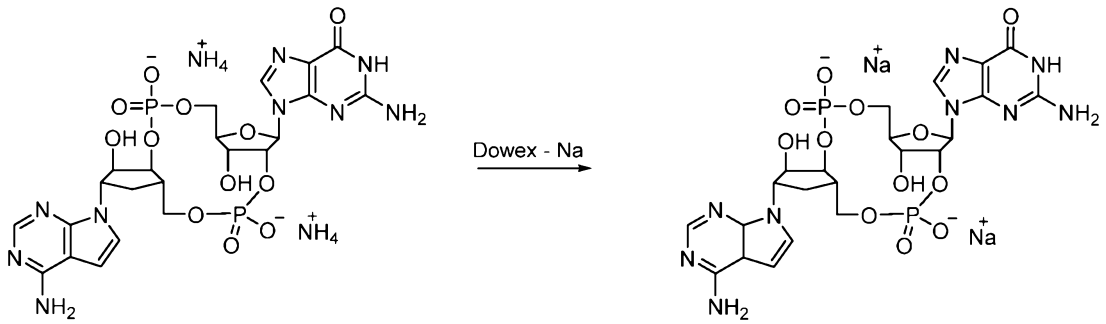

NHBz
DMTrO
OH OTBS
3f
+
NHBz
DMTrO
TBSO OH
3g
DIPEA, P(NiPr2)(OCE)Cl
THF, RT
NHBz
DMTrO
(iPr)2N
OTBS
CN
3h
+
NHBz
DMTrO
TBSO
N(iPr)2
CN
3i


DMTrO
TBSO
N(iPr)2
CN
1o
1) TFA•Py, water, MeCN, RT
2) t-BuNH2, RT
DMTrO
TBSO
OH
3j
DCA (3% in DCM), H2O
DCM, RT

5



5 **Compound 3**, ammonium salt

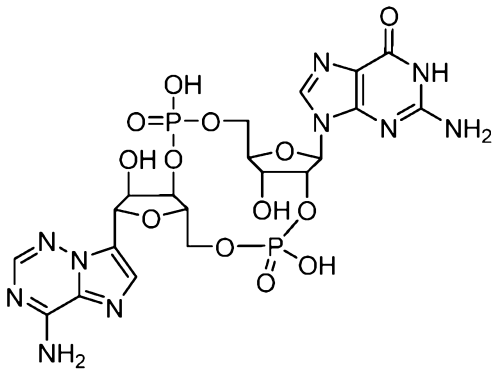


Compound 3, Ammonium salt

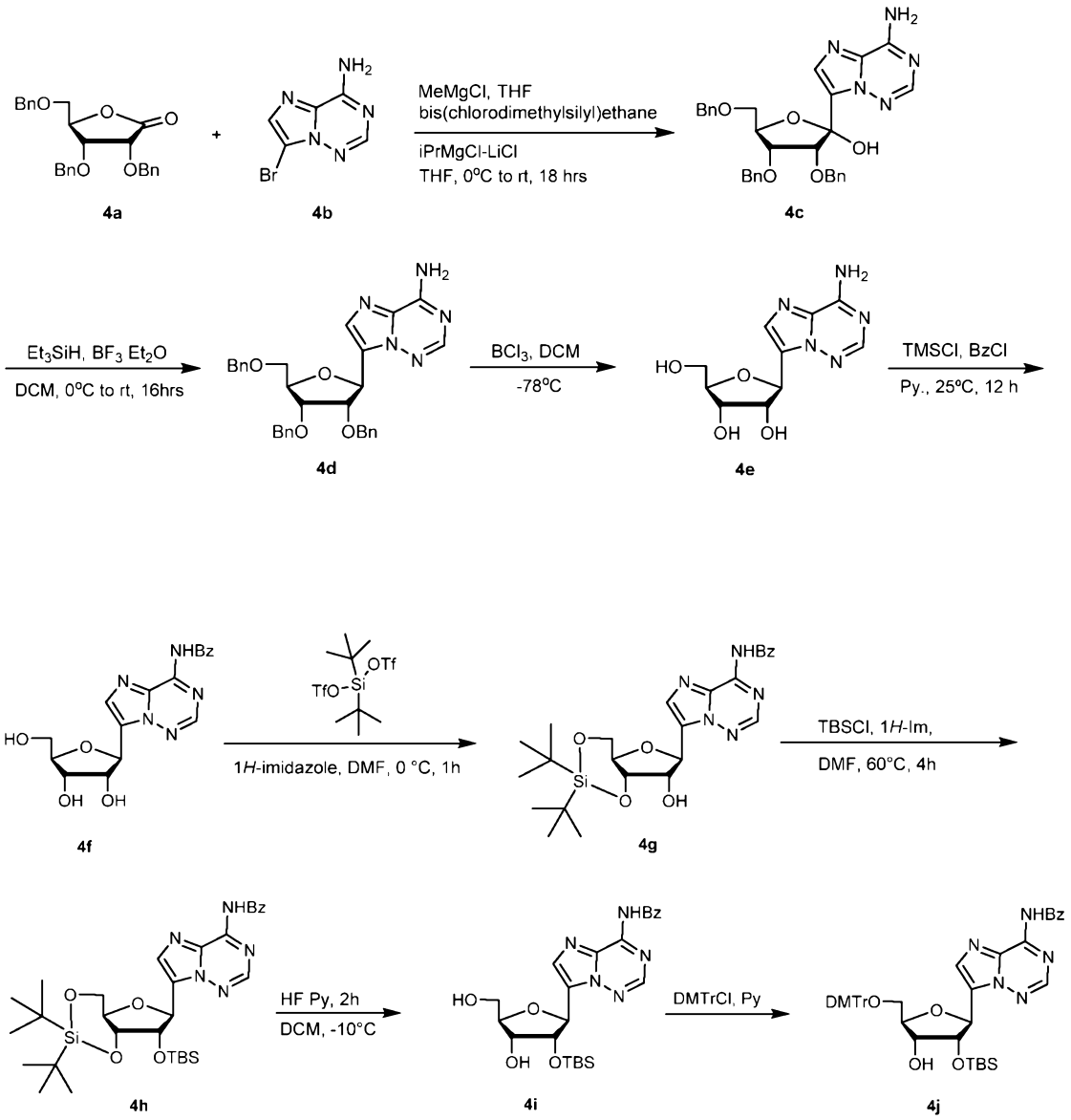
Compound 3, Sodium salt

The reaction scheme illustrated in Example 4 describes one possible route to the preparation of compound **4**, and pharmaceutically acceptable salt forms thereof, of the present invention.

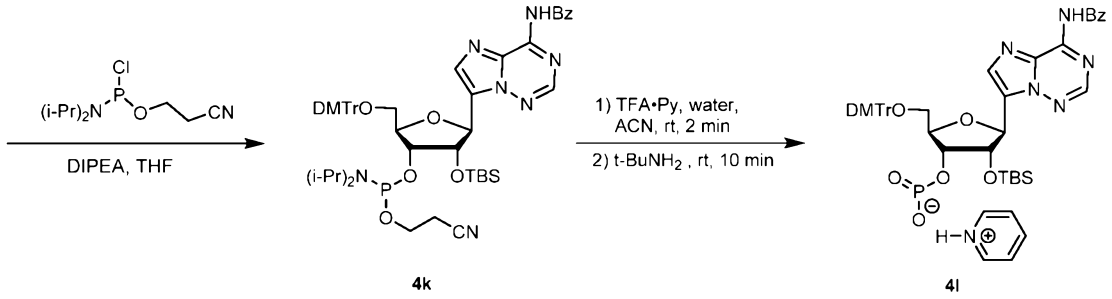
Example 4



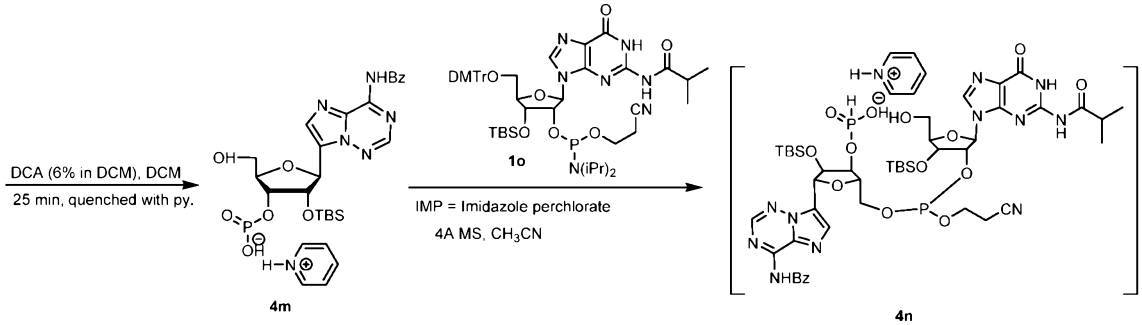
Compound 4


MeMgCl, THF
bis(chlorodimethylsilyl)ethane
iPrMgCl-LiCl
THF, 0°C to rt, 18 hrs
4a
4b
4c
Et3SiH, BF3 Et2O
DCM, 0°C to rt, 16hrs
4d
BCl3, DCM
-78°C
4e
TMSCl, BzCl
Py., 25°C, 12 h
4f
1H-imidazole, DMF, 0 °C, 1h
4g
TBSCl, 1H-Im,
DMF, 60°C, 4h
4h
HF Py, 2h
DCM, -10°C
4i
DMTrCl, Py
4j

5

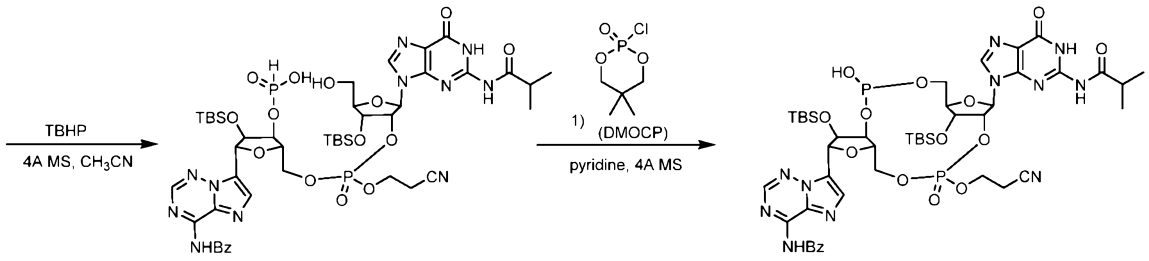


4k 4l

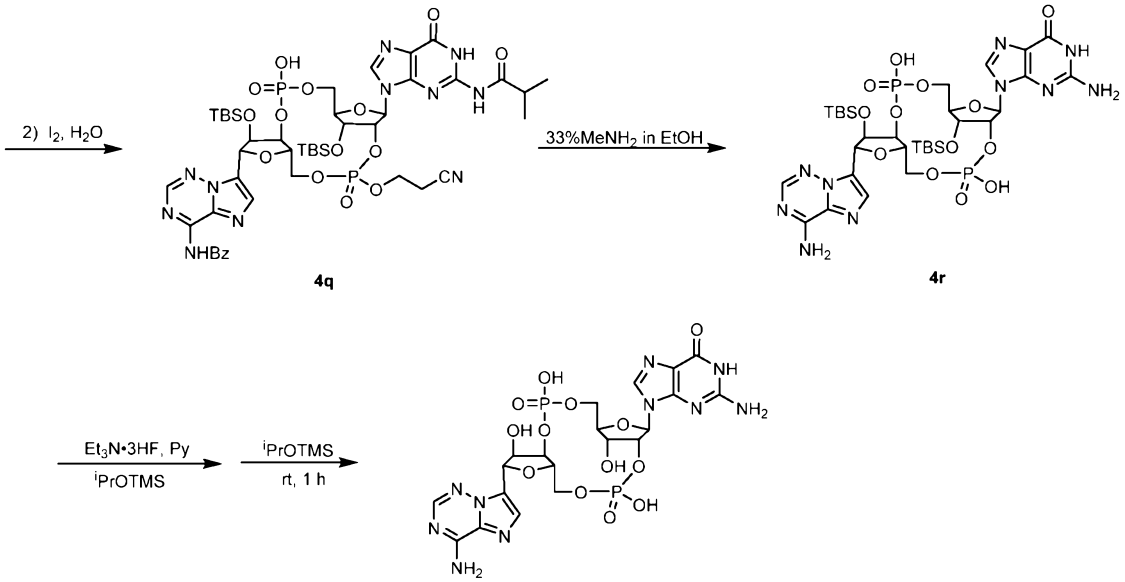


4m 1o 4n

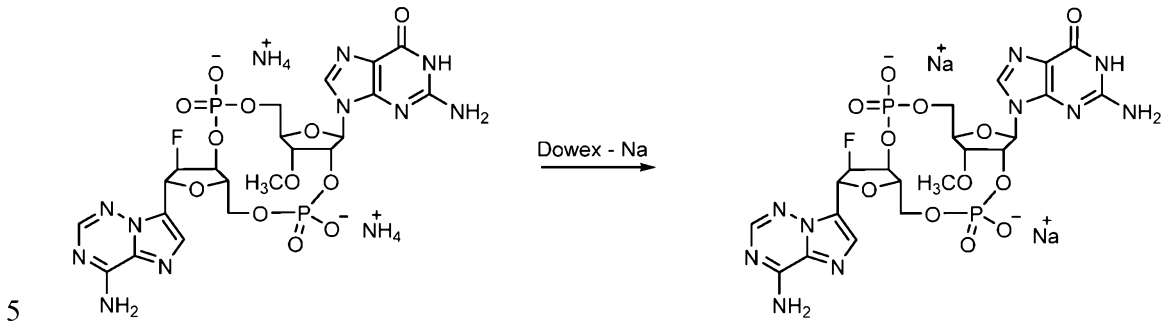
5



4o 4p



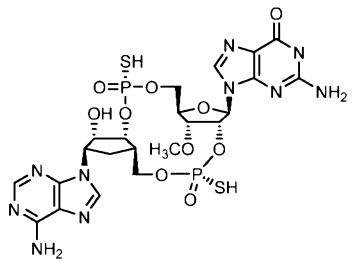
Compound 4, ammonium salt



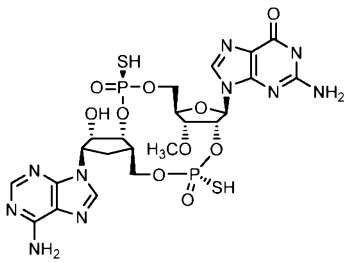
Compound 4, Ammonium salt

Compound 4, Sodium salt

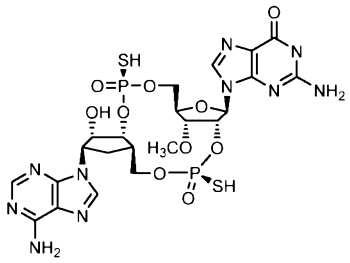
Example 5



Compound 5

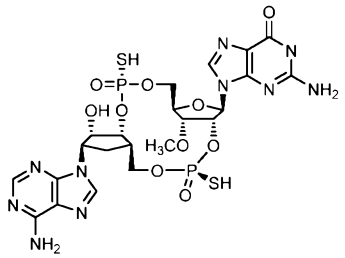


Compound 6

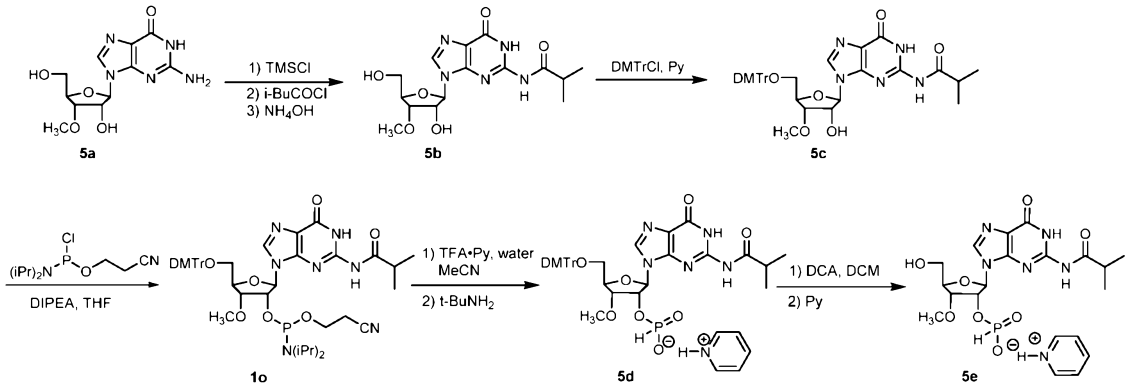


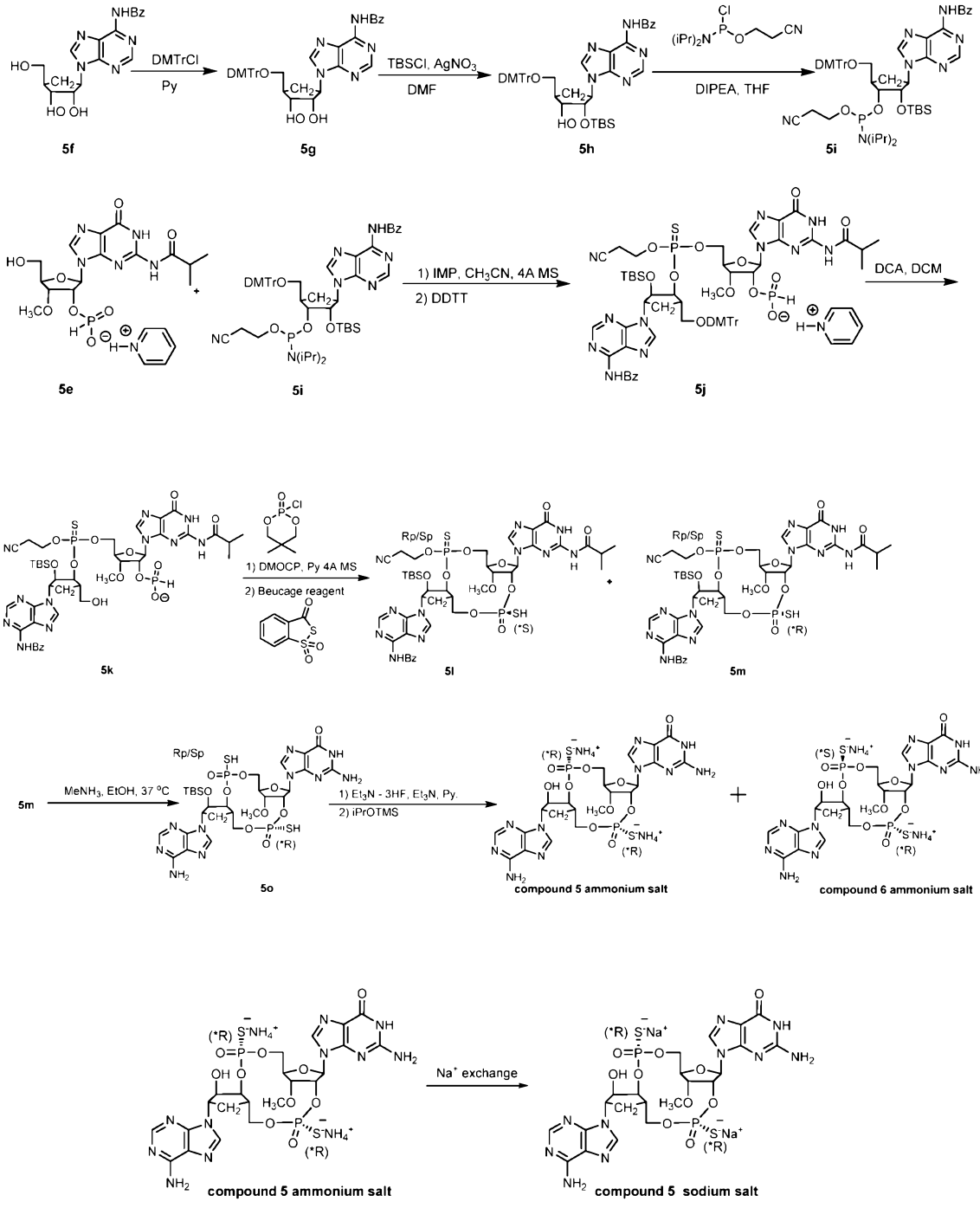
Compound 7

and



Compound 8

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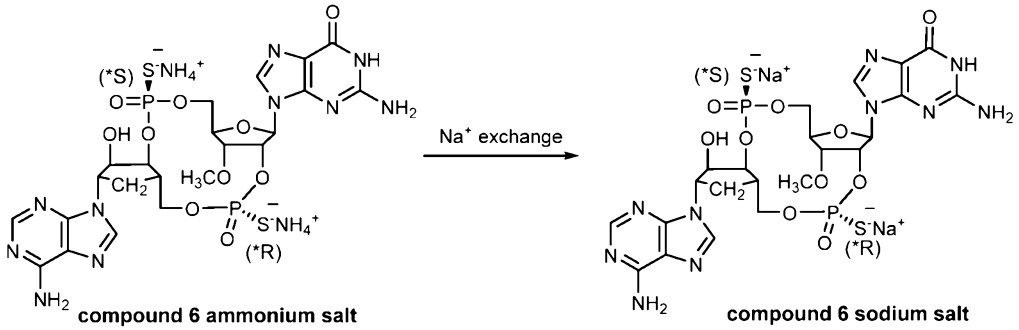
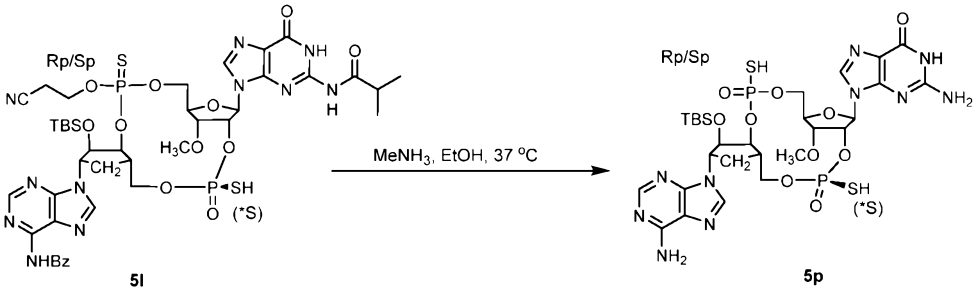
Py
TBSCl, AgNO3
DMF
DIPEA, THF
1) IMP, CH3CN, 4A MS
2) DDTT
DCA, DCM
1) DMOCP, Py 4A MS
2) Beucage reagent
MeNH3, EtOH, 37 °C
1) Et3N - 3HF, Et3N, Py.
2) iPrOTMS
Na+ exchange
5f
5g
5h
5i
5e
5j
5k
5l
5m
5o
compound 5 ammonium salt
compound 6 ammonium salt
compound 5 sodium salt

5



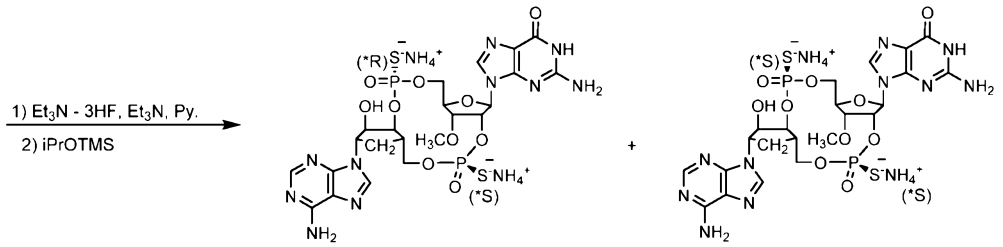
compound 6 ammonium salt

compound 6 sodium salt



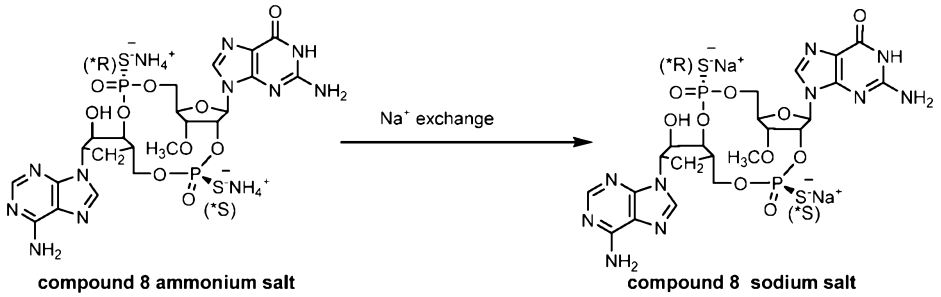
5l

5p



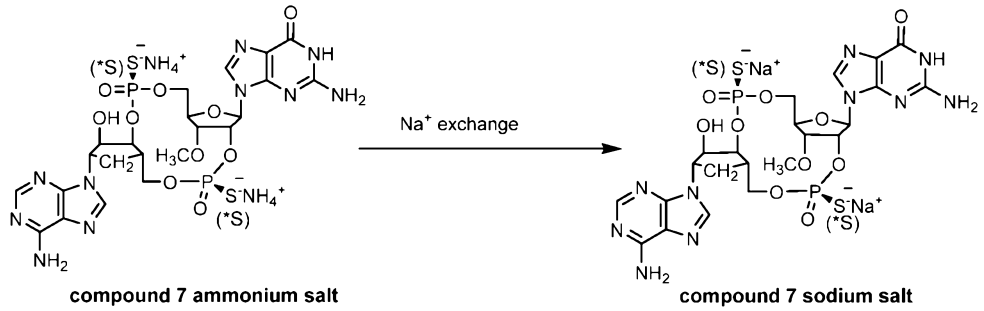
compound 8 ammonium salt

compound 7 ammonium salt



compound 8 ammonium salt

compound 8 sodium salt



compound 7 ammonium salt compound 7 sodium salt

Step 1: preparation of compound **5b**

To a solution of compound **5a** (10.0 g, 33.64 mmol) in pyridine (250 ml) was added TMSCl (18.27 g, 168.20 mmol) dropwise at 0 °C; after stirring at 15 °C for 1 h, isobutyryl chloride (4.30 g, 40.37 mmol) was added dropwise at 15 °C. After stirring at 15 °C for 2 h, the mixture was quenched with H_2O (50 mL) at 0 °C and $NH_3 \cdot H_2O$ (50 mL) was added at 0 °C. After 10 mins, the mixture was stirred at 15 °C for 0.5 h. The above procedure was repeated (20g scale of compound**5a**) and the two reaction mixtures combined. The mixtures were then concentrated under reduced pressure and the residue was purified by flash column chromatography (DCM/MeOH=100/1 to 10/1) to afford compound **5b** (25.5 g) as a white solid.

Step 2: preparation of compound **5c**

To a solution of compound **5b** (24.5 g, 66.69 mmol) in pyridine (500 ml) was added dimethoxy trityl chloride (24.85 g, 73.36 mmol) at 0 °C. After stirring at 25 °C for 2 h, the solution was concentrated under reduced pressure to give a residue. The residue was diluted with ethyl acetate (500 mL) and washed with water (300 mL x 3). The organic layer was concentrated under reduced pressure to give a residue. The residue was combined with another batch (generated from a 2g scale of compound **5b**) and purified by flash column chromatography (DCM/MeOH =100/1 to 10/1) to afford compound **5c** (30.0 g) as a yellow solid. LCMS: m/z 670.2 $[M+H]^+$.

Step 3: preparation of compound **1o**

To a solution of compound **5c** (1 g, 1.49 mmol) in THF (20 mL) was added N-ethyl-N-isopropylpropan-2-amine (1.16 g, 8.96 mmol,) and 3-((chloro(diisopropylamino)-phosphino)oxy)propanenitrile (1.11 g, 4.48 mmol, 3 eq.) at 0 °C. After stirring for 2 h at 25 °C, the reaction mixture was quenched by addition water (30 mL) and diluted with ethyl acetate (30 mL). Aqueous layer was extracted with ethyl acetate (20 mL x 2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under pressure to give a residue. The residue was purified by flash column chromatography (DCM/EA=10/1 to 2/1) to afford compound **1o** (0.99 g) as a white solid. LCMS:ESI-MS: *m/z* 787.4 $[M + H]^+$.

Step 4: preparation of compound **5d**

To a solution of compound **1o** (0.99 g, 1.14 mmol) in acetonitrile (7 mL) was added water (0.041 g, 2.28 mmol) and pyridinium trifluoroacetate (0.263 g, 1.37 mmol). Tert-butylamine (7 mL) was added and the reaction mixture was stirred for 15 min at 25 °C. The mixture was then concentrated under reduced pressure to give a foam. The foam was dissolved in CH_3CN (10.0 mL), concentrated under reduced pressure to afford compound **5d** (0.925 g) as a white foam which was used into the next step without any further purification.

Step 5: preparation of compound **5e**

To a solution of compound **5d** (0.925 g, 1.14 mmol) in dichloromethane (20mL) and water (0.205 g, 11.38 mmol) was added 2,2-dichloroacetic acid (20 mL, 6% in DCM). After stirring at 25 °C for 20 min, the mixture was quenched with pyridine (3 mL) and concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography (DCM/MeOH=10/1 to 5/1) to afford compound **5e** (0.43 g, 0.84 mmol) as a white foam. LCMS:ESI-MS: *m/z* 432.2 $[M + H]^+$.

Step 6: preparation of compound **5g**

To a solution of compound **5f**(4 g, 10.83 mmol) in pyridine (40 mL) was added 4,4'-(chloro(phenyl)methylene)bis(methoxybenzene) (4.4 g, 12.99 mmol) at 25 °C. After stirring at 25 °C for 2 h the reaction mixture was quenched with methanol (5 mL) and then concentrated under reduced pressure to afford a residue. The residue was purified by flash column chromatography (DCM/MeOH = 50/1 to 20/1) to give compound **5g** (6.3 g) as a yellow foam. LCMS:ESI-MS: *m/z* 672.3 $[M + H]^+$.

Step 7: preparation of compound **5h**

To a solution of compound **5g** (0.5 g, 0.74 mmol) in N,N-dimethylformamide (8 mL) was added tert-butylchlorodimethylsilane (0.17 g, 1.12 mmol) and 1H-imidazole (0.15 g, 2.23 mmol) at 25 °C. After stirring at 25 °C for 12 h, the reaction mixture was quenched with methanol (2 mL) and diluted with ethyl acetate (40 mL); the organic layer was successively washed with H_2O (20 mL x 2), brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography (DCM/MeOH = 50/1 to 10/1) to afford compound **5h** (0.14 g) as a yellow oil. LCMS:ESI-MS: *m/z* 786.8 $[M + H]^+$.

Step 8: preparation of compound **5i**

To a solution of compound **5h** (0.5 g, 0.64 mmol) in THF (9 mL) was added at 0 °C N-ethyl-N-isopropylpropan-2-amine (0.49 g, 3.82 mmol) and 3-((chloro(diisopropyl-amino)phosphino)oxy)propanenitrile (0.45 g, 1.91 mmol). After stirring for 12 h at 25 °C, the reaction mixture was quenched with MeOH (3 mL), diluted with ethyl acetate (30 mL) and water (30 mL). Aqueous layer was extracted with ethyl acetate (20 mL x 2). The combined organic layers were successively dried over anhydrous Na_2SO_4, filtered and concentrated under pressure to give a residue. The residue was purified by flash column chromatography (DCM/EA=10/1 to 1/1) to afford compound **5i** (0.48 g, 0.49 mmol) as a white solid. LCMS:ESI-MS: *m/z* 903.5 $[MH\text{-}iPr_2]^+$.

Step 9: preparation of compound **5j**

To a solution of compound **5e** (0.25 g, 0.49 mmol) in acetonitrile (10 mL) was added 4A molecular sieves; the resulting mixture was stirred at 25 °C for 10 mins. 1H-imidazole perchlorate (0.42 g, 2.45 mmol) was added and the mixture stirred for another 10 mins at 25 °C. Compound **5i** (0.48 g, 0.49 mmol) was added and the mixture stirred at 25 °C for 1 h. N,N-dimethyl-N'-(5-sulfanylidene-1,2,4-dithiazol-3-yl)- methanimidamide (DDTT) (0.5 g, 2.45 mmol) was then added and the mixture stirred at 25 °C for another 1 h. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to afford a residue. The residue was purified by flash column chromatography (DCM/Methanol=10/1 to 3/1) to afford compound **5j** (0.42 g) as a white solid.

Step 10: preparation of compound **5k**

To a solution of compound **5j** (0.42 g, 0.31 mmol) in dichloromethane (20 mL) and water (0.056 g, 3.115 mmol, 10 eq.) was added 2,2-dichloroacetic acid (6% in DCM, 20 mL). After stirring at 25 °C for 20 min, the mixture was then quenched with pyridine (3 mL), then concentrated under reduced pressure to afford a residue. The residue was purified by flash column chromatography (DCM/MeOH=10/1 to 2/1) to afford compound **5k** (0.29 g) as a white solid. LCMS:ESI-MS: m/z= 1046.5$[M+1]^+$;

Step 11: preparation of compound **5l** and **5m**

To a solution of compound **5k** (0.34 g, 0.32 mmol) in pyridine (80 mL) was added 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide (0.18 g, 0.97 mmol); the resulting mixture was stirred at 25 °C for 1 h. It was then added 3H-benzo[c][1,2]dithiol-3-one 1,1-dioxide (0.325 g, 1.62 mmol) and the mixture stirred at 25 °C for 1 h. The reaction mixture was concentrated under reduced pressure; the residue was purified by preparative HPLC (Column: Agela DuraShell 150mm x 25mm x 5μm; water (10mM NH_4HCO_3)- v/v) (A) - CH_3CN (B); from 37% to 67% of B; Flow Rate: 25 mL/min) to afford two fractions including a mixture of analogues **5m** (46 mg) and **5l** (59 mg) as white solids. Each fraction

was then carried out separately. LCMS:ESI-MS: *m/z* 1060.3 $[M + H]^+$.(compound **5m**); LCMS:ESI-MS: *m/z* 1060.4 $[M + H]^+$.(compound **5l**).

Step 12: preparation of compound **5o**

Compound **5m** (80 mg, 0.075 mmol) was treated with methanamine (8 mL, 35% in EtOH); after stirring at 25°C for 12 h, the reaction mixture was concentrated under reduced pressure to afford compound **5o** (62.8 mg) which was used into the next step without any further purification.

Step 13: preparation of compounds **5** and **6**

To a solution of compound **5o** (62.8 mg, 0.075 mmol) in pyridine (8 mL), was added triethylamine (0.46 g, 4.53 mmol) and triethylamine trihydrofluoride (0.37 g, 2.26 mmol); the resulting mixture was stirred at 50 °C for 10 h. Isopropoxytrimethylsilane (0.99 g, 7.55 mmol) was added at 15 °C and the mixture stirred for another 2 h. The mixture was concentrated under reduced pressure and the residue purified by Prep-HPLC (Column: Agela DuraShell 150mm x 25mm x 5μm; water (0.05% ammonia hydroxide v/v) (A) - CH_3CN (B); from 1% to 16% of B; Flow Rate: 25 mL/min) to afford compound **5** ammonium salt (27 mg) and compound **6** ammonium salt (11 mg) as white solids. LCMS:ESI-MS: *m/z* 718.8 $[M + H]^+$. (compound **5**); LCMS: ESI-MS: *m/z* 718.8 $[M + H]^+$. (compound **6**).

Step 14: preparation of **5 sodium salt**

A 20 mL volume of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 58 mg of compound **5**) and washed with DI H_2O (2X). It was then added to the resin 15% H_2SO_4 in DI H_2O (50 mL), the mixture was stirred for 15 min and decanted (1X). The resin was transferred to a column with 15% H_2SO_4 in DI H_2O and washed with 15% H_2SO_4 (at least 4 Column Volume), and then with DI H_2O until it was neutral. The resin was transferred back into the beaker and 15% NaOH in H_2O solution (50 mL) was added; the mixture was stirred for 15 min and decanted (1X). The resin was transferred to the

column and washed with 15% NaOH in H_2O (at least 4 Column Volume), and then with H_2O until it was neutral (at least 4 Column Volume). Compound **5 ammonium salt** was dissolved in DI water (58 mg in 5 mL), added to the top of the column, and eluted with DI water. **5** was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to afford **compound 5 sodium salt** (55.1 mg) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ = 8.39 (s, 1H), 8.35 (s, 1H), 8.19 (s, 1H), 5.98 (d, J=8.5 Hz, 1H), 5.50 (ddd, J=4.3, 8.7, 12.9 Hz, 1H), 5.09-5.02 (m, 1H), 4.91 (q, J=7.4 Hz, 1H), 4.74 (dd, J=4.8, 6.3 Hz, 1H), 4.58 (br s, 1H), 4.32 (d, J=4.3 Hz, 1H), 4.30-4.17 (m, 3H), 4.05-3.97 (m, 1H), 3.63 (s, 3H), 2.82-2.68 (m, 2H), 1.93-1.82 (m, 1H); ^{31}P NMR (162 MHz, D_2O) δ = 57.599, 54.556; LCMS:ESI-MS: *m/z* 718.8 $[M + H]^+$.

Step 15: preparation of **6 sodium salt**

An 8 mL volume of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 22 mg of compound **6**) and washed with DI H_2O (2X). It was then added to the resin 15% H_2SO_4 in DI H_2O (50 mL), the mixture was stirred for 15 min and decanted (1X). The resin was transferred to a column with 15% H_2SO_4 in DI H_2O and washed with 15% H_2SO_4 (at least 4 Column Volume), and then with DI H_2O until it was neutral. The resin was transferred back into the beaker and 15% NaOH in H_2O solution (50 mL) was added; the mixture was stirred for 15 min and decanted (1X). The resin was transferred to the column and washed with 15% NaOH in H_2O (at least 4 Column Volume), and then with H_2O until it was neutral (at least 4 Column Volume). Compound **6 ammonium salt** was dissolved in DI water (39 mg in 5 mL), added to the top of the column, and eluted with DI water. **6** was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to afford **compound 6 sodium salt** (10.1 mg) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ = 8.47 (s, 1H), 8.26 (s, 1H), 7.98 (s, 1H), 5.95 (d, J=8.5 Hz, 1H), 5.77 (ddd, J=4.3, 8.4, 12.7 Hz, 1H), 5.13 - 5.05 (m, 1H), 4.97 - 4.90 (m, 1H), 4.58 (br s, 1H), 4.53 (t, J=3.9 Hz, 1H), 4.47 - 4.38 (m, 1H), 4.29 (d, J=4.5 Hz, 1H), 4.24 (td, J=5.4, 10.6 Hz, 1H), 4.12 (br d, J=12.3 Hz, 1H), 4.05 (br d, J=10.3 Hz, 1H), 3.62 (s, 3H), 2.91 - 2.69 (m, 2H),

2.17 - 2.01 (m, 1H); ^{31}P NMR (162 MHz, D_2O) δ = 55.896, 54.936; LCMS:ESI-MS: *m/z* 718.8 $[M + H]^+$.

Example 5a

Step 1: preparation of compound **5p**

Compound **5l** (60 mg, 0.057 mmol) was treated with methanamine (6 mL, 35% in EtOH); after stirring at 25°C for 12 h, the reaction mixture was concentrated under reduce pressure to afford compound **5p** (47.14 mg) which was used for next step without any futher purification.

Step 2: preparation of compound **7** and **8**

To a solution of compound **5p** (47.14 mg, 0.06 mmol) in pyridine (5 mL), was added triethylamine (0.34 g, 3.4 mmol) and triethylamine trihydrofluoride (0.27 g, 1.7 mmol); the resulting mixture was stirred at 50 °C for 10 h. Isopropoxytrimethylsilane (0.75 g, 5.66 mmol) was added at 15 °C and the mixture stirred for another 2 h. The mixture was concentrated under reduced pressure and the residue purified by Prep-HPLC (Column: Agela DuraShell 150mm x 25mm x 5μm; water (0.05% ammonia hydroxide v/v) (A) - CH_3CN (B); from 1% to 16% of B; Flow Rate: 25 mL/min) to afford compound **7** ammonium salt (8 mg) and compound **8** ammonium salt (19 mg) as white solids. LCMS:ESI-MS: *m/z* 718.8 $[M + H]^+$. (compound **8**); LCMS: ESI-MS: *m/z* 718.8 $[M + H]^+$. (compound **7**).

Step 3: preparation of **7 sodium salt**

An 8 mL volume of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 20 mg of compound **7**) and washed with DI H_2O (2X). It was then added to the resin 15% H_2SO_4 in DI H_2O (50 mL), the mixture was stirred for 15 min and decanted (1X). The resin was transferred to a column with 15% H_2SO_4 in DI H_2O and washed with 15% H_2SO_4 (at least 4 Column Volume), and then with DI H_2O until it was neutral. The resin was transferred back into the beaker and 15% NaOH in H_2O solution (50 mL) was added;

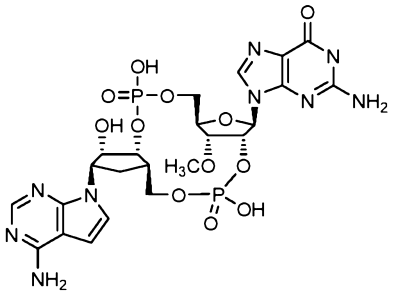
the mixture was stirred for 15 min and decanted (1X). The resin was transferred to the column and washed with 15% NaOH in H_2O (at least 4 Column Volume), and then with H_2O until it was neutral (at least 4 Column Volume). Compound **7 ammonium salt** was dissolved in DI water (39 mg in 5 mL), added to the top of the column, and eluted with DI water. **7** was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to afford **compound 7 sodium salt** (15.5 mg) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ = 8.32 (s, 1H), 8.25 (s, 2H), 6.00 (d, J=8.5 Hz, 1H), 5.57 - 5.42 (m, 1H), 4.98 - 4.83 (m, 3H), 4.63 (br s, 1H), 4.44 (d, J=4.0 Hz, 1H), 4.24 (br s, 2H), 4.14 (br d, J=5.0 Hz, 2H), 3.62 (s, 3H), 2.85 - 2.64 (m, 2H), 2.04 - 1.91 (m, 1H); ^{31}P NMR (162 MHz, D_2O) δ 54.121, 52.853; ESI-MS: *m/z* 718.8 $[M + H]^+$.

Step 4: preparation of **8 sodium salt**

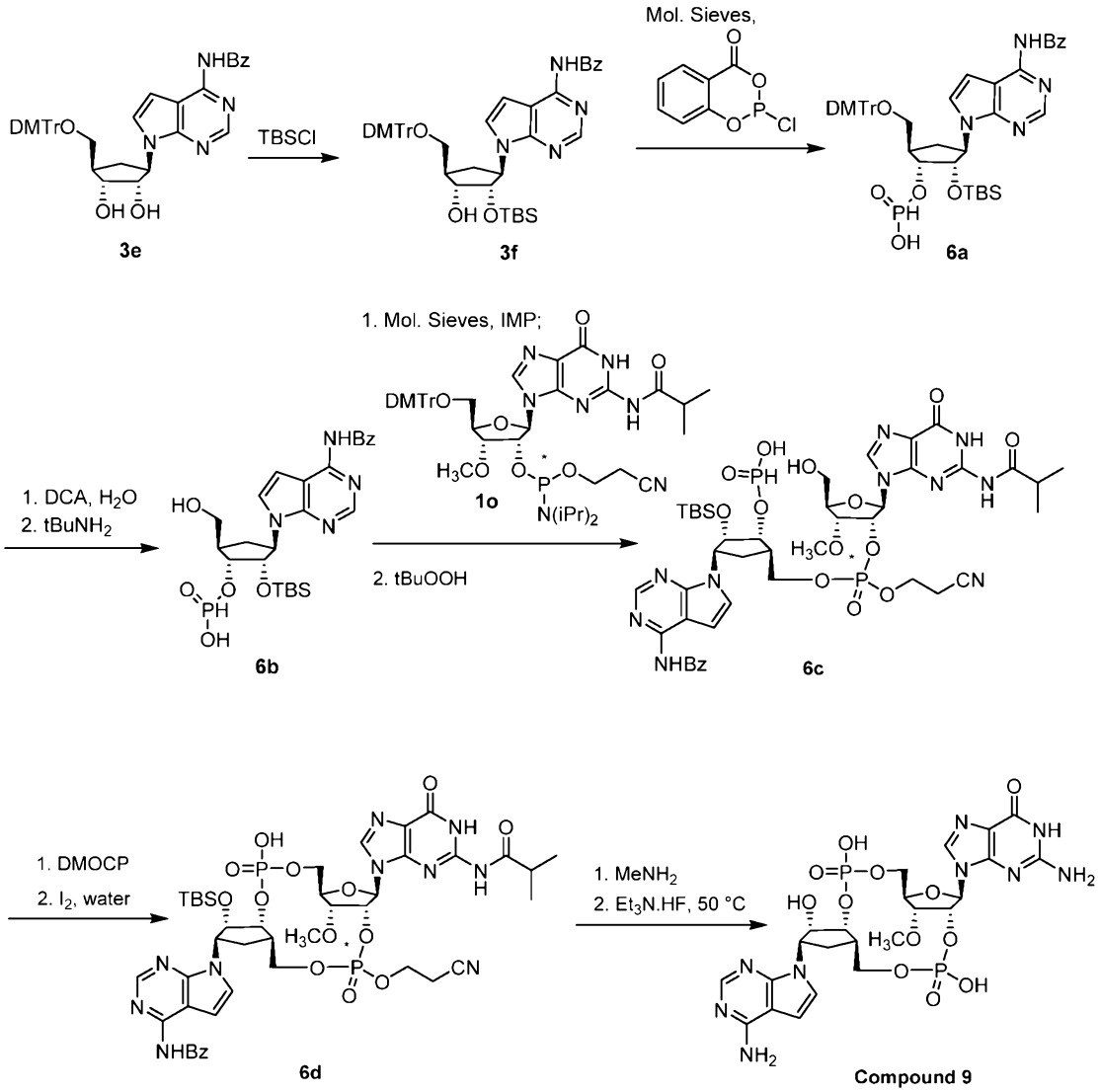
An 8 mL volumne of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 39 mg of compound **8**) and washed with DI H_2O (2X). It was then added to the resin 15% H_2SO_4 in DI H_2O (50 mL), the mixture was stirred for 15 min and decanted (1X). The resin was transferred to a column with 15% H_2SO_4 in DI H_2O and washed with 15% H_2SO_4 (at least 4 Column Volume), and then with DI H_2O until it was neutral. The resin was transferred back into the beaker and 15% NaOH in H_2O solution (50 mL) was added; the mixture was stirred for 15 min and decanted (1X). The resin was transferred to the column and washed with 15% NaOH in H_2O (at least 4 Column Volume), and then with H_2O until it was neutral (at least 4 Column Volume). Compound **8 ammonium salt** was dissolved in DI water (39 mg in 5 mL), added to the top of the column, and eluted with DI water. **8** was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to afford **compound 8 sodium salt** (25.8 mg) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ = 8.21 (d, *J*= 2.3 Hz, 2H), 7.91 (s, 1H), 5.90 (d, *J*= 8.8 Hz, 1H), 5.83 - 5.74 (m, 1H), 5.01 (dt, *J*= 4.1, 8.1 Hz, 1H), 4.88 - 4.83 (m, 1H), 4.59 (br s, 1H), 4.49 - 4.38 (m, 2H), 4.32 (d, *J*= 4.3 Hz, 1H), 4.28 - 4.20 (m, 1H), 4.14 - 4.04 (m, 2H), 3.60 (s, 3H), 2.89 -

2.70 (m, 2H), 2.13 - 2.03 (m, 1H); ^{31}P NMR (162 MHz, D_2O) δ = 54.483, 52.291; LCMS: ESI-MS: *m/z* 718.8 $[M + H]^+$.

Example 6



Compound 9



Step 1: preparation of intermediate **3f**

Diol **3e** ([1834500-45-6], 27.2 g, 40.6 mmol) and imidazole (8.3 g, 121.7 mmol) were dissolved in DCM (600mL), followed by the addition of tert-butyldimethylsilyl chloride (10.4 g, 69.0 mmol). The reaction mixture was stirred at room temperature for 16 hours, after which it was poured into aqueous NaHCO3 and extracted with DCM. The combined organic layers were washed with brine, dried with anhydrous Na2SO4, filtered and evaporated under reduced pressure. The crude product was purified by silica column

chromatography (gradient elution: 0 – 50% EtOAc in petroleum ether) to give a mixture of intermediate 1b and its 3'-hydroxyl protected isomer (structure not shown) (25 g in total, yield: 79%). This regioisomeric mixture was separated by preparative reversed phase HPLC (Stationary phase: Phenomenex Synergi, 10 µm Max-RP, 250 x 50 mm; Mobile phase: H2O (A) - MeCN (B); isocratic elution: 92% B, flow rate: 110 mL/min) to give pure intermediate **3f** as the first eluding isomer (12 g, yield: 37%). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm -0.42 (s, 3 H), -0.21 (s, 3 H), 0.62 (s, 9 H), 1.89 - 2.02 (m, 1 H), 2.17 - 2.30 (m, 2 H), 3.07 - 3.15 (m, 1 H), 3.15 - 3.23 (m, 1 H), 3.73 (s, 6 H), 3.83 - 3.90 (m, 1 H), 4.41 (dd, J=8.8, 5.3 Hz, 1 H), 4.45 (d, J=5.3 Hz, 1 H), 5.03 - 5.15 (m, 1 H), 6.64 (d, J=3.5 Hz, 1 H), 6.91 (d, J=8.8 Hz, 4 H), 7.23 (t, J=7.5 Hz, 1 H), 7.27 - 7.38 (m, 6 H), 7.45 (d, J=7.1 Hz, 2 H), 7.49 (d, J=3.5 Hz, 1 H), 7.54 (t, J=7.5 Hz, 2 H), 7.63 (t, J=7.1 Hz, 1 H), 8.06 (d, J=7.1 Hz, 2 H), 8.48 (s, 1 H), 11.05 (br s, 1 H); ESI-MS: m/z 785.3 [M+H]+

Step 2: preparation of intermediate **6a**

2 g activated 4Å MS was added to a solution of intermediate **3f** (2.5 g, 3.2 mmol) in pyridine (12.5 mL) and 1,4-dioxane (35 mL), the resulting reaction mixture was stirred at room temperature for 15 min. A solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (1.26 g, 6.2 mmol) in dry 1,4-dioxane (2.5 mL) was added and stirring was continued for 30 min. The reaction mixture was quenched by adding 15 mL of a 1/1 mixture of water and pyridine, followed by an extra amount of water (50 mL). The obtained mixture was filtered and extracted with EtOAc. The combined organic phases were washed with aqueous NaHCO3, dried with Na2SO4, filtered and concentrated under vacuum to give crude intermediate **6a** as a white solid (3.3 g). The crude product was used as such in the next step without purification.

ESI-MS: m/z 849.2 [M+H]+

Step 3: preparation of intermediate **6b**

To a solution of crude intermediate **6a** (6.5 g, 5.7 mmol) in DCM (80 mL) was added H2O (1.0 mL, 56.7 mmol) and dichloroacetic acid (1.46 g, 11.3 mmol). The reaction

mixture was stirred at room temperature for 30 min. An extra amount of dichloroacetic acid (472 µL, 5.7 mmol) was added and stirring was continued for 20 min. The reaction was quenched by the addition of tert-butylamine (26 mL, 248.1 mmol) and concentrated under vacuum. The obtained residue was purified by silica column chromatography (gradient elution: 0 – 30% MeOH in DCM) to give intermediate 1d as a white solid (3 g, yield: 82%). Intermediate **6b** was converted into the corresponding tert-butylammonium salt by having it stirred as a solution in DCM (30 mL) for 1 hour presence of tert-butylamine, followed by concentration under reduced pressure and drying under high vacuum. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm -0.4 (s, 3 H), -0.1 (s, 3 H), 0.6 (s, 9 H), 1.3 (s, 9 H), 1.7 - 1.8 (m, 1 H), 2.2 (dt, J=12.9, 8.6 Hz, 1 H), 2.3 - 2.4 (m, 1 H), 3.5 - 3.6 (m, 2 H), 4.2 - 4.4 (m, 2 H), 5.1 - 5.2 (m, 1 H), 5.8 (br s, 1 H), 6.7 (d, J=583.9 Hz, 1 H), 6.7 (d, J=3.7 Hz, 1 H), 7.5 (t, J=7.7 Hz, 2 H), 7.6 - 7.7 (m, 2 H), 8.0 (br s, 3 H), 8.1 (d, J=7.3 Hz, 2 H), 8.5 (s, 1 H), 11.0 (br s, 1 H); ESI-MS: m/z 547.0 [M+H]+ .

Step 4: preparation of intermediate **6c**

1H-imidazolium perchlorate (IMP, 2.4 g, 14.0 mmol) was added to a solution of intermediate **6b** (500 mg, 0.81 mmol) in anhydrous MeCN (23 mL) under nitrogen. The resulting mixture was stirred at room temperature for 10 min, after which a solution of 5'-O-(4,4-dimethoxytrityl)-N2-isobutyryl-3'-O-methylguanosine-2'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) ([179479-04-0], 1.4 g, 1.56 mmol) in anhydrous MeCN (2 mL) was added. The reaction mixture was stirred at room temperature for 2 hours, tBuOOH (5.5 M in decane, 0.71 mL, 3.9 mmol) was added and stirring was continued for 1 hour. The reaction mixture was filtered and concentrated under reduced pressure to give a colorless oil which was purified by preparative reversed phase HPLC (Stationary phase: Waters XBridge, 5 µm, 150 x 25 mm; Mobile phase: H_2O (A) - MeCN (B); gradient elution: 18% – 48% B in A, flow rate: 25 mL/min) to afford intermediate **6c** (250 mg, yield: 31%). ESI-MS: m/z 515.4 $[(M+H)/2]^+$.

Step 5: preparation of intermediate **6d**

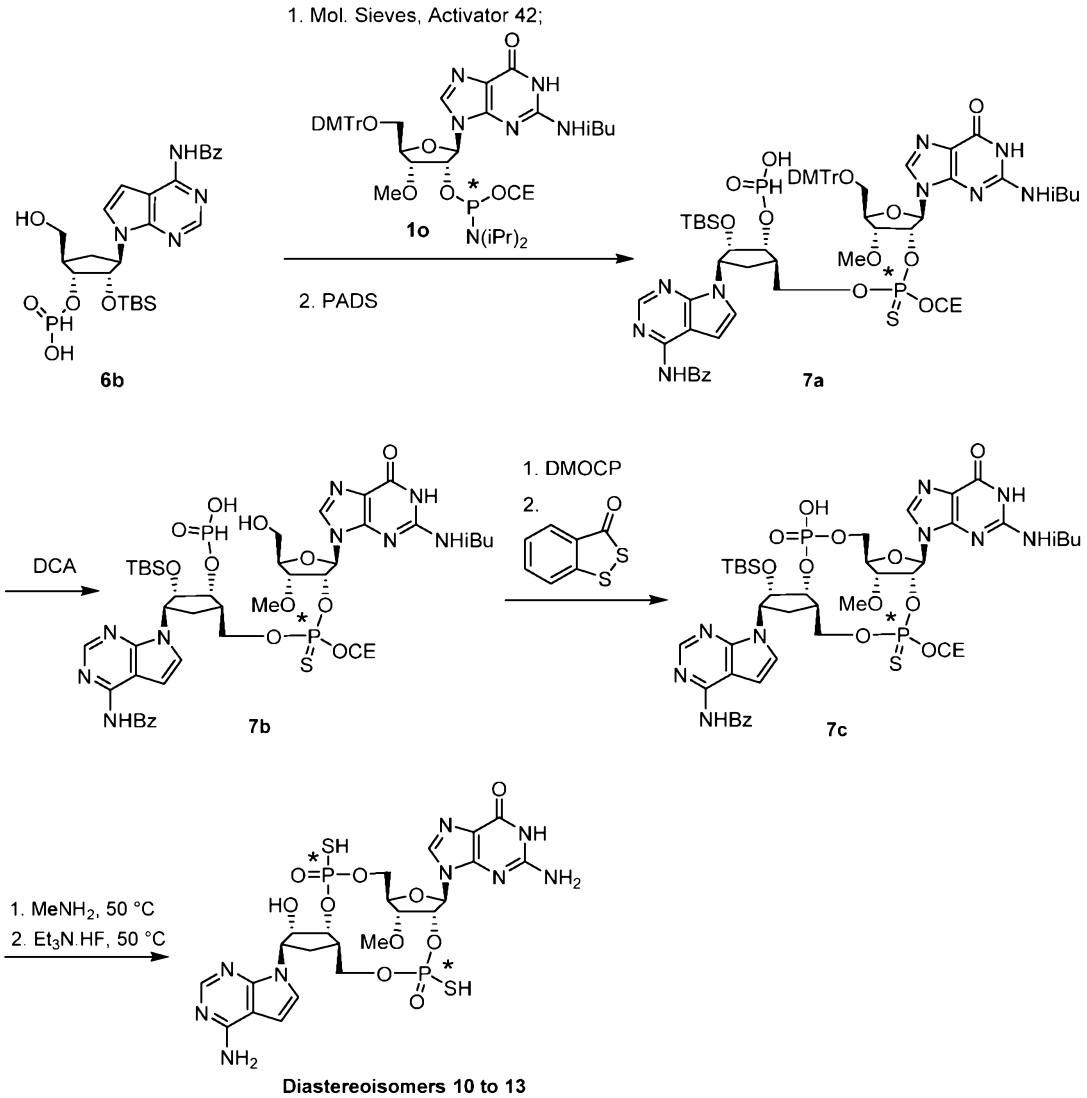
DMOCP (52 mg, 0.29 mmol) was added to a solution of intermediate **6c** (100 mg, 0.095 mmol) in pyridine (60 mL), the reaction mixture was stirred for 3 hours at room temperature. Water (17 mg, 0.95 mmol) and I2 (120 mg, 0.48 mmol) were added, and stirring was continued for 1 hour. The reaction was quenched with aqueous Na2SO3 (8 mL), filtered and concentrated under reduced pressure. The residue was purified by preparative reversed phase HPLC (Stationary phase: Waters XBridge, 5 □m, 150 x 25 mm; Mobile phase: 10 mM aqueous ammonia bicarbonate (A) - MeCN (B); gradient elution 17 – 47% B in A; flow rate: 25 mL/min) to give intermediate 1f (250 mg, 0.238 mmol) as a white solid. The previous procedure was repeated using a similar scale to generate a total amount of 100 mg (yield: 46%) of intermediate **6d**. ESI-MS: m/z 1027.4 $[M+H]^+$.

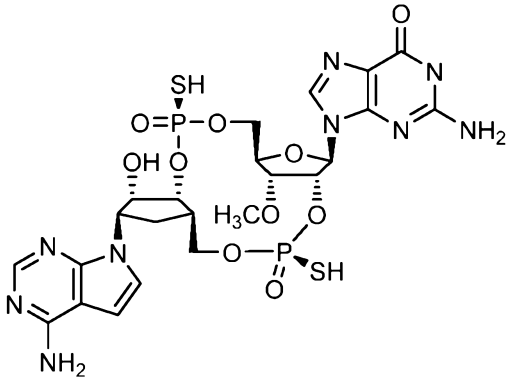
Step 6: preparation of **compound 9**

Intermediate **6d** (100 mg, 0.97 mmol) was stirred in a 33% methylamine solution in ethanol (40 mL) at room temperature for 6 hours, after which the reaction mixture was concentrated under reduced pressure. This procedure was repeated on 68 mg scale. The total amount of crude product obtained was dissolved in pyridine (8 mL), followed by the addition of triethylamine (1.1 g, 10.5 mmol) and triethylamine trihydrofluoride (847 mg, 5.25 mmol). The reaction mixture was stirred at 50 °C for 12 hours, after which it was cooled to room temperature; isopropoxytrimethylsilane (2.8 g, 21.0 mmol) was added and stirring was continued for another 12 hours. The residue obtained after concentration under reduced pressure was purified by preparative reversed phase HPLC (Stationary phase: Agela Durashell C18, 5 µm, 150 x 25 mm; Mobile phase: 0.05% aqueous ammonia hydroxide (A) - MeCN (B); gradient elution: 0 – 15% B in A, flow rate: 35 mL/min) to give **compound 9** as the ammonium salt (60 mg, 50%). Conversion into **compound 9 sodium salt** was done by elution of an aqueous solution over a column packed with Dowex® 50WX8 Na+ form resin. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 1.28 - 1.41 (m, 1 H), 2.22 - 2.39 (m, 2 H), 3.49 (s, 3 H), 3.55 - 3.65 (m, 1 H), 3.75 (br d, J=11.4 Hz, 1 H),

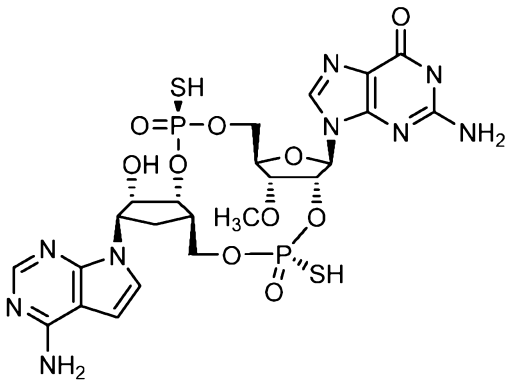
3.90 - 4.00 (m, 1 H), 4.03 (d, J=4.1 Hz, 1 H), 4.14 (br s, 1 H), 4.36 (dd, J=10.0, 3.9 Hz, 1 H), 4.76 - 4.84 (m, 1 H), 4.90 (q, J=9.5 Hz, 1 H), 5.35 (td, J=9.2, 4.1 Hz, 1 H), 5.81 (d, J=8.5 Hz, 1 H), 6.57 (br s, 2 H), 6.65 (d, J=3.3 Hz, 1 H), 7.37 (d, J=3.3 Hz, 1 H), 7.65 (br s, 2 H), 7.98 (s, 1 H), 8.00 (s, 1 H), 10.73 (s, 1 H); 31P NMR (162 MHz, DMSO-d6) δ ppm 0.83 (s, 1 P), 1.63 (s, 1 P); ESI-MS: m/z 685.9 $[M+H]^+$.

Example 7

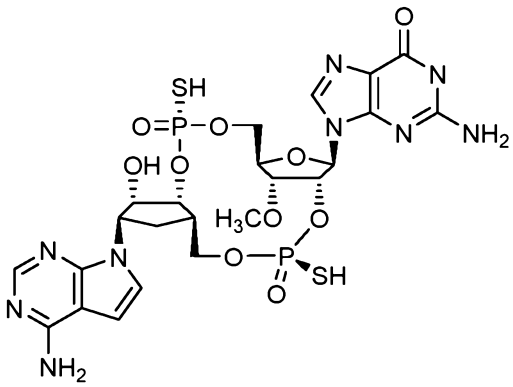




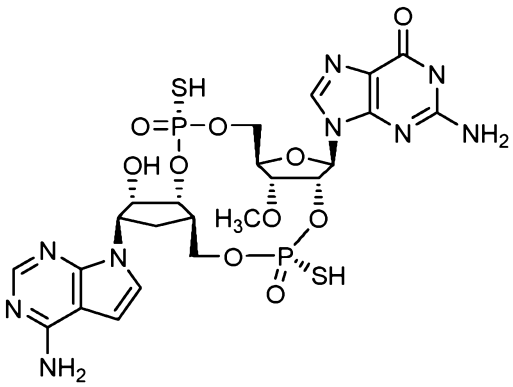
Compound 10



Compound 11



Compound 12



Compound 13

Step 1: preparation of intermediate **7a**

To the tert-butyl ammonium salt of intermediate **6b** (700 mg, 1.13 mmol) and activated molecular sieves was added a MeCN solution of Activator 42 ([175205-09-1], 9.9 mL of a 0.25 M solution, 2.49 mmol). The resulting mixture was shaken for 75 min under nitrogen, after which a solution of 5'-O-(4,4-dimethoxytrityl)-N2-isobutyryl-3'-O-methylguanosine-2'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) **1o** (1.28 g in anhydrous MeCN (9 mL), 1.4 mmol, solution dried on molecular sieves before use) was added. The reaction mixture was shaken for 1 hour followed by the addition of an extra portion of **1o** (248 mg in MeCN, 0.282 mmol, solution dried on molecular sieves before

use), after an extra hour of shaking a third portion of 5'-O-(4,4-dimethoxytrityl)-N2-isobutyryl-3'-O-methylguanosine-2'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (98 mg in MeCN, 0.113 mmol, solution dried on molecular sieves before use) was added. Shaking was continued for one hour after which the reaction mixture was concentrated under reduced pressure. Pyridine (10 mL) was added, followed by the addition of phenylacetyl disulfide (PADS, 854 mg, 2.82 mmol), the mixture was shaken for 1 hour. The molecular sieves were removed by filtration and washed with DCM, the filtrate was concentrated and the resulting residue co-evaporated with MeCN. The crude product **7a** was used as such in the next step. ESI-MS: m/z 672.8 $[M-H]^-$.

Step 2: preparation of intermediate **7b**

Water (204 µL, 11.3 mmol), triethylsilane (1.8 mL, 11.3 mmol) and dichloroacetic acid (370 µL, 4.5 mmol) were added to a solution of crude intermediate **7a** in DCM (10 mL). The reaction mixture was stirred for 1 hour, followed by pyridine (457 µL, 5.6 mmol) quench and concentration under reduced pressure. The crude product was purified by silica column chromatography (gradient elution: 5 – 20% MeOH in DCM) to give intermediate **7b** (700 mg, purity 75%, yield: 44%). ESI-MS: m/z 1045.3 $[M+H]^+$.

Step 3: preparation of intermediate **7c**

DMOCP (1.1 g, 6.0 mmol) was added to a solution of intermediate **7b** (625 mg, 0.60 mmol) in pyridine (22 mL), the reaction mixture was stirred at room temperature for 90 minutes. Next, water (78 mg, (59.8 mmol) and 3H-1,2-benzodithiol-3-one (503 mg, 3.0 mmol) were added and stirring was continued for 40 minutes. The reaction was quenched by the addition of brine and extracted with EtOAc. The combined organic phases were washed with aqueous NaHCO3, dried with MgSO4, filtered and concentrated under reduced pressure to give crude intermediate **7c** which was used as such in the next step. ESI-MS: m/z 1059.3 $[M+H]^+$.

Step 4: preparation of **diastereoisomer compounds 10 to 13**

Crude intermediate **7c** was stirred in a 33% methylamine solution in ethanol (30 mL) at 50 °C for 4 hours. The residue obtained after concentration under reduced pressure was dissolved in a mixture of triethylamine (4.2 mL) and pyridine (4.8 mL), to which triethylamine trihydrofluoride (2.5 mL, 14.9 mmol) was added. The resulting reaction mixture was stirred at 50 °C for 3 hours and thereafter cooled to room temperature, followed by the addition of isopropoxytrimethylsilane (3.2 mL, 17.9 mmol), stirring was continued for one hour. Next, water was added and the resulting aqueous phase was washed with EtOAc and lyophilized. Methanol was added to the oily lyophilizate resulting in the precipitation of crude **diastereoisomers 10 to 13**. Purification by preparative reversed phase HPLC (Stationary phase: XBridge C18 OBD, 5 µm, 250 x 30 mm; Mobile phase: aqueous 0.25% ammonia bicarbonate (A) - MeCN (B); gradient elution: 0 – 15% B in A over 45 min, flow rate: 30 mL/min) gave all four diastereoisomers: compound **10** (20 mg, yield: 4.5% from intermediate **7b**), compound **11** (18 mg, yield: 4% from intermediate **7b**), compound **12** (44 mg, yield: 10% from intermediate **7b**) and compound **13** (60 mg, yield: 13.5% from intermediate **7b**). All were converted into the corresponding sodium salt by elution of an aqueous solution over a column packed with Amberlite IR Na+ form resin.

Compound **10**. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 1.31 - 1.43 (m, 1 H), 2.26 (dt, J=13.2, 9.1 Hz, 1 H), 2.36 - 2.48 (m, 1 H), 3.50 (s, 3 H), 3.57 (m, J=10.6 Hz, 1 H), 3.76 - 3.91 (m, 2 H), 4.01 - 4.14 (m, 2 H), 4.18 (d, J=4.1 Hz, 1 H), 4.37 (ddd, J=8.8, 4.1, 2.4 Hz, 1 H), 4.90 (q, J=9.0 Hz, 1 H), 5.00 (dt, J=10.2, 3.7 Hz, 1 H), 5.33 (ddd, J=12.2, 8.8, 3.9 Hz, 1 H), 5.60 (d, J=2.9 Hz, 1 H), 5.81 (d, J=9.0 Hz, 1 H), 6.37 (br s, 2 H), 6.55 (d, J=3.3 Hz, 1 H), 6.87 (br s, 2 H), 7.23 (d, J=3.7 Hz, 1 H), 8.04 (s, 1 H), 8.23 (s, 1 H), 10.64 (br s, 1 H); 31P NMR (162 MHz, DMSO-d6) δ ppm 53.49 (s, 1 P), 55.64 (s, 1 P); ESI-MS: m/z 717.1 $[M+H]^+$.

Compound **11**: ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 1.31 - 1.42 (m, 1 H), 2.22 (dt, J=13.3, 8.7 Hz, 1 H), 2.52 - 2.60 (m, 1 H), 3.53 (s, 3 H), 3.71 - 3.80 (m, 2 H), 3.83 - 3.90 (m, 2 H), 4.12 (br q, J=2.0 Hz, 1 H), 4.31 (d, J=3.7 Hz, 1 H), 4.41 (dd, J=8.0, 4.7 Hz, 1 H), 4.82 (dt, J=9.0, 4.1 Hz, 1 H), 4.92 (q, J=9.0 Hz, 1 H), 5.32 (ddd, J=11.0, 9.4, 4.1 Hz, 1 H),

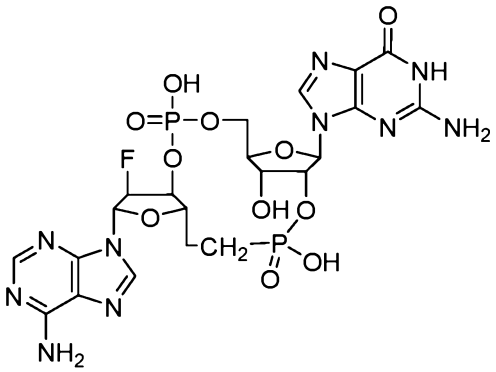
5.45 (br s, 1 H), 5.81 (d, J=9.0 Hz, 1 H), 6.45 (br s, 2 H), 6.59 (d, J=3.7 Hz, 1 H), 7.05 (br s, 2 H), 7.29 (d, J=3.3 Hz, 1 H), 8.07 (s, 1 H), 8.23 (s, 1 H), 10.56 (s, 1 H); 31P NMR (162 MHz, DMSO-d6) δ ppm 54.03 (s, 1 P), 56.86 (s, 1 P); ESI-MS: m/z 717.1 $[M+H]^+$.

Compound **12**: ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 1.21 - 1.33 (m, 1 H), 2.20 - 2.39 (m, 2 H), 3.50 (s, 3 H), 3.46 - 3.53 (m, 1 H), 3.65 (br d, J=11.0 Hz, 1 H), 3.92 (d, J=4.4 Hz, 1 H), 3.99 (br t, J=11.0 Hz, 1 H), 4.11 - 4.26 (m, 2 H), 4.34 (dd, J=10.5, 3.9 Hz, 1 H), 4.92 (q, J=9.9 Hz, 1 H), 5.23 (dd, J=8.8, 3.7 Hz, 1 H), 5.36 (ddd, J=12.7, 8.7, 4.3 Hz, 1 H), 5.81 (d, J=9.0 Hz, 1 H), 6.46 (br s, 2 H), 6.65 (d, J=3.5 Hz, 1 H), 7.34 (d, J=3.5 Hz, 1 H), 7.31 (br s, 2 H), 8.08 (s, 1 H), 8.12 (s, 1 H), 10.56 (s, 1 H); 31P NMR (162 MHz, DMSO-d6) δ ppm 56.36 (s, 1 P), 59.35 (s, 1 P); ESI-MS: m/z 717.1 $[M+H]^+$.

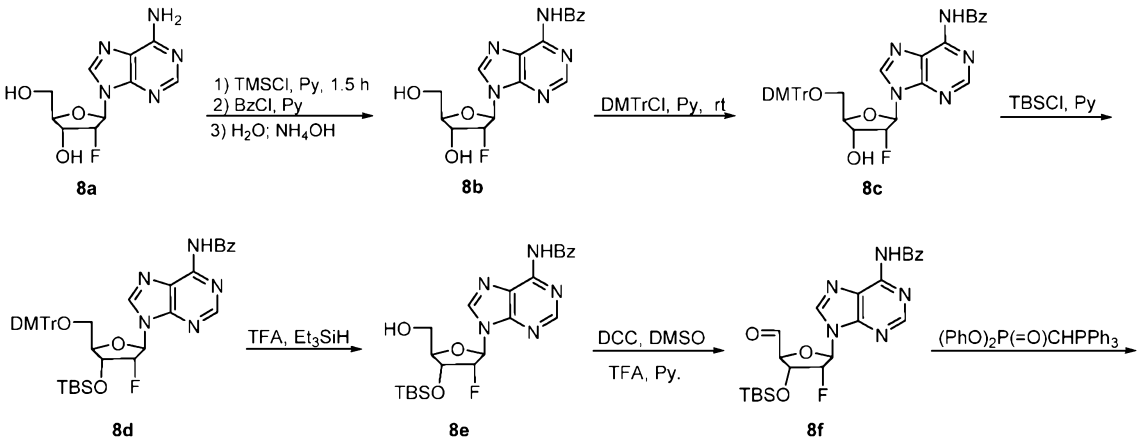
Compound **13**: ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 1.32 (br t, J=8.8 Hz, 1 H), 2.24 - 2.41 (m, 2 H), 3.52 (s, 3 H), 3.49 - 3.59 (m, 1 H), 3.68 (br d, J=11.4 Hz, 1 H), 3.85 (q, J=11.0 Hz, 1 H), 3.93 - 4.05 (m, 2 H), 4.95 (br s, 1 H), 4.36 (dd, J=9.8, 4.1 Hz, 1 H), 4.97 (dd, J=8.6, 4.1 Hz, 1 H), 4.93 (q, J=9.0 Hz, 1 H), 5.36 - 5.50 (m, 1 H), 5.80 (d, J=9.0 Hz, 1 H), 6.49 (br s, 2 H), 6.74 (d, J=3.7 Hz, 1 H), 7.50 (d, J=3.7 Hz, 1 H), 7.83 (br s, 2 H), 8.01 (s, 1 H), 8.20 (s, 1 H), 10.57 (s, 1 H); 31P NMR (162 MHz, DMSO-d6) δ ppm 54.46 (s, 1 P), 58.63 (s, 1 P), 58.66 (s, 1 P); ESI-MS: m/z 717.1 $[M+H]^+$.

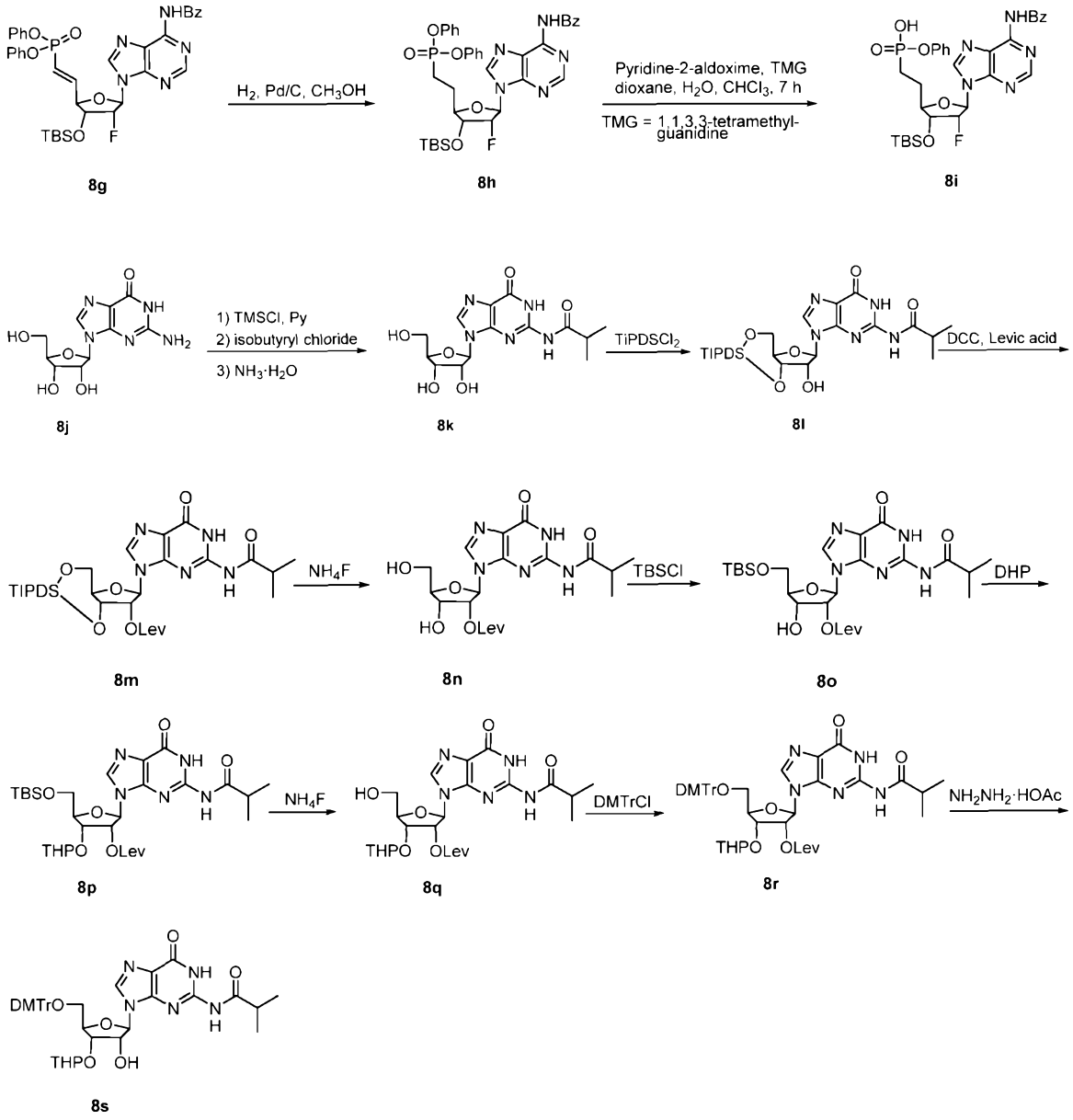
The reaction scheme illustrated in Example 8 describes one possible route for the preparation of compound **14** and pharmaceutically acceptable salt forms thereof, of the present invention.

Example 8



Cpd 14

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PhO
NHBz
TBSO
F
8g
H2, Pd/C, CH3OH
OPh
8h
Pyridine-2-aldoxime, TMG
dioxane, H2O, CHCl3, 7 h
TMG = 1,1,3,3-tetramethyl-guanidine
OH
8i
HO
NH2
8j
1) TMSCl, Py
2) isobutyryl chloride
3) NH3·H2O
8k
TiPDSCl2
TIPDS
8l
DCC, Levic acid
NH4F
OLev
8m
8n
TBSCl
TBSO
8o
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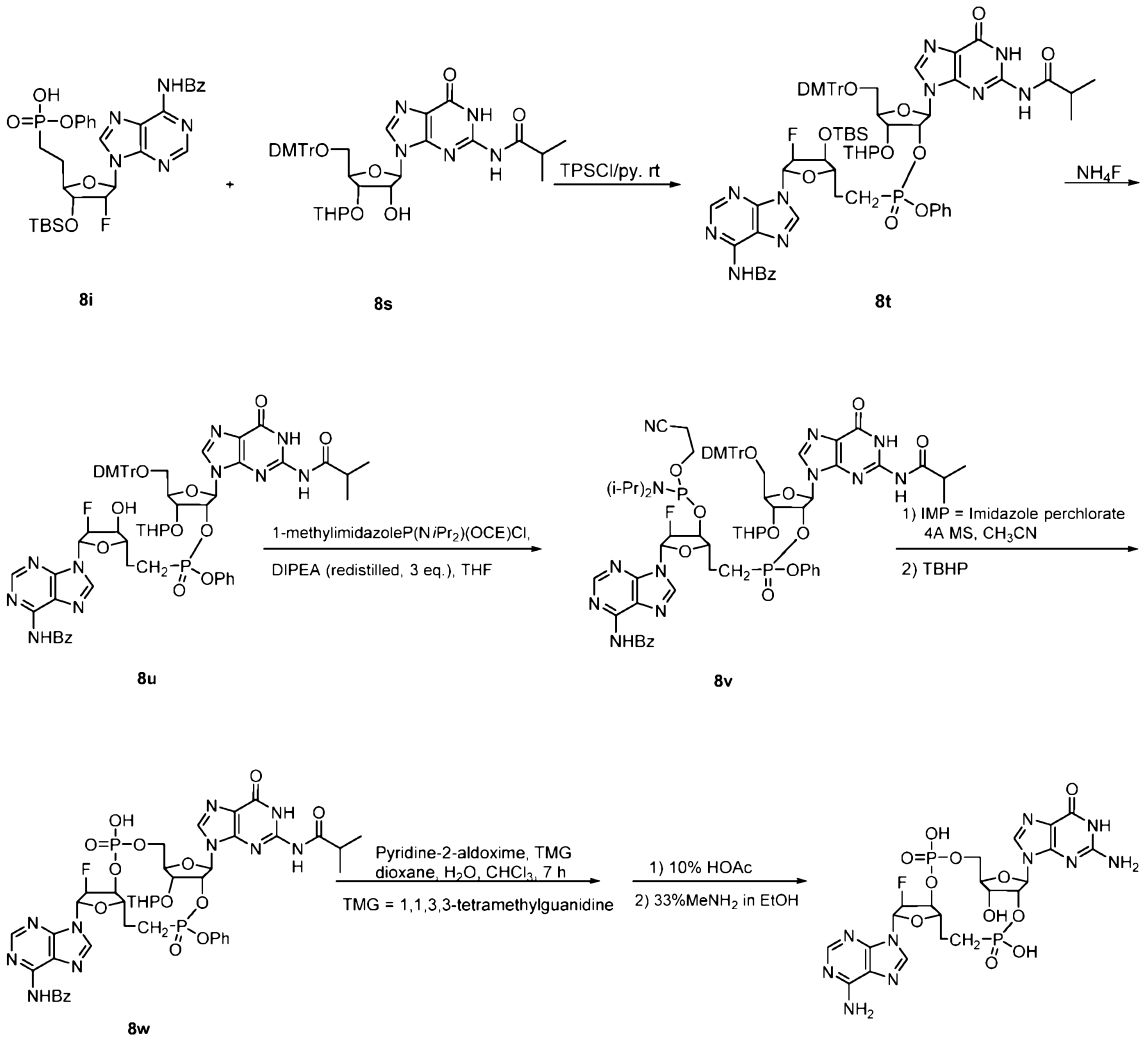
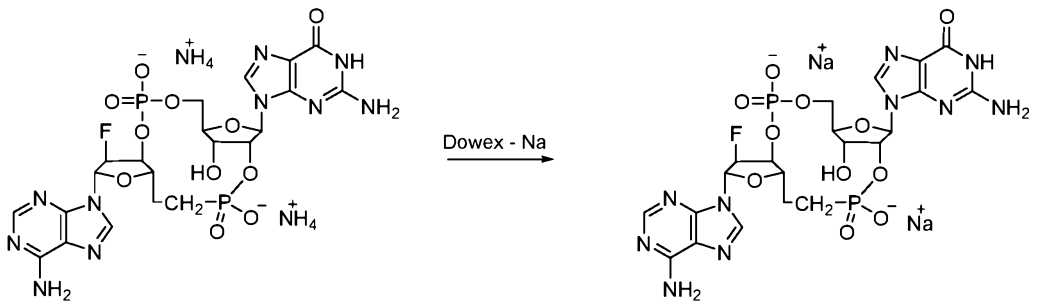


8i 8s 8t 8u 8v 8w

Cpd 14, ammonium salt

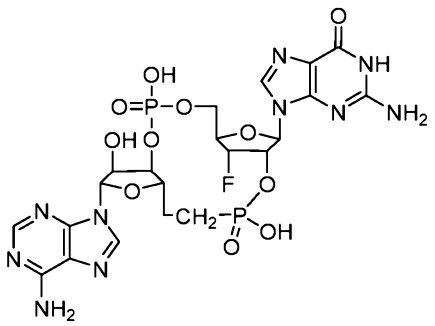


Compound 14, ammonium salt

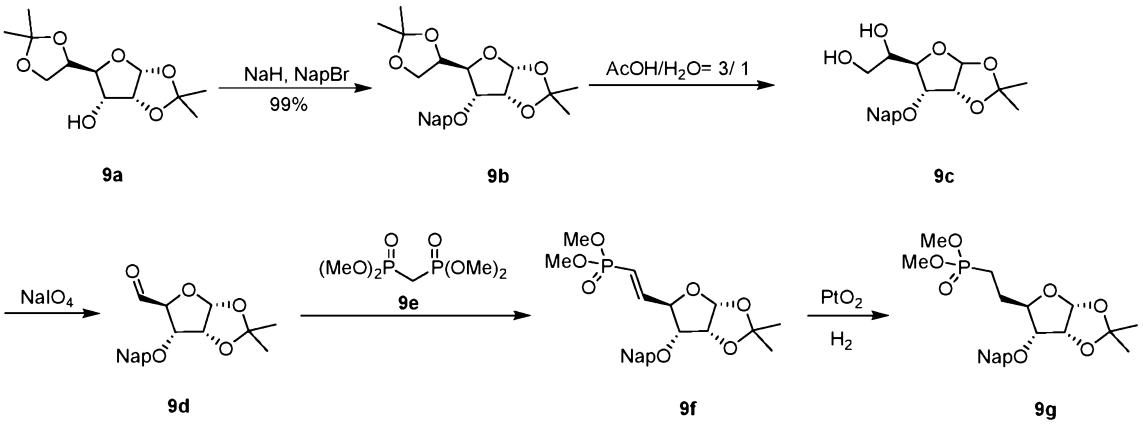
Compound 14, sodium salt

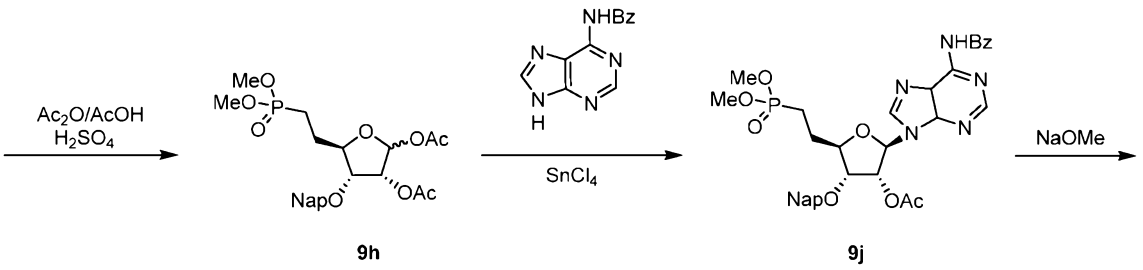
The reaction scheme illustrated in Example 9 describes one possible route for the preparation of compound **15** and pharmaceutically acceptable salt forms thereof, of the present invention.

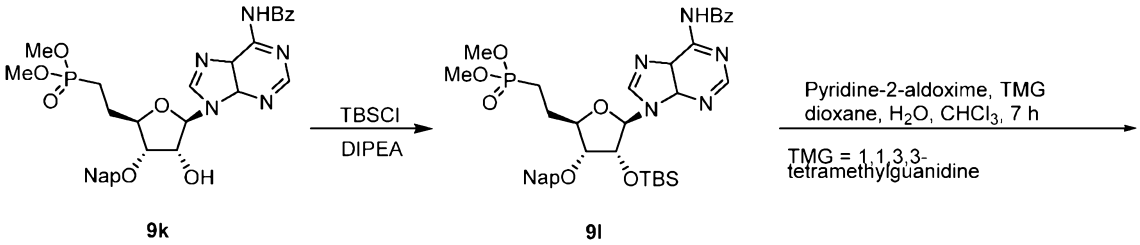
Example 9



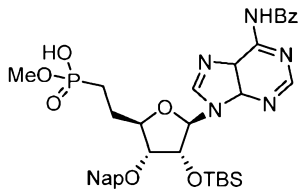
Cpd 15



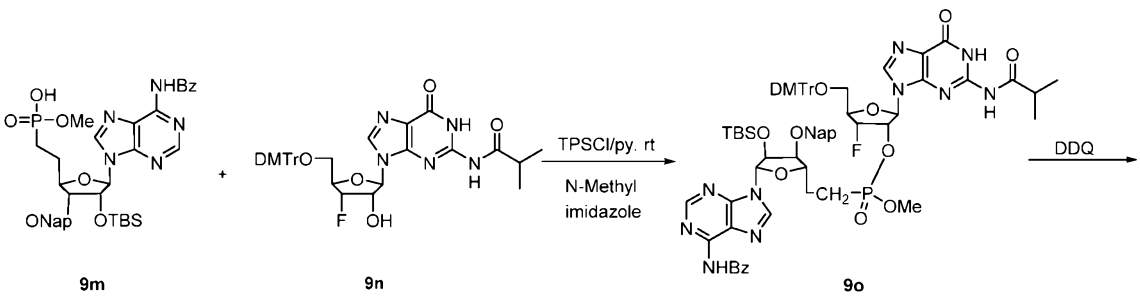




9k 9l

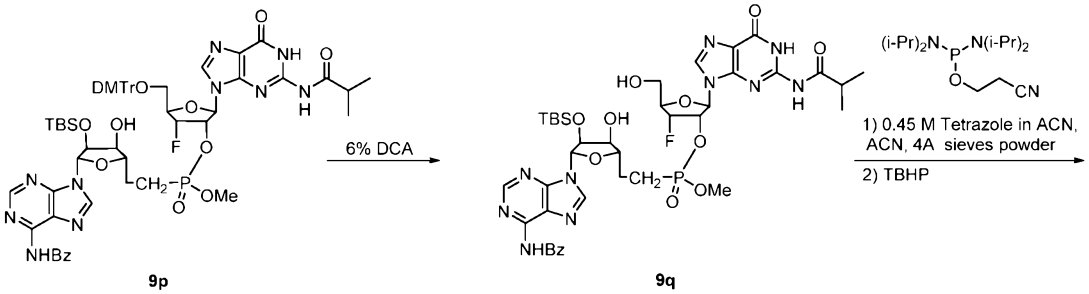


9m

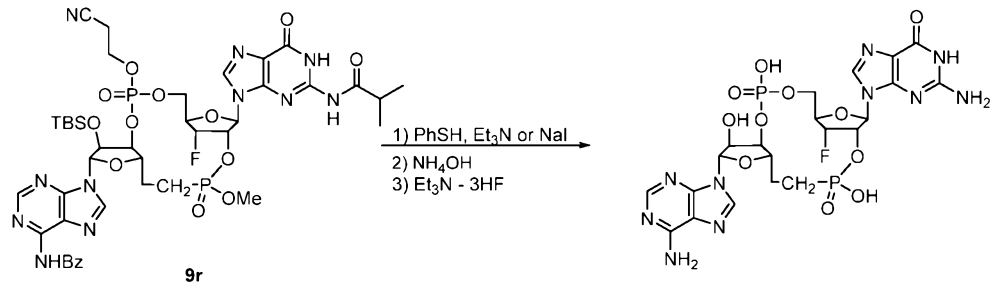


9m 9n 9o

5

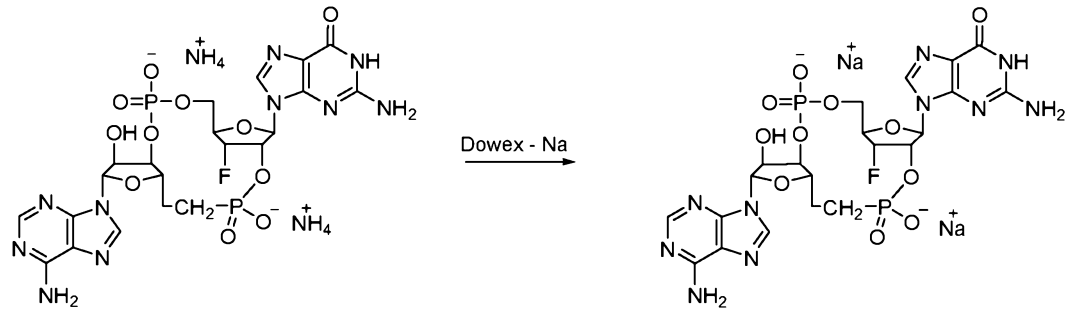


9p 9q



9r

Compound 15, ammonium salt

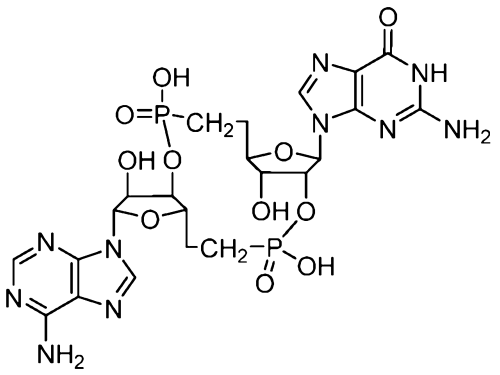


Compound 15, ammonium salt

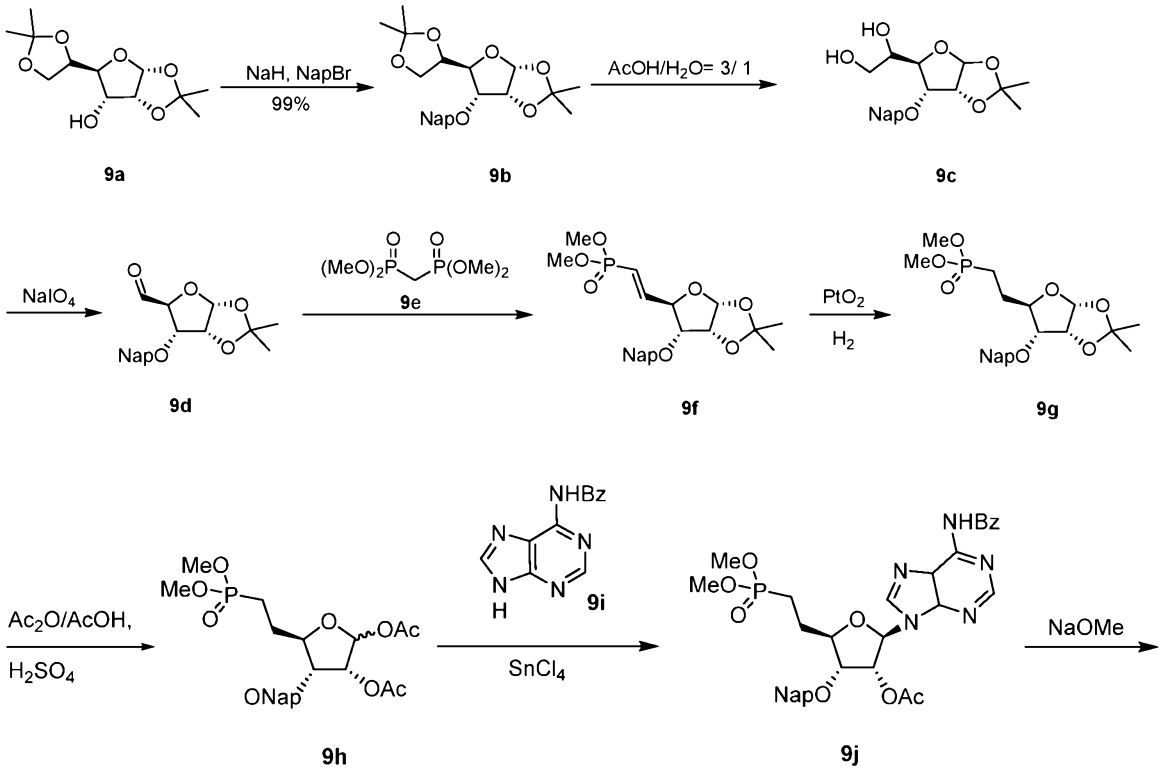
Compound 15 sodium salt

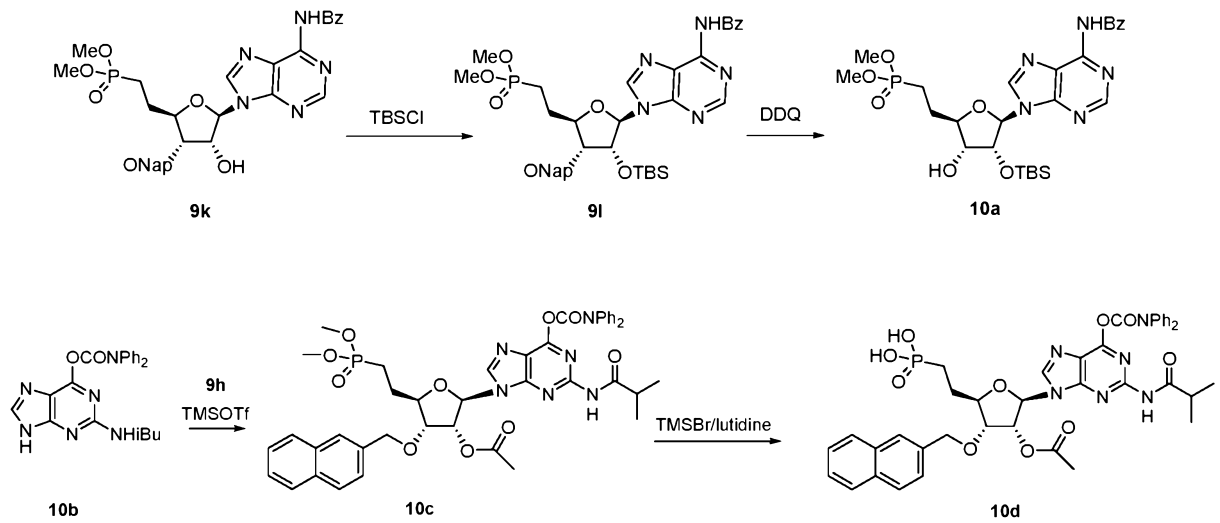
The reaction scheme illustrated in Example 10 describes one possible route for the preparation of compound **16** and pharmaceutically acceptable salt forms thereof, of the present invention.

Example 10



Cpd 16

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MeO
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O
ONap
OH
TBSCl
ONap
OTBS
DDQ
HO
OTBS
9k
9l
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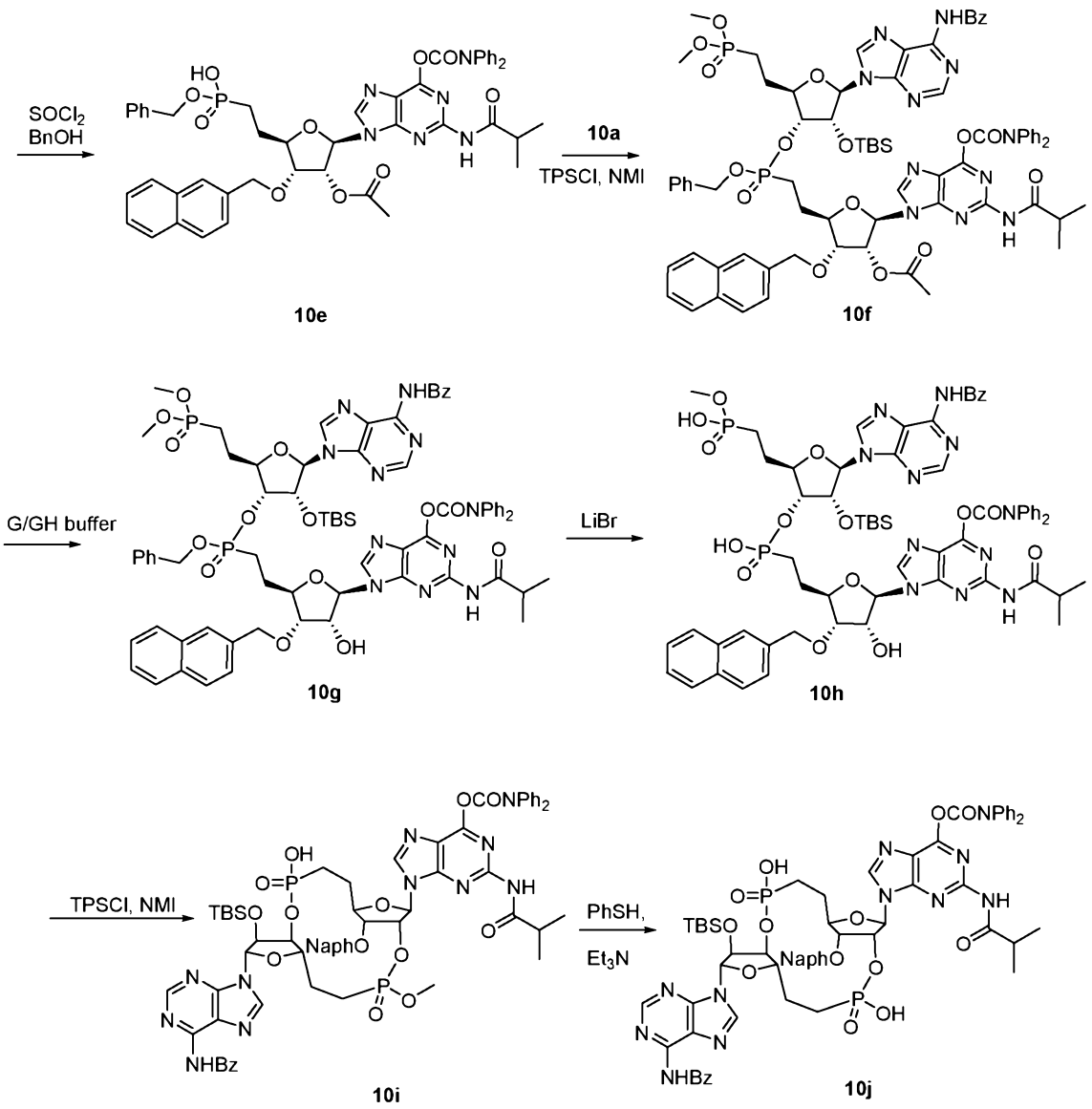
9h
TMSOTf
TMSBr/lutidine
HO
10b
10c
10d

5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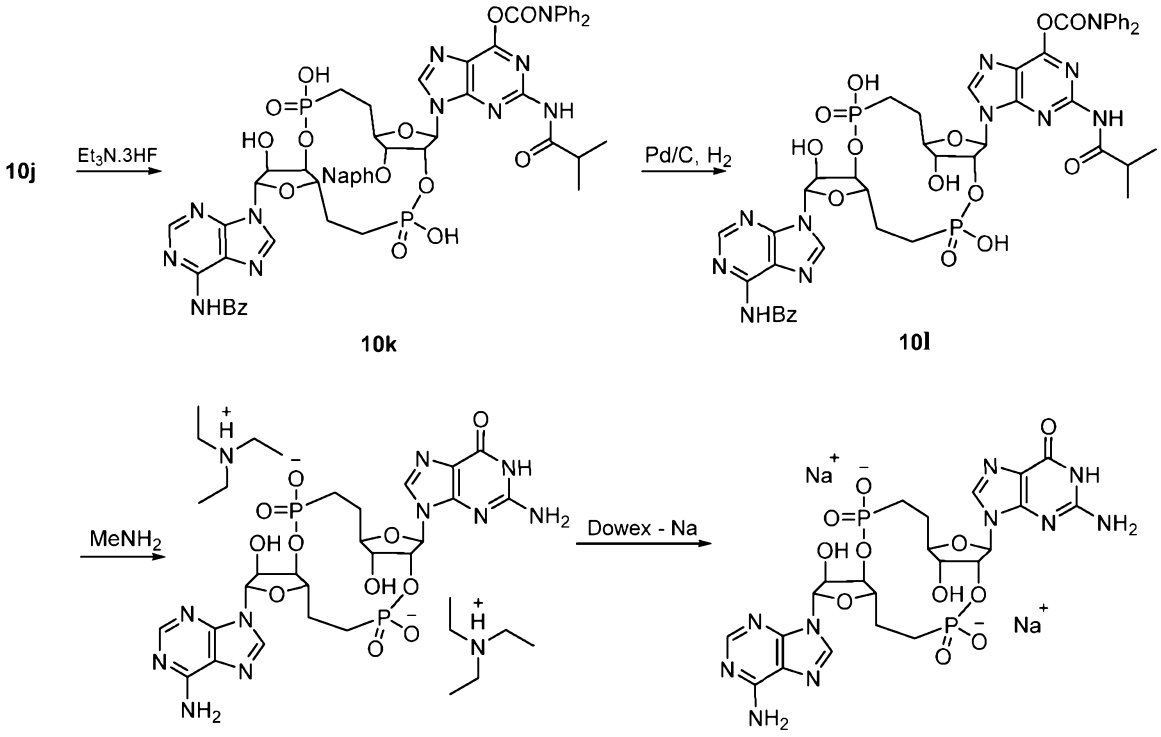
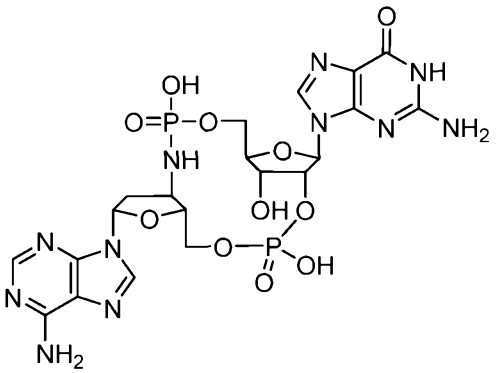
TPSCl, NMI
10e
10f
G/GH buffer
LiBr
10g
10h
TPSCl, NMI
PhSH,
Et3N
10i
10j



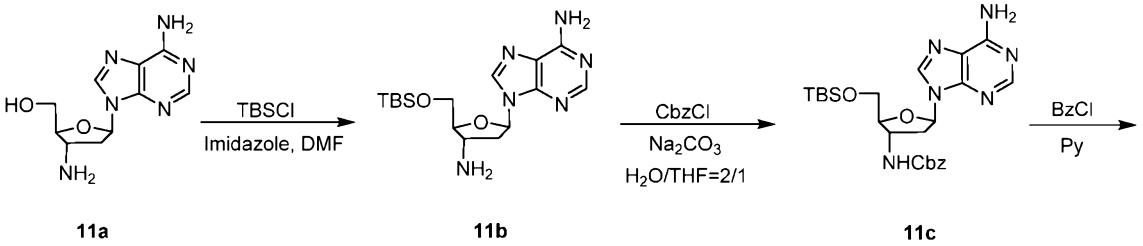
Compound 16, triethylammonium salt

Compound 16, sodium salt

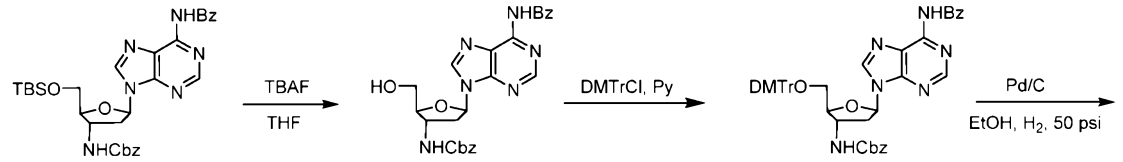
Example 11



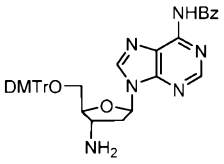
Cpd 17



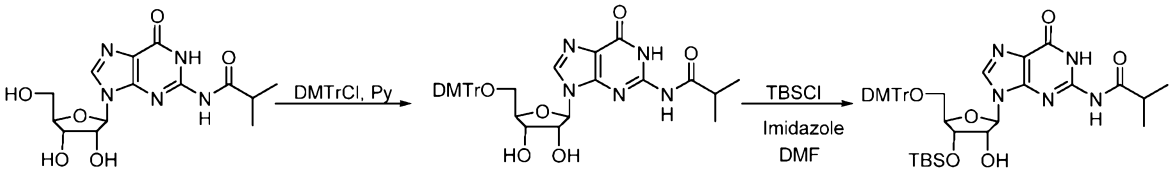
11a 11b 11c



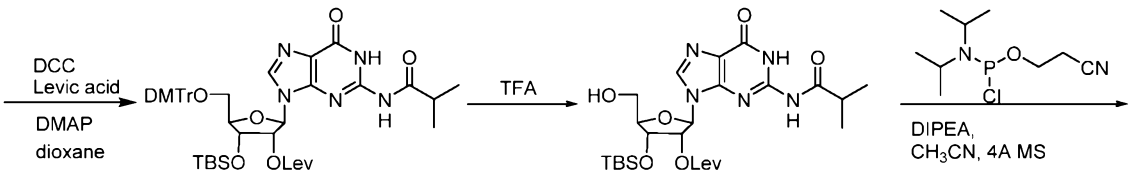
11d 11e 11f



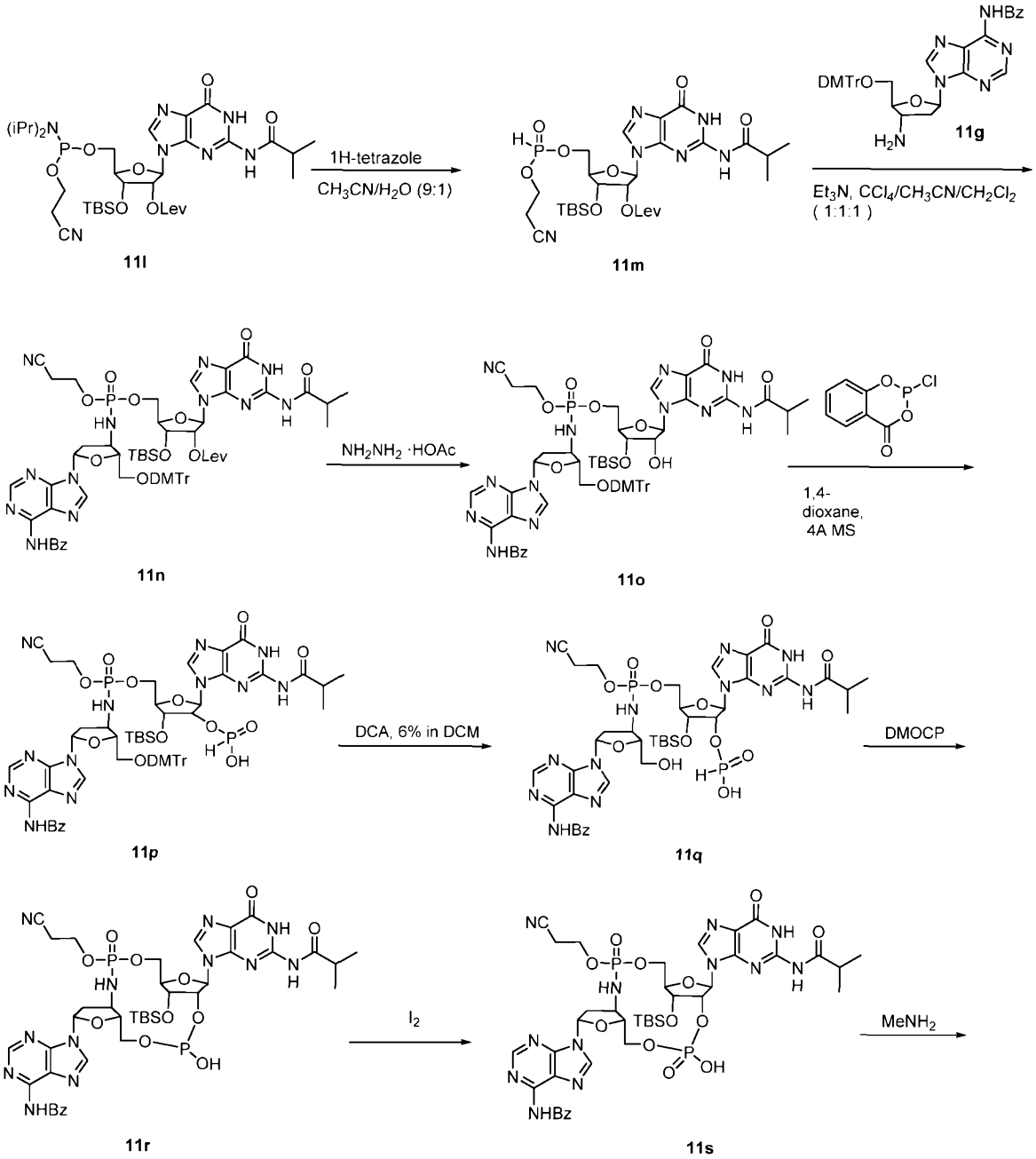
11g



8k 11h 11i



11j 11k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OLev
CN
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CH3CN/H2O (9:1)
DMTrO
NHBz
H2N
11g
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(1:1:1)
11l
11m
ODMTr
NH2NH2 ·HOAc
OH
1,4-
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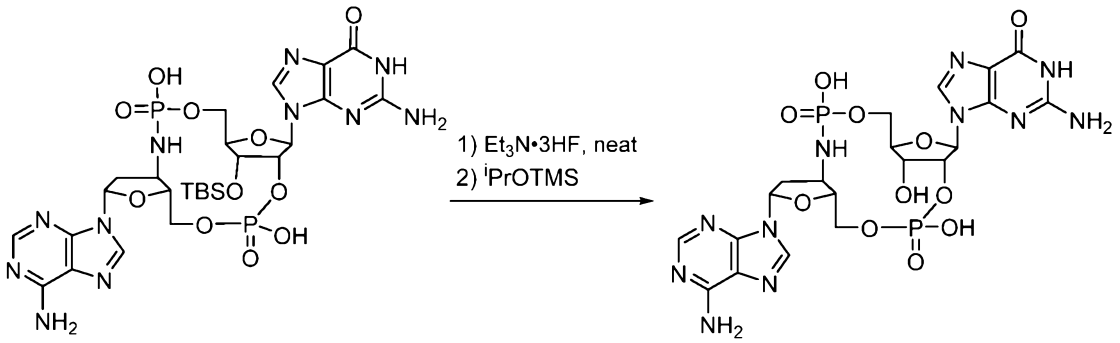
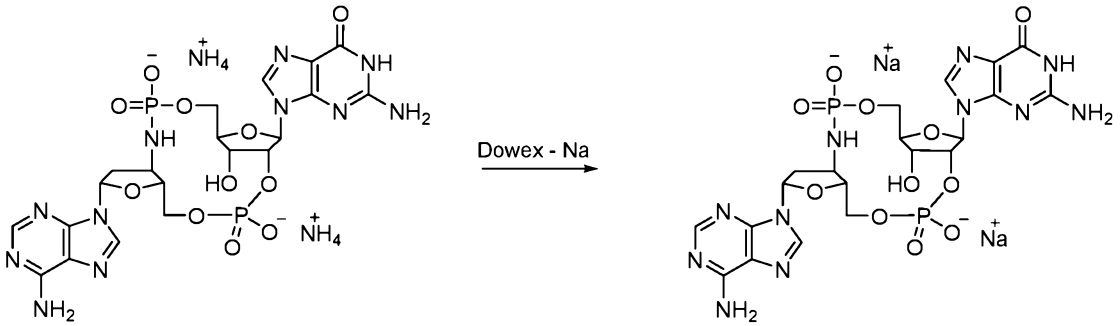
DMOCP
11p
11q
I2
MeNH2
11r
11s



11t

Compound 17, ammonium salt



Compound 17, ammonium salt

Compound 17, sodium salt

Step 1: preparation of **compound 11b**

To a solution of compound **11a** (4.1 g, 16.383 mmol, 1.0 eq) in pyridine (50 mL) was added 4-dimethylaminopyridine (0.4 g, 3.277 mmol, 0.2 eq) and tert-butyldimethylsilyl chloride (3.704 g, 24.575 mmol, 1.5 eq) at 25 °C. Then the reaction mixture was stirred at 25 °C for 12 h. The solvent was concentrated under reduced pressure to give a residue that was purified by column chromatography on silica gel (DCM/MeOH=50/1 to 10/1) to afford compound **11b** (2.86 g, 48 % yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) 8.32 (s, 1H), 8.21 (s, 1H), 6.47 (dd, *J* =5.2, 7.2 Hz, 1H), 4.31 - 4.18 (m, 2H), 4.06 - 3.96 (m, 1H), 3.94 - 3.84 (m, 1H), 3.18 - 3.06 (m, 1H), 2.80 - 2.70 (m, 1H), 0.85 (s, 9H), 0.03 (d, *J* =9.6 Hz, 6H); ESI-MS *m/z* 365.1 $[M+1]^+$.

Step 2: preparation of **compound 11c**

To a solution of compound **11b** (3.5 g, 9.602 mmol, 1.0 eq) in water/THF (v/v 2/1, 150 mL) was added sodium carbonate (1.348 g, 12.482 mmol 1.3 eq) and benzyloxycarbonyl chloride (2.129 g, 12.482 mmol, 1.3 eq). The mixture was stirred at 25 °C for 24 h. The reaction mixture was quenched by the addition of NH_4Cl (aq.), then diluted with EtOAc (50 mL). The organic portion was washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography (Petroleum ether/EtOAc = 10/1 to EtOAc) to afford compound **11c** (2.72 g, 57 % yield) as a white solid. ^1H NMR (400MHz, $CDCl_3$) 8.34 (s, 1H), 8.20 (s, 1H), 7.43 - 7.29 (m, 5H), 6.42 (t, J =5.8 Hz, 1H), 5.62 (s, 2H), 5.19 - 4.99 (m, 3H), 4.53 (t, J =6.0 Hz, 1H), 4.04 (s, 1H), 3.99 - 3.77 (m, 2H), 2.86 - 2.73 (m, 1H), 2.60 - 2.44 (m, 1H), 0.91 (s, 9H), 0.09 (s, 6H); ESI-MS m/z 521.1 $[M+Na]^+$.

Step 3: preparation of **compound 11d**

To a solution of compound **11c** (2.7 g, 5.415 mmol, 1.0 eq) in pyridine (30 mL) was added benzoyl chloride (1.142 g, 8.122 mmol, 1.5 eq) at 25 °C. The reaction mixture was stirred at 25 °C for 12 h. The reaction mixture was quenched by addition of water (5 mL), then NH_4OH (5 mL) was added. The mixture was stirred at 25 °C for 1 h. The reaction mixture was diluted with EtOAc (100 mL), washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography (Petroleum ether/EtOAc = 10/1 to EtOAc) to afford compound **11d** (2.42 g, 74 % yield) as a white solid. ^1H NMR (400MHz, $CDCl_3$) 9.03 (s, 1H), 8.79 (s, 1H), 8.40 (s, 1H), 8.02 (d, J =7.6 Hz, 2H), 7.66 - 7.57 (m, 1H), 7.56 - 7.47 (m, 2H), 7.42 - 7.30 (m, 5H), 6.62 - 6.37 (m, 1H), 5.24 - 4.98 (m, 3H), 4.55 (t, J =6.0 Hz, 1H), 4.24 - 4.03 (m, 1H), 4.02 - 3.76 (m, 2H), 2.97 - 2.73 (m, 1H), 2.70 - 2.46 (m, 1H), 0.90 (s, 9H), 0.09 (s, 6H); ESI-MS m/z 603.2 $[M+1]^+$.

Step 4: preparation of **compound 11e**

To a solution of compound **11d** (2.4 g, 3.982 mmol, 1.0 eq) in THF (20 mL) was added tetra-butylammonium fluoride (1 M in THF, 19.91 mL, 19.909 mmol, 5.0 eq) at 25 °C. The reaction mixture was stirred at 25 °C for 2 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was dissolved in EtOAc (50 mL), washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography (acetone/EtOAc = 3/1 to 1/1) to afford compound **11e** (1.89 g, 97 % yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) 8.72 (s, 2H), 8.09 (d, J =7.6 Hz, 2H), 7.78 - 7.47 (m, 3H), 7.44 - 7.20 (m, 5H), 6.53 (t, J =5.6 Hz, 1H), 5.11 (s, 2H), 4.60 (s, 1H), 4.14 - 3.99 (m, 1H), 3.94 - 3.64 (m, 2H), 2.91 (m, 1H), 2.67 - 2.47 (m, 1H); LCMS: ESI-MS m/z 489.1 $[M+1]^+$.

Step 5: preparation of **compound 11f**

To a solution of compound **11e** (1.4 g, 2.866 mmol, 1.0 eq) in pyridine (20 mL) was added 4,4'-dimethoxytrityl chloride (1.457 g, 4.299 mmol, 1.5 eq) at 25 °C. The reaction mixture was stirred at 25 °C for 2 h. The reaction mixture was quenched by the addition of MeOH (5 mL), and the mixture was stirred for 30 min. The mixture was diluted with EA (50 mL), washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography (Petroleum ether/EtOAc = 10/1 to EtOAc) to afford compound **11f** (2.03 g, 89 % yield) as a white solid. ^{1}H NMR (400MHz, $CDCl_3$) 8.97 (s, 1H), 8.76 (s, 1H), 8.18 (s, 1H), 8.02 (d, J =7.2 Hz, 2H), 7.67 - 7.58 (m, 1H), 7.58 - 7.48 (m, 2H), 7.45 - 7.14 (m, 15H), 6.78 (m, 4H), 6.44 (s, 1H), 5.10 (s, 2H), 4.94 (s, 1H), 4.59 (s, 1H), 3.76 (s, 6H), 3.45 (s, 2H), 3.11 - 2.94 (m, 1H), 2.62 (s, 1H); ESI-MS m/z 791.2 $[M+1]^+$.

Step 6: preparation of **compound 11g**

To a solution of compound **11f** (2.03 g, 2.567 mmol, 1.0 eq) in MeOH (200 mL) was added 10% Pd/C (2.0 g) under an argon atmosphere. Then the reaction mixture was replaced with a hydrogen atmosphere (3x). The reaction mixture was stirred at 25 °C for 18 h (50 psi). The reaction mixture was filtered and the filter cake was washed with MeOH (100 mL x 3). The organic layer was concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (DCM/MeOH = 100/1 to 10/1) to afford compound **11g** (1.24 g, 74 % yield) as a white solid. ^{1}H NMR (400MHz, $CDCl_3$) 9.10 (s, 1H), 8.74 (s, 1H), 8.19 (s, 1H), 8.00 (d, *J* =7.2 Hz, 2H), 7.57 (m, 1H), 7.54 - 7.46 (m, 2H), 7.42 - 7.33 (m, 2H), 7.30 - 7.15 (m, 8H), 6.78 (d, *J* =8.4 Hz, 4H), 6.42 (dd, *J* =3.6, 6.8 Hz, 1H), 3.91 - 3.80 (m, 2H), 3.75 (s, 6H), 3.40 (m, 2H), 2.82 (m, 1H), 2.45 - 2.30 (m, 1H); LCMS: ESI-MS *m/z* 657.2 $[M+1]^+$.

Step 7: preparation of **compound 11h**

To a solution of compound **8k** (16.50 g, 46.698 mmol, 1.00 eq) in pyridine (200 mL) was added 4,4'-(chloro(phenyl)methylene)bis(methoxybenzene) (23.734 g, 70.048 mmol, 1.50 eq) at 25 °C. The reaction mixture was stirred at 25 °C overnight. The reaction mixture was quenched with methanol (10 mL) and then concentrated to afford a residue. The residue was purified by column chromatography on silica gel (DCM/MeOH = 50/1 to 10/1) to afford compound **11h** (23.9 g, 78 % yield) as a yellow solid. LCMS: ESI-MS: *m/z* 656.1 $[M + H]^+$.

Step 8: preparation of **compound 11i**

To a solution of compound **11h** (23.9 g, 36.450 mmol, 1.00 eq) in N,N-dimethylformamide (120 mL) was added tert-butylchlorodimethylsilane (8.241 g, 54.675 mmol, 1.50 eq) and 1*H*-imidazole (6.204 g, 91.124 mmol, 2.50 eq) at 25 °C. The reaction mixture was stirred at 25 °C overnight. The reaction mixture was quenched with methanol (5 mL) and concentrated to afford a residue. The residue was purified by Prep-HPLC

(water (0.225 % formic acid-CH_3CN) to afford compound **11i** (6.7 g, 24 % yield) as a yellow solid. LCMS: ESI-MS: *m/z* 770.3 $[M + H]^+$.

Step 9: preparation of **compound 11j**

To a solution of compound **11i** (5.000 g, 6.494 mmol, 1.00 eq) in 1,4-dioxane (50 mL) was added DMAP (79.334 mg, 0.649 mmol, 0.1 eq), N,N'-methanediylidenedicyclohexanamine (4.020 g, 19.482 mmol, 3 eq) and 4-oxopentanoic acid (1.508 g, 12.988 mmol, 2.0 eq) at 25 °C. The mixture was stirred at 25 °C for 3 h. The reaction mixture was filtered, and the filter cake was washed with EtOAc (30 mL). The combined organic layers were concentrated under reduced pressure to give a residue. The residue was dissolved in EtOAc (100 mL), washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by column chromatography on silica gel (DCM/MeOH = 100/1 to 10/1) to afford compound **11j** (5.1 g, 90 % yield) as a white foam. LCMS: ESI-MS: *m/z* 868.4 $[M + H]^+$.

Step 10: preparation of **compound 11k**

To a solution of compound **11j** (5.1 g, 5.875 mmol, 1.00 eq) in DCM (12 mL) was added 2,2,2-trifluoroacetic acid (300 uL) and triethylsilane (6 mL). The mixture was stirred at 25 °C for 10 min. The reaction mixture was quenched with saturated solution of $NaHCO_3$ (20 mL), and then diluted with DCM (30 mL x 3). The combined organic layers were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (DCM/MeOH=50/1 to 10/1) to afford compound **11k** (3.1 g, 93 % yield) as a yellow solid. ^1H NMR (400MHz, $CDCl_3$) 12.11 (s, 1H), 11.64 (s, 1H), 8.42 - 8.22 (m, 1H), 6.00 (d, *J* =6.8 Hz, 1H), 5.74 (s, 1H), 5.54 (dd, *J* =5.0, 6.7 Hz, 1H), 5.26 (t, *J* =5.2 Hz, 1H), 4.55 (dd, *J* =2.4, 4.9 Hz, 1H), 3.98 (br d, *J* =2.4 Hz, 1H), 3.67 (td, *J* =4.9, 11.9 Hz, 1H), 3.61 - 3.51 (m, 1H), 2.77 (spt, *J* =6.8 Hz, 1H), 2.70 - 2.60 (m, 2H), 2.02 (s, 3H), 1.11 (d, *J* =6.6 Hz, 6H), 0.89 (s, 9H), 0.10 (s, 3H), 0.06 (s, 3H).

Step 11: preparation of **compound 11l**

To a solution of compound **11k** (3.1 g, 5.480 mmol, 1.00 eq) in THF (40 mL) was added N-ethyl-N-isopropylpropan-2-amine (4.250 g, 32.880 mmol, 6.0 eq) and 3-((chloro(diisopropylamino)phosphino)oxy)propanenitrile (3.891 g, 16.440 mmol, 3.0 eq) at 0 °C. The mixture was stirred at 0 °C for 2 h. The reaction mixture was quenched by the addition of MeOH (3 mL), then diluted with EA (50 mL). The organic layer was washed with $NaHCO_3$ (40 mL) and brine (40 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (DCM/EtOAc=10/1 to 1/1) to afford compound **11l** (3.1 g, 74 % yield) as a colorless oil. LCMS: ESI-MS: *m/z* 683.1 $[M- N(iPr)_2+18]^+$.

Step 12: preparation of **compound 11m**

To a solution of compound **11l** (2.1 g, 2.742 mmol, 1.0 eq) in acetonitrile (16 mL) was added a solution of 1*H*-tetrazole (0.45 M) in acetonitrile/water (9/1, 18/2 mL) at 25 °C. The solution was stirred at 25 °C for 2 h. The reaction mixture was cooled to 0 °C, diluted with ethyl acetate (50 mL), washed successively with cold water (20 mL), cold 5% sodium bicarbonate (20 mL), cold water (20 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give compound **11m** (1.62 g, 87 % yield) as a colorless oil. ^{31}P NMR (162 MHz, $CDCl_3$) 11.43 (s, 1P), 7.10 (s, 1P); LCMS: ESI-MS *m/z* 683.1 $[M+1]^+$.

Step 13: preparation of **compound 11n**

A solution of compound **11g** (1.2 g, 1.827 mmol, 1.0 eq) in a mixture of acetonitrile/dichloromethane/tetrachloromethane (1/1/1, 30 mL) and triethylamine (900 uL) was added to a sealed flask containing compound **11m** (1.62 g, 2.375 mmol, 1.3 eq). The resulting solution was stirred at 25 °C for 2 h. The reaction mixture was concentrated to dryness under reduced pressure to give a residue. The residue was purified by flash column chromatography (DCM/MeOH=100/1 to 100/2) to afford compound **11n** (1.21 g,

50 % yield) as a white foam. ^{31}P NMR (162 MHz, CD_3OD) 9.13 (s, 1P), 8.73 (s, 1P); LCMS: ESI-MS *m/z* 1337.5 $[M+1]^+$.

Step 14: preparation of **compound 11o**

To a solution of compound **11n** (1.1 g, 0.822 mmol, 1.0 eq) in MeCN (30 mL) was added hydrazine acetate (757.462 mg, 8.225 mmol, 10.0 eq) at 25 °C. The reaction mixture was stirred at 25 °C for 2 h. Then the reaction mixture was diluted with EA (50 mL), washed with water (50 mL) and brine (30 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography (DCM/MeOH = 100/1 to 100/5) to afford compound **11o** (833 mg, 82 % yield) as a white solid. LCMS: ESI-MS *m/z* 1261.2 $[M+23]^+$.

Step 14: preparation of **compound 11p**

To a solution of compound **11o** (653 mg, 0.527 mmol, 1.0 eq) and 4Å molecular sieves in 1,4-dioxane (9.0 mL) and pyridine (3.0 mL) was added a solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (160.068 mg, 0.790 mmol, 1.5 eq) in 1,4-dioxane (5 mL). The reaction mixture was stirred at 25 °C for 30 min. The reaction mixture was quenched by the addition water/pyridine (1/1, 5 mL), and the resulting mixture was poured into $NaHCO_3$ solution (20 mL). The mixture was extracted with EtOAc (30 x 3 mL) and the layers were partitioned. The combined EtOAc extracts were concentrated to dryness under reduced pressure to give compound **11p** (723 mg, crude) as a colorless foam, which was used in the next step without further purification.

Step 15: preparation of **compound 11q**

To a solution of compound **11p** (723 mg, crude) in DCM (5 mL) was added water and DCA (6% in DCM, 5 mL). The reaction mixture was stirred at 25 °C for 30 min. The reaction mixture was quenched by addition of pyridine (3 mL) with stirring for 10 min. The reaction mixture was concentrated to dryness under reduced pressure to afford a residue. The resultant residue was purified by Prep-HPLC (column: Waters Xbridge

150*25 5u; mobile phase: water (10mM NH_4HCO_3)-ACN from 22% to 42%, flow rate: 25 mL/min, Gradient Time: 8 min) to afford compound **11q** (197 mg, 35 % yield for two steps) as a white solid. ^{31}P NMR (162 MHz, D_2O) 9.23 (s, 1P), 8.62 (s, 1P), 4.48 (s, 1P); LCMS: ESI-MS *m/z* 1001.5 $[M+1]^+$.

Step 16: preparation of **compound 11r**

To a solution of compound **11q** (197 mg, 0.197 mmol, 1.0 eq) and 4Å molecular sieves in pyridine (60 mL) was added 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide (127.131 mg, 0.689 mmol, 3.5 eq) at 25 °C. The reaction mixture was stirred at 25 °C for 1 h. The reaction mixture (0.00328 M in Py, 60 mL), containing compound **11r**, was used for next step without further purification.

Step 17: preparation of **compound 11s**

A solution of compound **11r** (0.00328 M in Py, 60 mL) was quenched by the addition of water (36.49 mg, 1.97 mmol, 10.0 eq), then I_2 (250 mg, 0.985 mmol, 5.0 eq) was added to the solution. The mixture was stirred at 25 °C for 1 h. This mixture was then quenched by the addition of an aqueous Na_2SO_3 solution (2 mL), filtered, and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by Prep-HPLC (column: Waters Xbridge 150*25 5u; mobile phase: water (10mM NH_4HCO_3)-ACN from 28% to 48%, flow rate: 25 mL/min, Gradient Time: 8 min) to afford compound **11a** (68 mg, 35% yield for two steps) as a white solid. LCMS: ESI-MS m/z 999.1 $[M+1]^+$.

Step 18: preparation of **compound 11t**

Compound **11s** (35 mg, 0.035 mmol, 1.0 eq) in a solution of $MeNH_2$ (33% in EtOH, 2 mL) was stirred at 25 °C for 12 h. The reaction mixture was concentrated under reduced pressure to afford compound **11t** (crude, 32 mg) as a yellow solid.

Step 18: preparation of **compound 17**

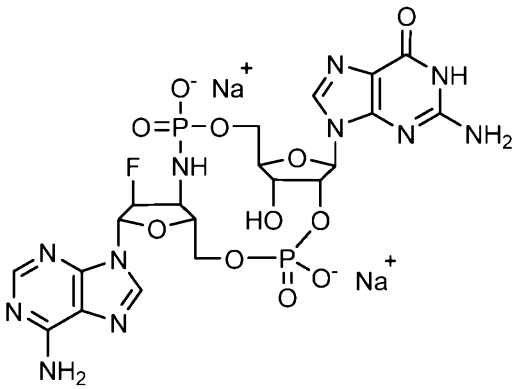
To a solution of compound **11t** (32 mg, crude) in pyridine (2 mL) was added triethylamine (209.805 mg, 2.073 mmol, 50 eq) and triethylamine trihydrofluoride (167.124 mg, 1.037 mmol, 25 eq) at 25 °C. The reaction mixture was stirred at 50 °C for 12 h. To the reaction mixture was added THF (1 mL) and isopropoxytrimethylsilane (548.516 mg, 4.147 mmol, 100 eq) at 25 °C and the mixture was stirred for 12 h. The mixture was concentrated under reduced pressure to afford a residue. The residue was purified by Prep-HPLC (column: Agela Durashell C18 150*25 5u; mobile phase: water (0.05% ammonia hydroxide v/v)-ACN from 0% to 15%, flow rate: 35 mL/min, Gradient Time:10 min) to afford **compound 17** as an ammonium salt (9.5 mg, 35 % yield for two steps) as a white solid. ^{1}H NMR (400 MHz, D_2O) 8.66 - 7.98 (m, 2H), 7.83 (s, 1H), 6.24 (s, 1H), 6.04 - 5.50 (m, 2H), 4.60 (s, 1H), 4.42 - 3.93 (m, 7H), 2.76 (s, 1H), 2.44 (s, 1H); ^{31}P NMR (162 MHz, D_2O) 7.62 (s, 1P), 7.49 (s, 1P), -3.40 (s, 1P).

Step 19: preparation of **compound 17 sodium salt**

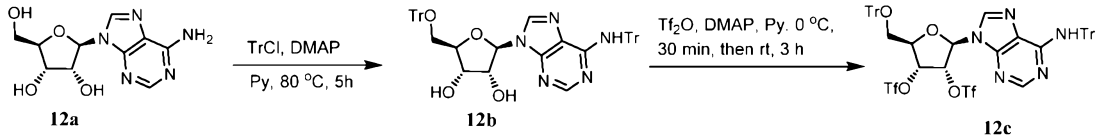
A 5 mL volume of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 9.5 mg of compound **17 ammonium salt**) and washed with deionized water (2x). Then to the resin was added 15% H_2SO_4 in DI water (50 mL), the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to a column with 15% H_2SO_4 in DI water and washed with 15% H_2SO_4 (at least 4 CV), and then with DI water until it was pH neutral. The resin was transferred back into the beaker, and an aqueous 15% NaOH solution (50 mL) was added, the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to the column and washed with aqueous 15% NaOH (at least 4 CV), and then with water until it was pH neutral (at least 4 CV). Compound **17 ammonium salt** was dissolved in DI water (9.5 mg in 2 mL), added to the top of the column, and eluted with DI water. A product eluted in early fractions as detected by TLC (UV). Product was lyophilized to afford **compound 17 sodium salt** (7.4 mg, 98 % purity, 71.819% yield) as a white solid. ^{1}H NMR (400 MHz, D_2O) 8.31 (s, 1H), 8.17 (s, 1H), 7.92 (s, 1H), 6.32 (d, J =6.4 Hz, 1H), 5.95 (d, J =8.0 Hz, 1H), 5.69 (m, 1H), 4.66 (d, J =4.0 Hz, 1H), 4.40 - 4.31

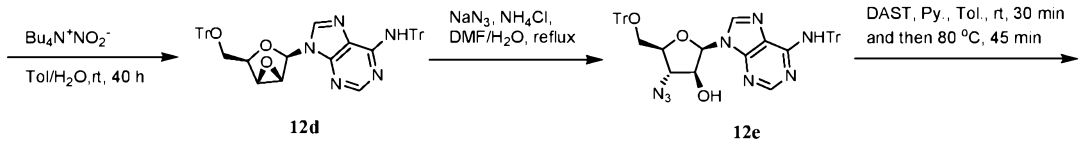
(m, 2H), 4.24 - 4.06 (m, 5H), 2.89-2.84 (m, 1H), 2.51-2.46 (m, 1H); ^{31}P NMR (162 MHz, D_2O) 7.56 (s, 1P), -1.86 (s, 1P); LCMS: ESI-MS m/z 657.8 $[M+1]^+$.

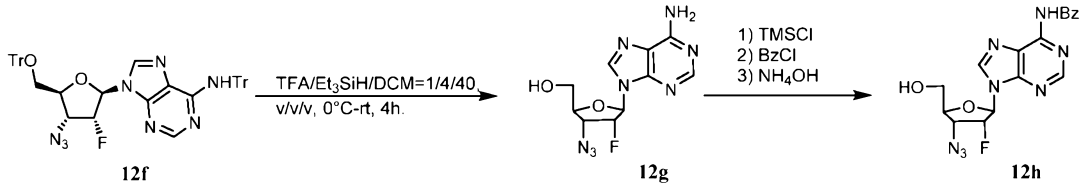
Example 12

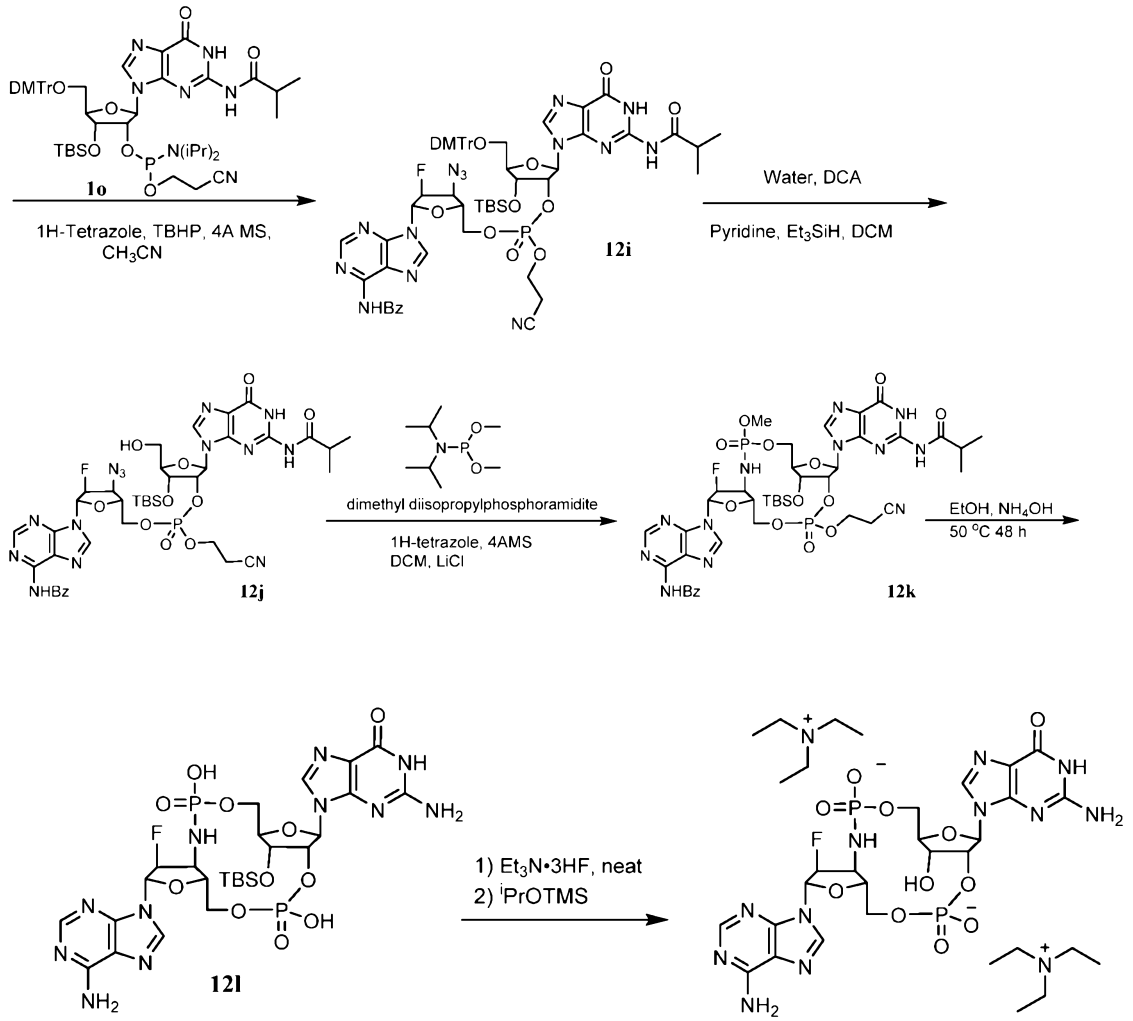


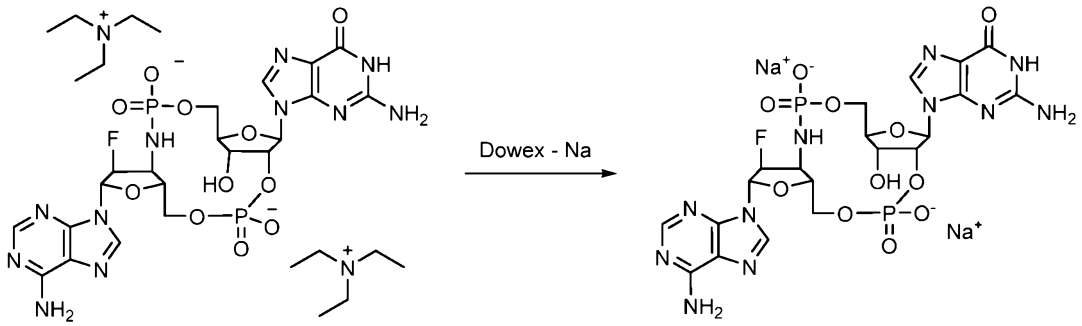
Cpd 18








1o
1H-Tetrazole, TBHP, 4A MS, CH3CN
12i
Water, DCA
Pyridine, Et3SiH, DCM
12j
dimethyl diisopropylphosphoramidite
1H-tetrazole, 4AMS
DCM, LiCl
12k
EtOH, NH4OH
50 °C 48 h
12l
1) Et3N•3HF, neat
2) iPrOTMS
Cpd 18 triethylammonium salt



Cpd 18 triethylammonium salt **Cpd 18 sodium salt**

Step 1: preparation of **compound 12b**

To a solution of compound **12a** (7.8 g, 29.187 mmol) and DMAP (2.995 g, 24.517 mmol) in pyridine (240 mL) was added TrCl (27.665 g, 99.236 mmol). After heating the reaction mixture at 80 °C for 5 h, the reaction was then cooled to ambient temperature and quenched with EtOH (150 mL). The reaction mixture was concentrated and purified by flash column chromatography on silica gel (DCM : EA = 0/1~1:1) to give compound **12b** as a white solid (10.45 g, 13.899 mmol). ESI-MS: *m/z* 774.1 $[M+Na]^+$.

Step 2: preparation of **compound 12c**

Compound **12b** (11.5 g, 15.295 mmol) and DMAP (4.671 g, 38.238 mmol) were dissolved in pyridine (300 mL). This solution was cooled to 0 °C and a solution of triflic anhydride (20.672 mL, 122.362 mmol) was added dropwise. The reaction mixture was held at 0 °C for 30 min and then allowed to warm to room temperature over a period of 3 h. The reaction mixture was diluted with CH_2Cl_2 (500 mL) and extracted with water (300 mL at 0 °C). The solvents were removed under reduced pressure and the residue purified by flash column chromatography on silica gel (Petroleum ether (PE)/EtOAc = 1:0~1:1) to give compound **12c** as a white amorphous solid (11 g, 10.827 mmol). ESI-MS: *m/z* 1037.8 $[M+Na]^+$; ^1H NMR (400MHz, $CDCl_3$) 7.89 (s, 1H), 7.84 (s, 1H), 7.39 - 7.29 (m, 12H), 7.28 - 7.17 (m, 18H), 6.98 (s, 1H), 6.45 (t, J = 5.2 Hz, 1H), 6.21 (d, J = 6.0 Hz, 1H), 5.86 -

5.81 (m, 1H), 4.48 (q, J = 3.6 Hz, 1H), 3.69 (dd, J = 4.3, 11.2 Hz, 1H), 3.36 (dd, J = 4.0, 11.2 Hz, 1H).

Step 3: preparation of **compound 12d**

Compound **12c** (9.5 g, 9.350 mmol) was dissolved in toluene (200 mL) containing tetrabutylammonium nitrite (21.579 g, 74.804 mmol) and water (26 mL). After vigorously stirring the reaction mixture for 40 h, the mixture was extracted with t-$BuOCH_3$ (200 mL) and water (2 x 100 mL). The organic layers were then combined, dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to afford the crude product. The residue was purified by flash column chromatography on silica gel (PE/EtOAc = 1:0~2:1) to give compound **12d** as a white amorphous solid (3.87 g, 5.274 mmol). ESI-MS: m/z 756.0 $[M+Na]^+$.

Step 4: preparation of **compound 12e**

A mixture of compound **12d** (3.87 g, 5.274 mmol), NH_4Cl (0.564 g, 10.547 mmol), NaN_3 (2.1 g, 32.303 mmol), DMF (16 mL) and water (2.4 mL) was heated at reflux (100 °C) for 1 h. The reaction mixture was then extracted with CH_2Cl_2 (100 mL) and water (100 mL). The organic layer was washed with water (3 x 100 mL), dried with anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (PE/EtOAc 1:0~3:1) to give compound **12e** as a white amorphous solid (2.86 g, 3.681 mmol), in addition to the other isomer (500 mg, 0.245 mmol). ESI-MS: m/z 799.1 $[M+Na]^+$; ^1H NMR (400MHz, $CDCl_3$) 8.00 (d, J = 9.3 Hz, 2H), 7.33 (dt, J = 1.5, 7.2 Hz, 12H), 7.26 - 7.17 (m, 18H), 7.05 (s, 1H), 6.05 (d, J = 5.2 Hz,1H), 5.53 (br d, J = 8.8 Hz, 1H), 4.58 - 4.49 (m, 1H), 4.43 (t, J = 6.0 Hz, 1H), 3.91 - 3.83 (m, 1H), 3.49 - 3.39 (m, 1H), 3.27 (dd, J = 4.3, 10.5 Hz, 1H).

Step 5: preparation of **compound 12f**

A solution of DAST (3.798 g, 23.561 mmol) was added dropwise to a solution of **12e** (2.86 g, 3.681 mmol) in toluene (50 mL) and pyridine (5.359 mL). After stirring at room

temperature for 30 min, the reaction mixture was heated at 80 °C for 1 h and then diluted with EtOAc (70 mL). The organic layer was successively washed with aqueous 7% $NaHCO_3$ (100 mL), water (100 mL), dried with anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (PE/EtOAc = 1:0~5:1) to afford compound **12f** as a yellowish amorphous solid (2.24 g, 2.876 mmol). ^1H NMR (400MHz, $CDCl_3$) 7.97 (s, 1H), 7.90 (s, 1H), 7.40 - 7.30 (m, 14H), 7.25 - 7.17 (m, 16H), 6.11 (d, J =19.6 Hz, 1H), 5.93 - 5.76 (m, 1H), 4.79 - 4.68 (m, 1H), 4.29 - 4.22 (m, 1H), 3.57 (dd, J = 3.0, 10.9 Hz, 1H), 3.33 (dd, J = 4.0, 11.0 Hz, 1H).

Step 6: preparation of **compound 12g**

To a solution of compound **12f** (1.7 g, 2.183 mmol) in DCM at 0 °C was added TFA, Et_3SiH and DCM (24 mL). After stirring the solution at 25 °C for 2 h, the reaction mixture was quenched with an aqueous saturated solution of $NaHCO_3$ (30 mL), diluted with DCM (20 mL) and extracted with DCM (50 mL x 2). The combined organic layers were then concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography on silica gel (DCM: MeOH = 1: 0 to 10: 1) to give compound **12g** as a white solid (440 mg, 1.495 mmol). ESI-MS: m/z= 295.0 $[M+1]^+$.

Step 7: preparation of **compound 12h**

To a solution of compound **12g** (440 mg, 1.196 mmol) in pyridine was added chlorotrimethylsilane at room temperature. After 2 h, the mixture was cooled to 0 °C and BzCl was added dropwise. After stirring at room temperature for 3 h, the reaction mixture was cooled in an ice bath and quenched with water at 0 °C. NH_4OH was then added and the reaction mixture was stirred overnight at room temperature. The mixture was then diluted with DCM (50 mL) and extracted with DCM (20 mL). The combined organic layers were then washed with brine (50 mL), dried over $NaSO_4$, filtered and the filtrate concentrated. The resultant residue was purified by flash column chromatography on silica

gel (DCM/MeOH: 1/0 to 10/1) to afford compound **12h** as a yellow solid (372 mg). ESI-MS: m/z= 399.1 $[M+1]^+$.

Step 8: preparation of **compound 12i**

A solution of compound **12h** (372 mg, 0.934 mmol) and 4Å MS (3 g) in CH_3CN (30 mL) was stirred at room temperature under N_2 atmosphere for 3 min. 1*H*-Tetrazole (12.451 mL, 5.603 mmol) was added. After 10 min, a solution of compound **1o** in CH_3CN (5 mL) (ChemGenes Corporation) (1.268 g, 1.307 mmol) was added. The mixture was stirred at 26 °C for 1 h. t-Butyl hydroperoxide (0.934 mL, 4.669 mmol) was then added. After stirring at 26 °C for 1 h, the mixture was filtered and the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (DCM: MeOH = 1: 0 to 10: 1) to give compound **12i** as a yellow solid (1.15 g). ESI-MS: m/z=1283.7 $[M+1]^+$.

Step 9: preparation of **compound 12j**

To a solution of compound **12i** (1.15 g, 0.896 mmol) in water and DCM was added dichloroacetic acid (406.140 mg, 8.961 mmol) at room temperature. Triethylsilane (5 mL) was then added. After stirring at room temperature for 48 h, pyridine (0.289 mL, 3.584 mmol) was added. After stirring for 10 min, the mixture was concentrated and the residue was purified by flash column chromatography on silica gel (DCM: MeOH=10 : 1) to afford compound **12j** as a white solid (607mg). ESI-MS: m/z= 981.4$[M+1]^+$; ^{1}H NMR (400MHz, $CDCL_3$) 8.76 (d, J = 8 Hz, 1H), 8.36 (s, 1H), 8.12 - 7.96 (m, 3H), 7.64 - 7.57 (m, 1H), 7.55 - 7.47 (m, 2H), 6.39 - 6.20 (m, 1H), 6.02 - 5.95 (m, 1H), 4.77 - 4.62 (m, 1H), 4.47 (br s, 1H), 4.43 - 4.29 (m, 1H), 4.28 - 4.16 (m, 2H), 4.15 - 4.06 (m, 2H), 4.15 - 4.06 (m, 1H), 4.15 - 4.06 (m, 1H), 4.05 - 3.96 (m, 1H), 3.96 - 3.87 (m, 1H), 3.67 (br t, J = 12.0 Hz, 1H), 3.47 - 3.37 (m, 1H), 2.73 - 2.58 (m, 3H), 1.28 - 1.13 (m, 7H), 0.88 (d, J = 14 Hz, 9H), 0.12 - 0.04 (m, 6H).

Step 10: preparation of **compound 12k**

To a solution of compound **12j** (607 mg, 0.619 mmol), 1*H*-tetrazole (1.031 mL, 0.464 mmol), LiCl (131.162 mg, 3.094 mmol) and 4Å MS in DCM (68 mL) was added dimethyl diisopropylphosphoramidite (125.54 mg, 0.650 mmol). After stirring at rt for 72 h, the mixture was filtered and purified by prep-HPLC (column: Phenomenex Gemini C18 250*50 10u; mobile phase: water (10mM NH_4HCO_3)-ACN from 28 % to 58 %, flow rate: 22 mL/min) to afford compound **12k** as a white solid (160 mg). ESI-MS: *m/z*= 978.2 $[M+1]^+$; ^1H NMR (400MHz, CD_3OD) 8.81 (s, 1H), 8.30 - 8.21 (m, 3H), 8.06 (s, 1H), 7.75 - 7.65 (m, 3H), 6.53 (d, *J* = 17.2 Hz, 1H), 6.24 (d, *J* = 8.4 Hz, 1H), 5.46 - 5.28 (m, 1H), 5.08 - 4.97 (m, 1H), 5.02 (dt, *J* = 3.8, 8.4 Hz, 1H), 4.76 (d, *J* = 3.2 Hz, 1H), 4.51 - 4.36 (m, 2H), 4.29 - 4.18 (m, 1H), 3.86 (d, *J* = 11.2 Hz, 3H), 2.80 - 2.69 (m, 1H), 1.28 - 1.24 (m, 6H), 1.00 (s, 9H), 0.32 (s, 3H), 0.27 - 0.24 (m, 3H).

Step 11: preparation of **compound 12l**

To a solution of compound **12k** (160 mg, 0.164 mmol) in EtOH (10 mL) was added concentrated NH_4OH (10 mL) while stirring. After stirring the reaction mixture at 50 °C for 2 days, it was concentrated under reduced pressure to afford compound **12l** as a white crude solid (125 mg). The crude product was used into the next step without further purification. ESI-MS: *m/z*= 790.5 $[M+1]^+$.

Step 12: preparation of **compound 18 triethyl ammonium salt**

A solution of compound **12l** (125 mg, 0.158 mmol), triethylamine (961.056 mg, 9.498 mmol) and triethylammonium fluoride (765.545, 4.749 mmol) in pyridine (4 mL) was stirred at 50 °C for 5 h. To the reaction mixture was then added isopropoxytrimethylsilane (3.141 g, 23.744 mmol), and the reaction mixture was stirred at room temperature overnight. The mixture was concentrated and the residue was purified by prep-HPLC (column: Agela Durashell C18 150*25 5u; mobile phase: water (10 mM NH_4HCO_3)-CH_3CN from 0% to 15 %, flow rate: 35 mL/min) to afford compound **5** as its triethyl ammonium salt (70 mg, 0.080 mmol) as a white solid. ^1H NMR (400 MHz, D_2O) 8.54 -

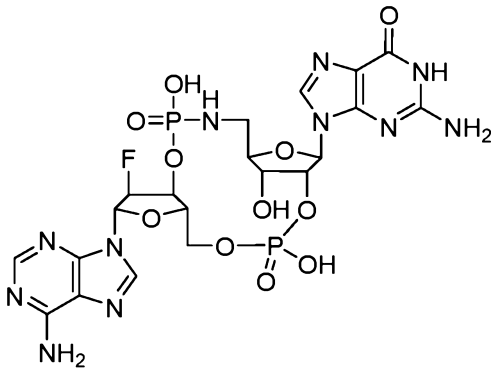
7.99 (m, 2H), 7.78 (br s, 1H), 6.25 (br s, 1H), 5.90 - 5.80 (m, 1H), 5.85 (br s, 1H), 5.71 (br s, 1H), 5.32 - 5.03 (m, 1H), 4.55 (br s, 1H), 4.32 - 4.01 (m, 6H), 3.94 (br s, 1H). ^{31}P NMR (162MHz, D_2O) 6.42 (br s, 1P), -3.47 (br s, 1P).

Step 13: preparation of **compound 18 triethyl sodium salt**

A 70 mL volume of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 70 mg of compound **18** triethyl ammonium salt) which was then washed with deionized water (2x). A volume of 15% H_2SO_4 in deionized water (100 mL) was added to the resin, and the mixture was stirred for 15 min, then decanted (1x). The resin was transferred to a column with 15% H_2SO_4 in deionized water and successively washed with 15% H_2SO_4 (at least 4 Column Volume) and then with deionized water until the resin was pH neutral. The resin was transferred back into the beaker and 15% NaOH in deionized water solution (100 mL) was added. The mixture was stirred for 15 min and decanted (1x). The resin was transferred to the column and washed with 15% NaOH in deionized water (at least 4 Column Volumes), and then with water until it was pH neutral (at least 4 Column Volumes). Compound **18 triethyl ammonium salt** was dissolved in deionized water (70 mg in 50 mL), then added to the top of the column and eluted with DI water. Compound **18** eluted in early fractions as detected by TLC (UV). The product was lyophilized to give compound **18 triethyl sodium salt** (49.9 mg, 0.068 mmol). ESI-MS: *m/z*= 675.8 $[M+1]^+$; ^{1}H NMR (400 MHz, D_2O) 8.39 - 8.06 (m, 2H), 7.78 (s, 1H), 6.28 (d, J = 13.6 Hz, 1H), 5.86 (d, J = .0 Hz, 1H), 5.69 (s, 1H), 5.35 - 5.08 (m, 1H), 4.56 (d, J = 4.5 Hz, 1H), 4.33 - 4.06 (m, 6H), 3.99 (d, *J* = 10.4 Hz, 1H). ^{19}F NMR (376MHz, D_2O) -200.54 (s, 1F). ^{31}P NMR (162 MHz, D_2O) 6.48 (s, 1P), -2.71 (s, 1P).

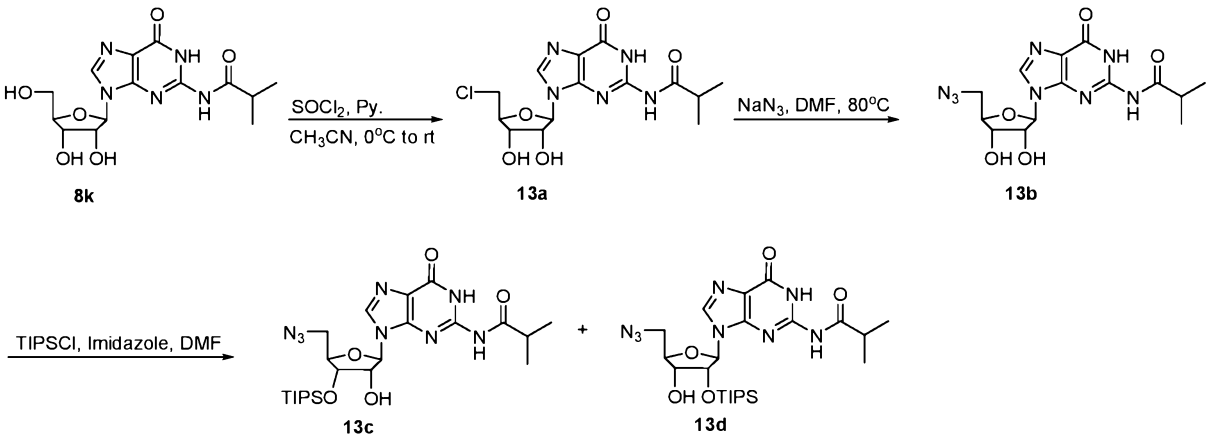
The reaction scheme illustrated in Example 13 describes one possible route for the preparation of compound **19** and pharmaceutically acceptable salt forms thereof, of the present invention.

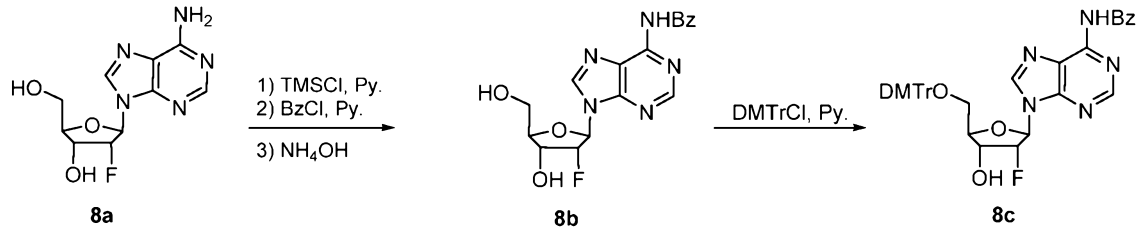
Example 13



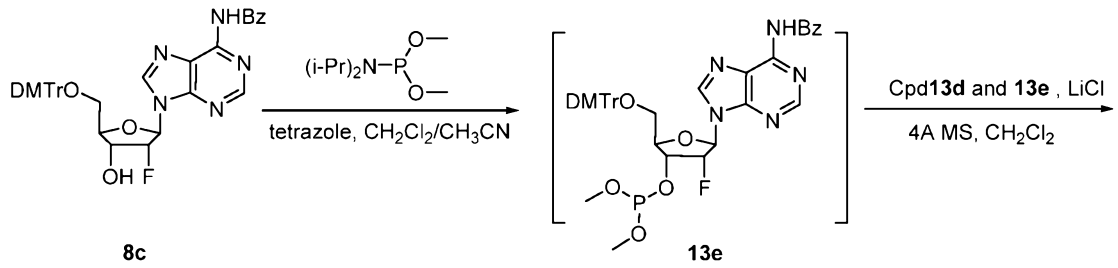
Cpd 19

5

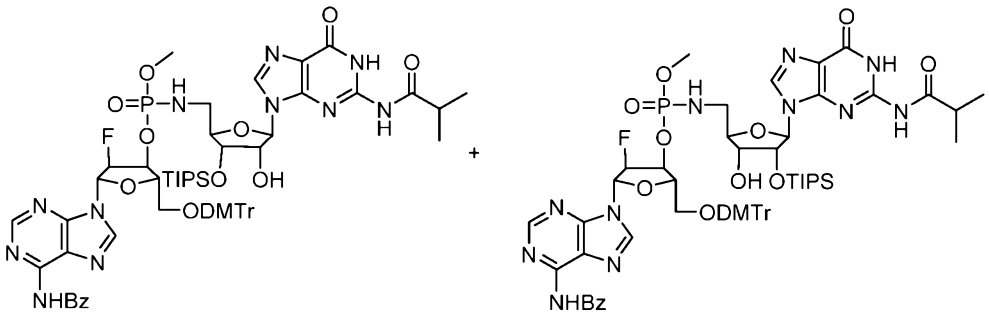




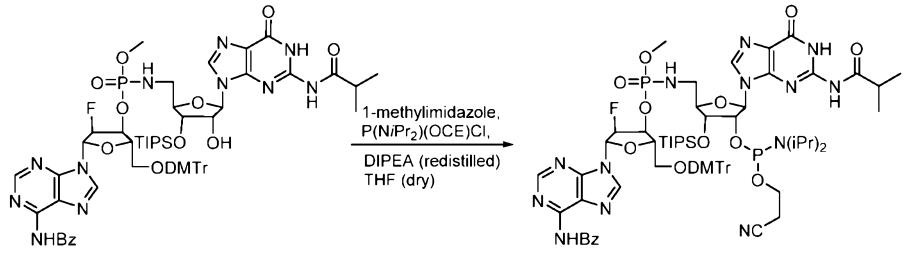
8a 8b 8c



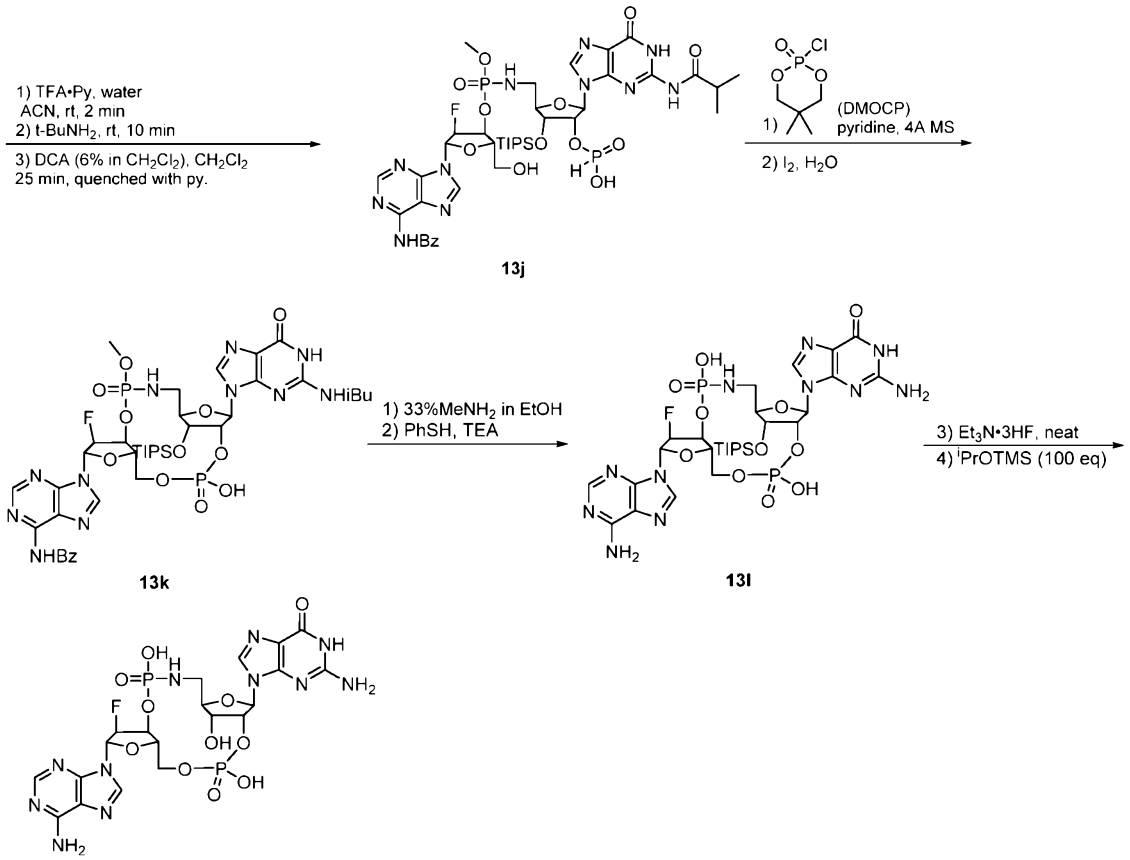
8c 13e



13f 13g



13h 13i



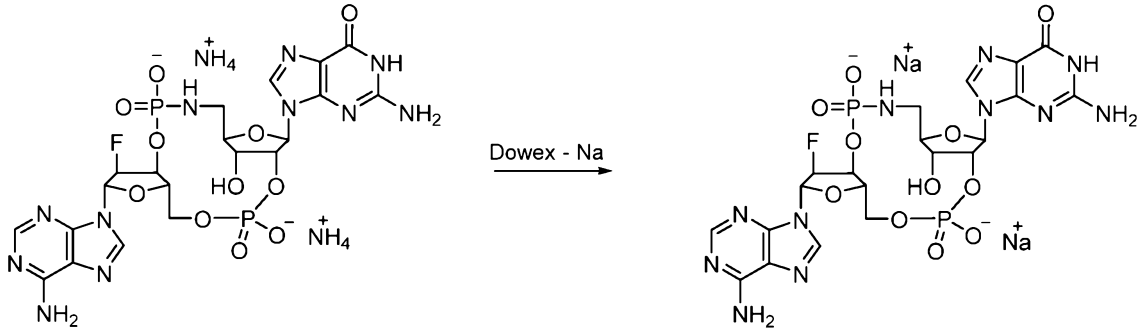
13j

13k

13l

Compound 19, ammonium salt

5

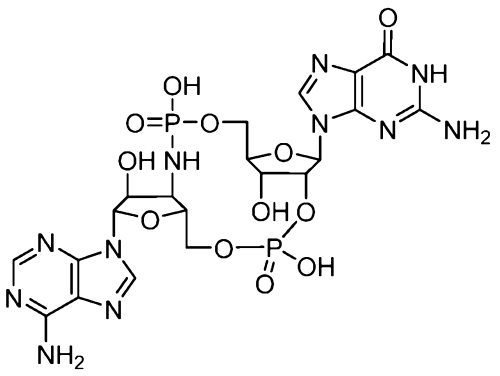


Compound 19, ammonium salt

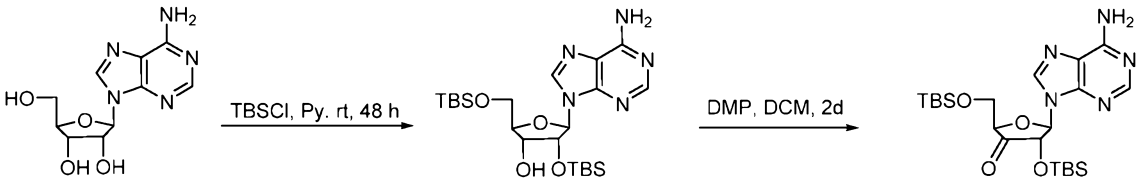
Compound 19, sodium salt

The reaction scheme illustrated in Example 14 describes one possible route for the preparation of compound **20** and pharmaceutically acceptable salt forms thereof, of the present invention.

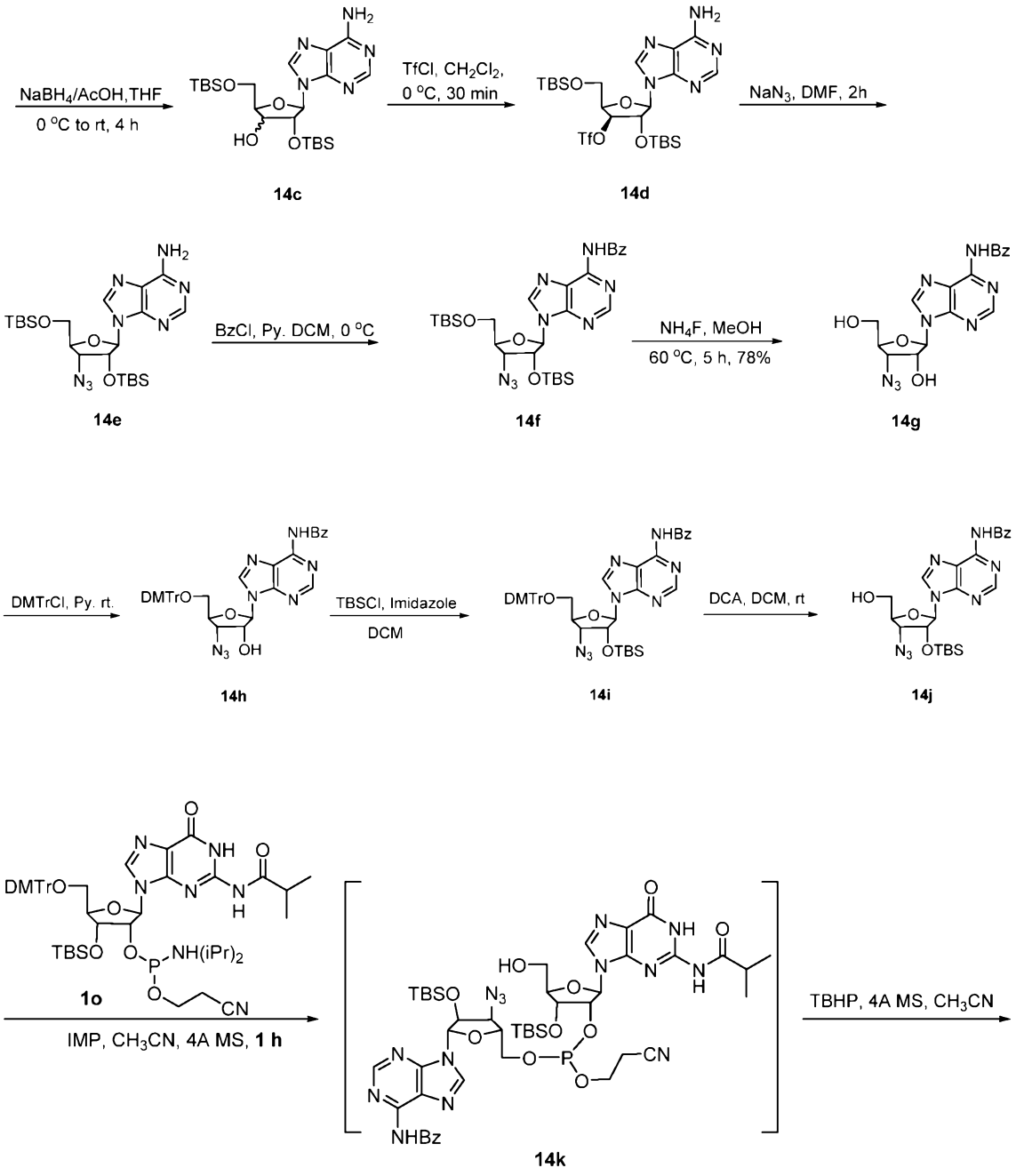
Example 14



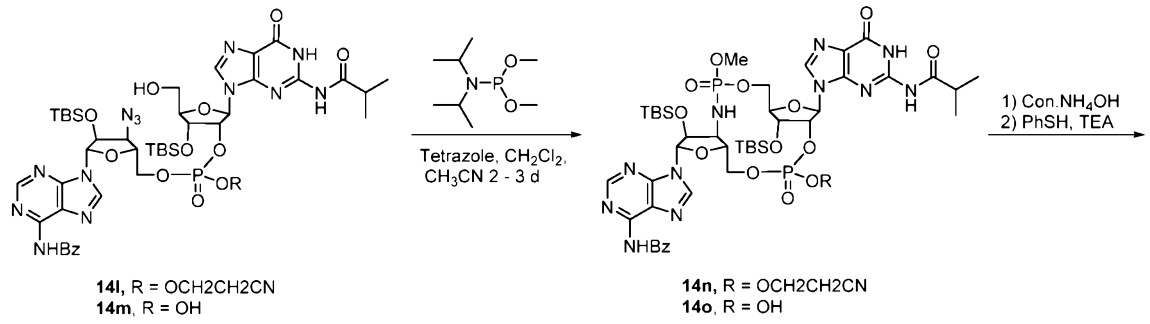
Cpd 20



12a **14a** **14b**

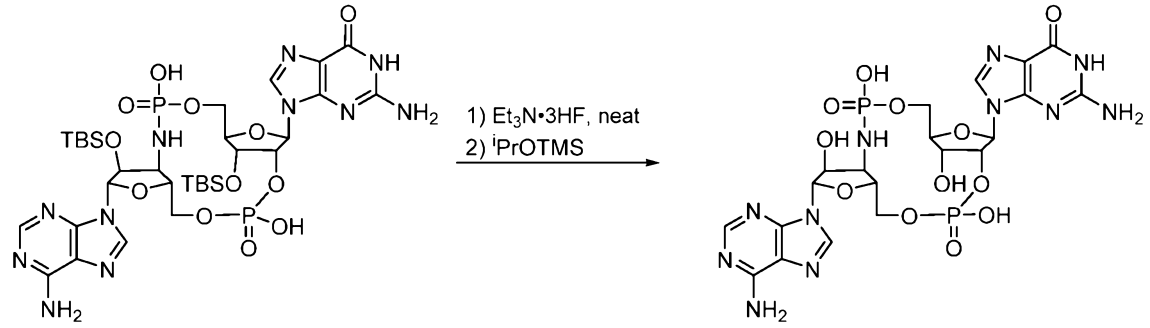


14c 14d 14e 14f 14g 14h 14i 14j 14k



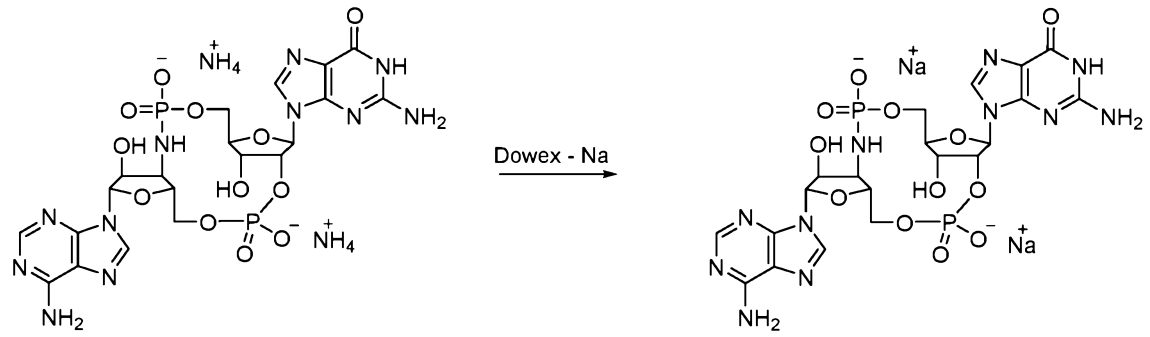
14l, R = OCH_2CH_2CN
14m, R = OH

14n, R = OCH_2CH_2CN
14o, R = OH



14p

Compound 20, ammonium salt

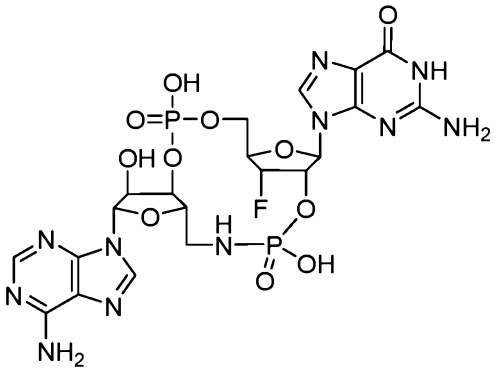


Compound 20, ammonium salt

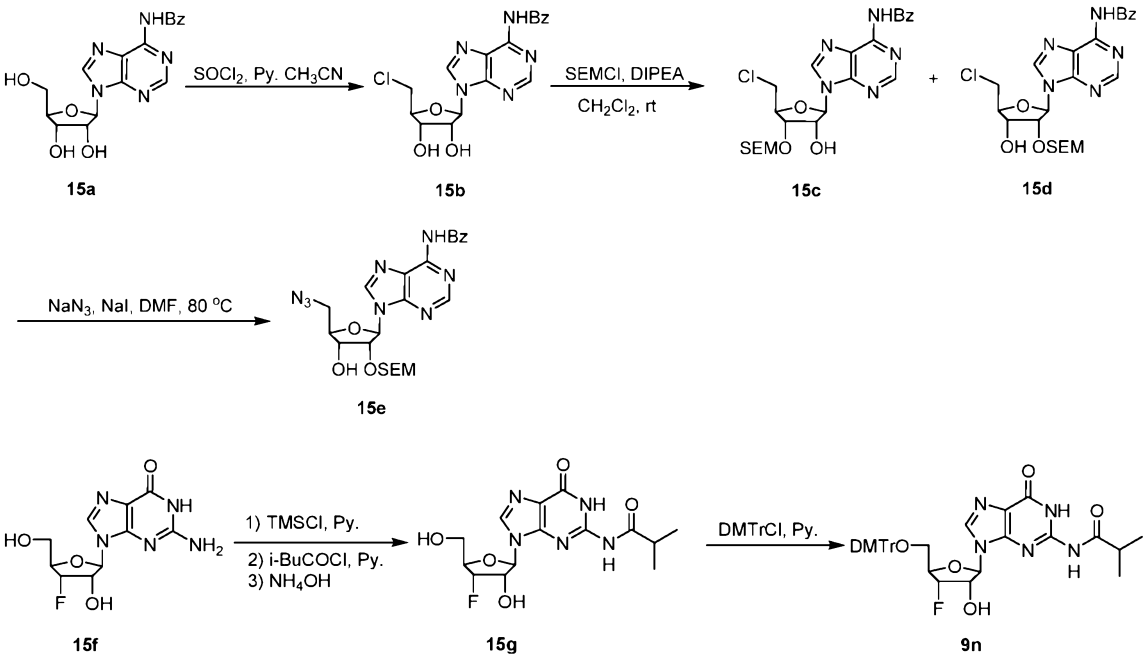
Compound 20, sodium salt

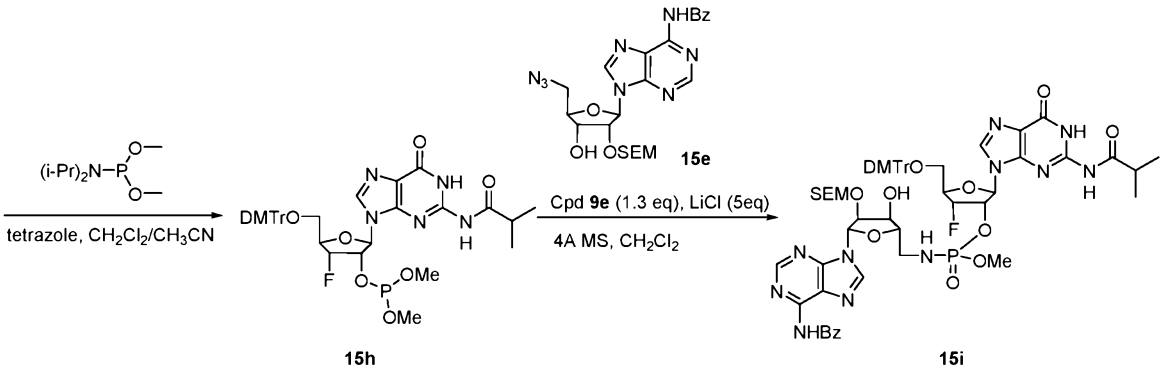
The reaction scheme illustrated in Example 15 describes one possible route for the preparation of compound **21** and pharmaceutically acceptable salt forms thereof, of the present invention.

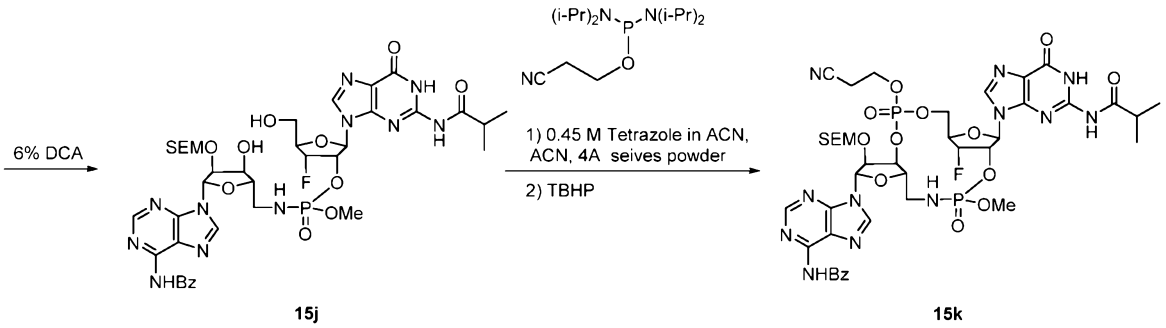
Example 15



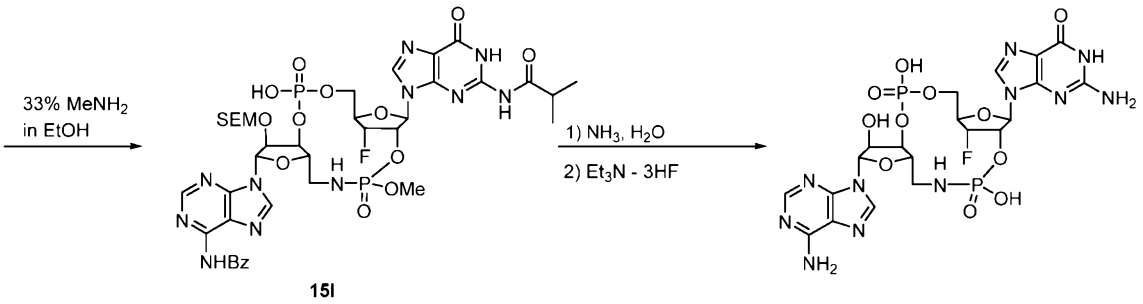
Cpd 21



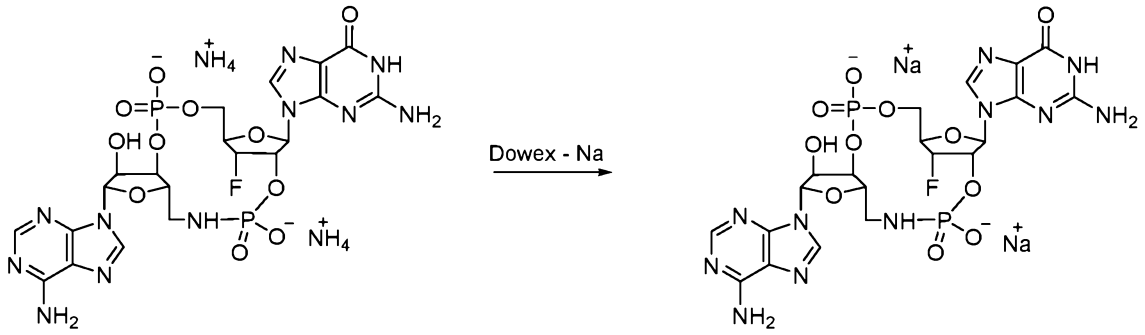




5



Compound 21, ammonium salt

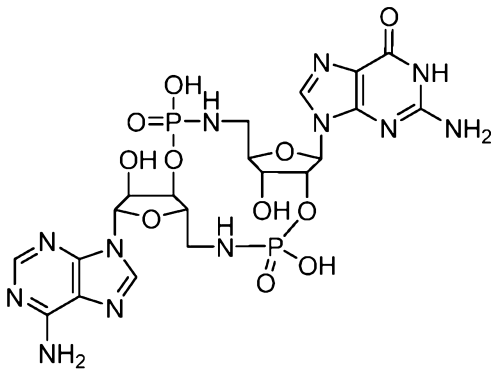


Compound 21, ammonium salt

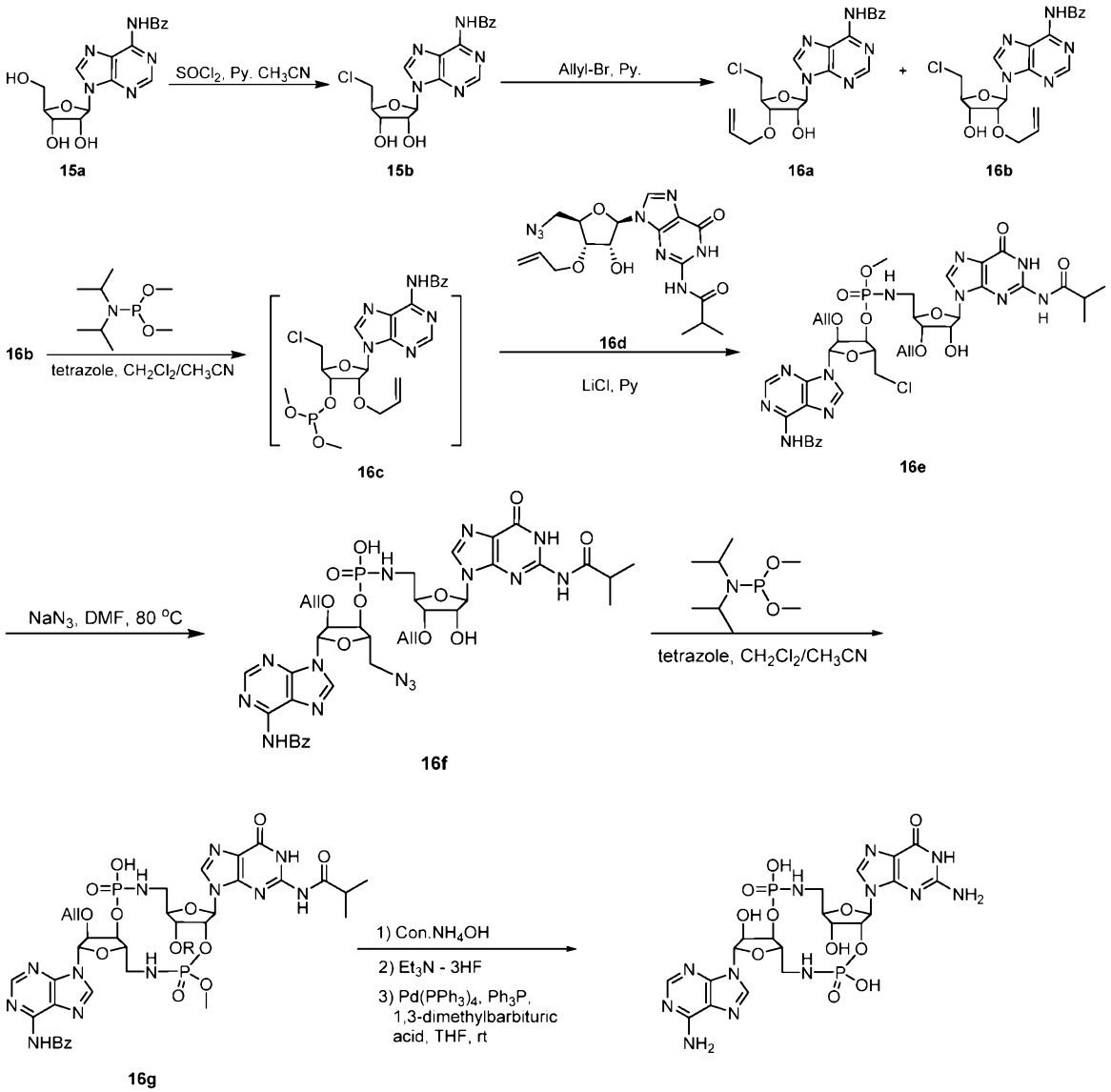
Compound 21, sodium salt

The reaction scheme illustrated in Example 16 describes one possible route for the preparation of compound **22** and pharmaceutically acceptable salt forms thereof, of the present invention.

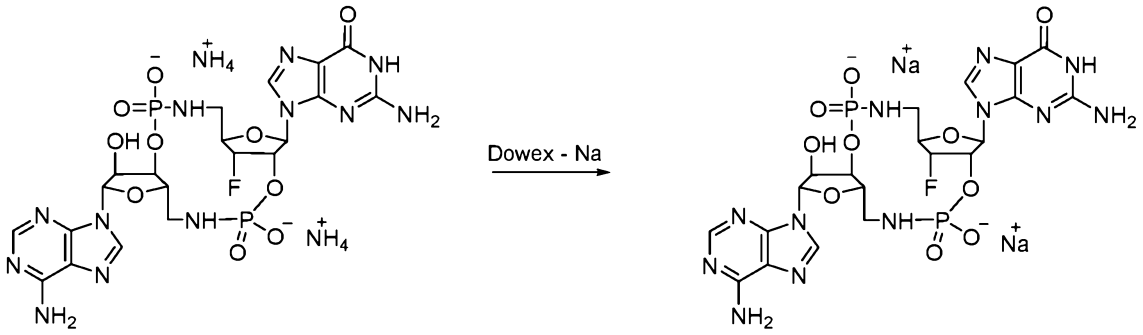
Example 16



Cpd 22



Compound 22, ammonium salt

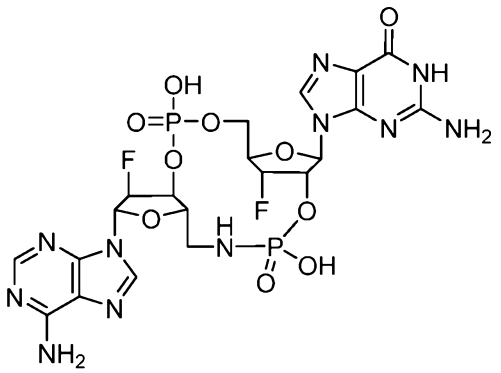


Compound 22, ammonium salt

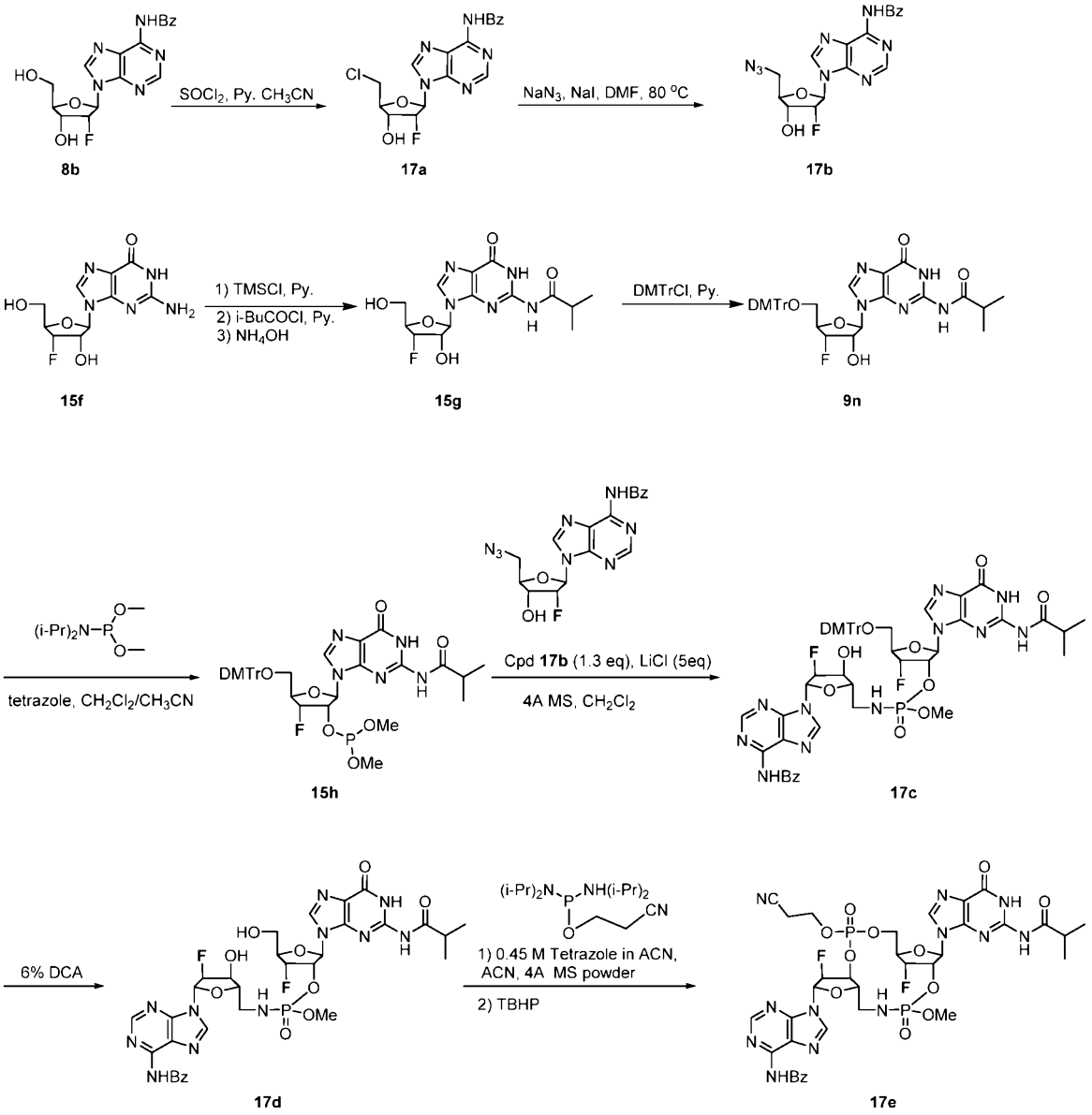
Compound 22, sodium salt

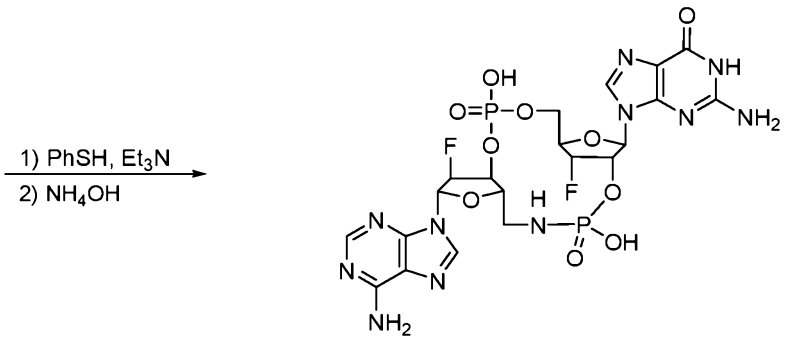
The reaction scheme illustrated in Example 17 describes one possible route for the preparation of compound **23** and pharmaceutically acceptable salt forms thereof, of the present invention.

Example 17

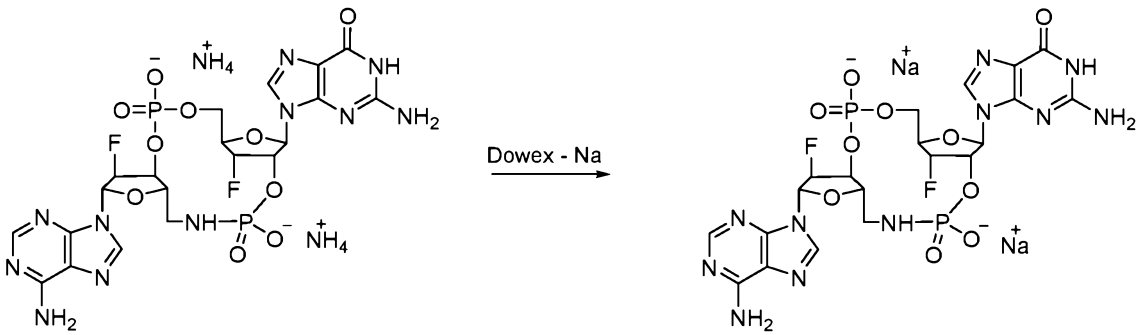


Cpd 23


NHBz
HO
OH F
8b
$SOCl_2$, Py. CH_3CN
Cl
17a
NaN_3, NaI, DMF, 80 °C
N_3
17b
1) TMSCl, Py.
2) i-BuCOCl, Py.
3) NH_4OH
NH_2
15f
15g
DMTrCl, Py.
DMTrO
9n
$(i\text{-}Pr)_2N-P(OMe)_2$
tetrazole, CH_2Cl_2/CH_3CN
15h
Cpd 17b (1.3 eq), LiCl (5eq)
4A MS, CH_2Cl_2
17c
6% DCA
17d
$(i\text{-}Pr)_2N-P(NH(i\text{-}Pr)_2)-O-CH_2CH_2CN$
1) 0.45 M Tetrazole in ACN,
ACN, 4A MS powder
2) TBHP
NC
17e



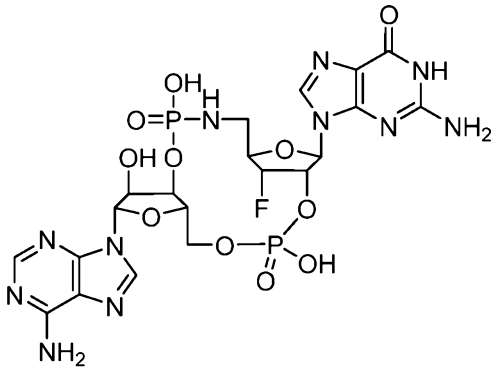
Compound 23, ammonium salt



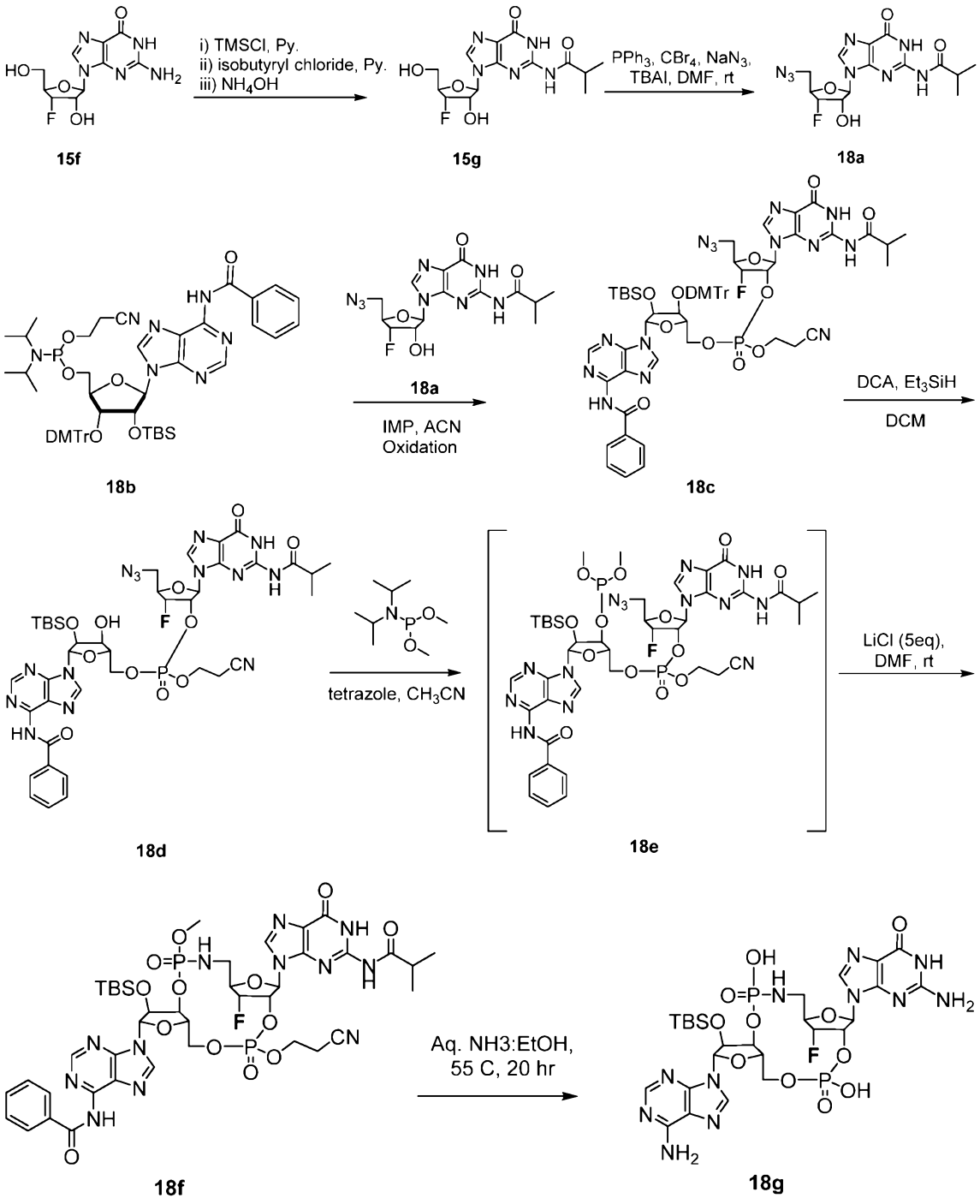
Compound 23, ammonium salt

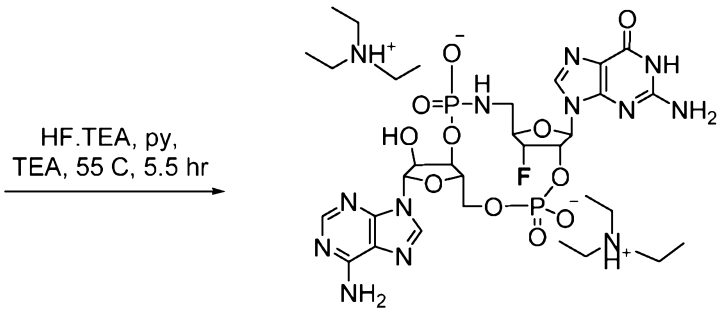
Compound 23, sodium salt

Example 18

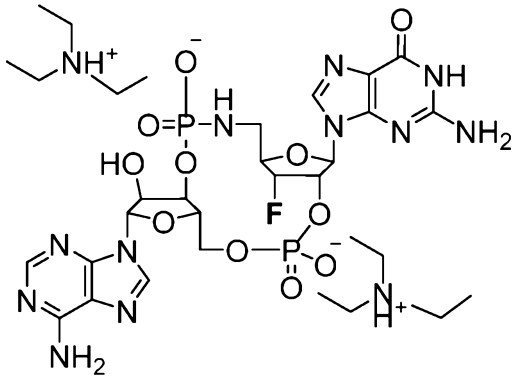


Cpd 24

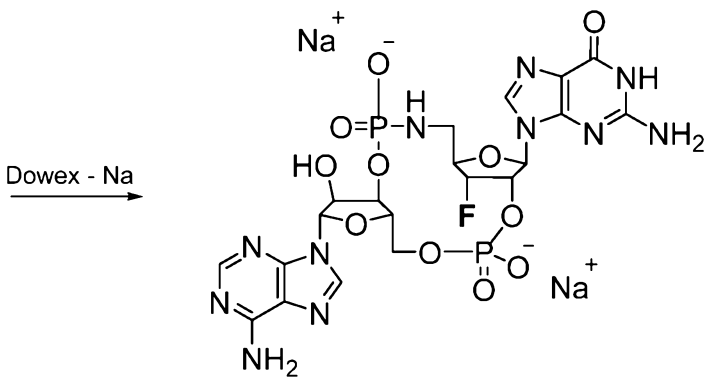

i) TMSCl, Py.
ii) isobutyryl chloride, Py.
iii) NH4OH
PPh3, CBr4, NaN3,
TBAI, DMF, rt
15f
15g
18a
18b
18a
IMP, ACN
Oxidation
18c
DCA, Et3SiH
DCM
18d
tetrazole, CH3CN
18e
LiCl (5eq),
DMF, rt
18f
Aq. NH3:EtOH,
55 C, 20 hr
18g



Compound 24



Compound 24, triethyl ammonium salt



Compound 24, sodium salt

Step 1: preparation of **compound 15g**

3'-Fluoro-guanosine, compound **15f** (2.0 g, 7.01 mmol) was co-evaporated with anhydrous toluene (3 × 20 mL), then suspended in anhydrous pyridine (35 mL). To this was added chlorotrimethylsilane (8.0 mL, 63.09 mmol) at 0 °C. After stirring the reaction mixture at rt for 2 h, the reaction was cooled to 0 °C. Then *iso*-butyrylchloride (1.46 mL, 14.02 mmol) was added. After stirring at rt for 2 h, the reaction mixture was cooled to 0 °C, then water (15 mL) and aq. NH_3 (15 mL) were added. The reaction mixture was stirred for 30 min, then evaporated to dryness to yield a crude residue which was purified by flash column chromatography (*N* 0-20% MeOH in CH_2Cl_2, *v/v*) to afford compound **15g** (1.8 g) as a white solid. LCMS: *m/z* 355.95 (M+1)+.

Step 2: preparation of **compound 18a**

3'-Fluoro-*N*-isobutyryl guanosine, **15g** (830 mg, 2.35 mmol) was co-evaporated with anhydrous toluene (2 x 20 mL) and dissolved in anhydrous DMF (14 mL). Triphenylphosphine (925 mg, 3.52 mmol), NaN_3 (458 mg, 7.05 mmol), tetrabutylammonium iodide (173 mg, 0.470 mmol), and CBr_4 (1.16 g, 3.52 mmol) were added at rt. After stirring the reaction mixture overnight at rt, the reaction mixture was evaporated to dryness and the resulting crude reaction mixture was purified by flash silica gel chromatography (0-20% MeOH in DCM, v/v) to afford compound **18a** (780 mg) as a white solid powder. ESI-MS: *m/z* 381.00 $[M+H]^+$.

Step 3: preparation of **compound 18c**

A solution of compound **18a** (800 mg, 2.10 mmol), 4Å molecular sieves powder (3 g) and 1*H*-imidazoleperchlorate (3.52 g, 21.0 mmol) in dry CH_3CN (80 mL) was stirred at room temperature under an $Ar_{(g)}$ atmosphere for 10 min. Amidite **18b** (commercially available, 2.1 g, 2.10 mmol) in dry CH_3CN (10 mL) was added. After stirring the reaction mixture at rt for 50 min, *tert*-butyl hydroperoxide (5.5 M, 1.90 mL, 10.5 mmol) was added, and the reaction mixture was stirred for 1 h. The reaction mixture was diluted with EtOAc (150 mL), washed with sat. aq. $NaHCO_3$ (1 x 30 mL) and sat. aq. NaCl (1 x 30 mL), and the organic phase was evaporated to dryness under reduced pressure. The resultant residue was purified by flash column chromatography (0-15% MeOH in CH_2Cl_2 *v/v*) to afford dimer **18c** (2.1 g) as a white solid. ESI-MS: *m/z* 1283.30 $[M+H]^+$.

Step 4: preparation of **compound 18d**

Compound **18c** (1.1 g, 0.86 mmol) was dissolved in dry CH_2Cl_2 (24 mL). To the mixture was added triethylsilane (0.7 mL, 5.14 mmol) and dichloroacetic acid (0.43 mL, 5.14 mmol). After stirring the reaction mixture for 35 min, the mixture was quenched with pyridine (0.83 mL, 10.28 mmol) and evaporated to dryness. The resulting crude product was purified by flash column chromatography (20-80% acetone in CH_2Cl_2, *v/v*) to afford dimer **18d** (0.75 g) as a white solid. ESI-MS: *m/z* 981.15 $[M+H]^+$.

Steps 5 and 6: preparation of **compound 18f**

Compound **18d** (570 mg, 0.581 mmol) was co-evaporated with anhydrous toluene (2 x 20 mL) and dissolved in anhydrous CH_3CN (10 mL). 4Å molecular sieves powder (2 g) and tetrazole (8 mL, 3.48 mmol, 0.45 M in CH_3CN) were added, and $Ar_{(g)}$ was bubbled into the reaction mixture for 2 min, then allowed to stir for 10 min at rt. Dimethyl-*N,N*-diisopropylphosphoramidite (0.3 mL, 1.16 mmol) was added. After stirring the reaction mixture for 1 h at rt, the reaction mixture was filtered, and the filtrate was diluted with EtOAc (150 mL), then washed sequentially with sat. aq. $NaHCO_3$ (1 x 30 mL) and sat. aq. NaCl (1 x 30 mL). The organic phase was dried over $MgSO_4$ (stirring for 10 min), filtered, and the filtrate concentrated to dryness under reduced pressure. The crude compound **18e** was used in the next step without further purification.

Step 6: Compound **18e** (0.581 mmol) was dissolved in anhydrous pyridine (25 mL) and LiCl (150 mg, 3.48 mmol) was added. After stirring the reaction mixture at 50 °C for 5.5 h, the reaction mixture was diluted with EtOAc (300 mL) and washed with water (1 x 25 mL). The aqueous phase was back-extracted with EtOAc (1 x 30 mL). The combined organic phases were concentrated to dryness and purified by flash column chromatography (0-15% MeOH in CH_2Cl_2 *v/v*) to afford compound **18f** (150 mg) as a white solid. ESI-MS: *m/z* 1031.15 $[M+H]^+$.

Step 7: preparation of **compound 18g**

Compound **18f** (125 mg, 0.121 mmol) was dissolved in aqueous NH_3:EtOH (8 mL, 3:1, *v/v*). After stirring the reaction for 20 h at 55 °C, the mixture was concentrated to dryness. The resulting precipitate was purified by prep-HPLC (mobile phase: Buffer A: 50 mM TEAA in water, Buffer B: 50 mM TEAA in CH_3CN from 25-65% gradient in 20 min) to afford compound **18g** (58 mg) as a white solid. ESI-MS: *m/z* 790.25 $[M+H]^+$.

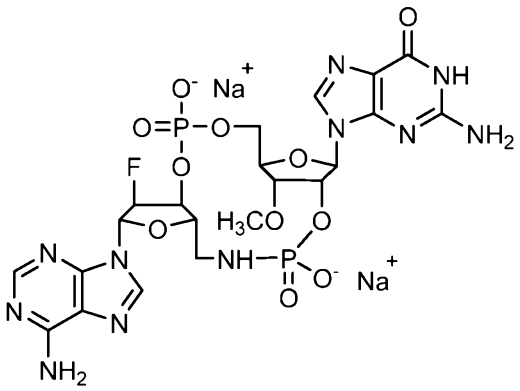
Step 8: preparation of **compound 24**

Compound **18g** (12 mg, 0.021 mmol) was dissolved in *N,N*-dimethylformamide (1 mL). To this was added tetrabutylammonium fluoride (0.15 mL, 1M TBAF in THF). After stirring the reaction for 2 h at rt, the reaction mixture was concentrated to dryness and purified by prep-HPLC (mobile phase: Buffer A: 50 mM TEAA in water, Buffer B: 50 mM TEAA in CH_3CN from 0-25% gradient in 20 min) to afford compound **24** (2.5 mg) as its **TEA salt**. ESI-MS: *m/z* 674.1 $[M-1]^-$.

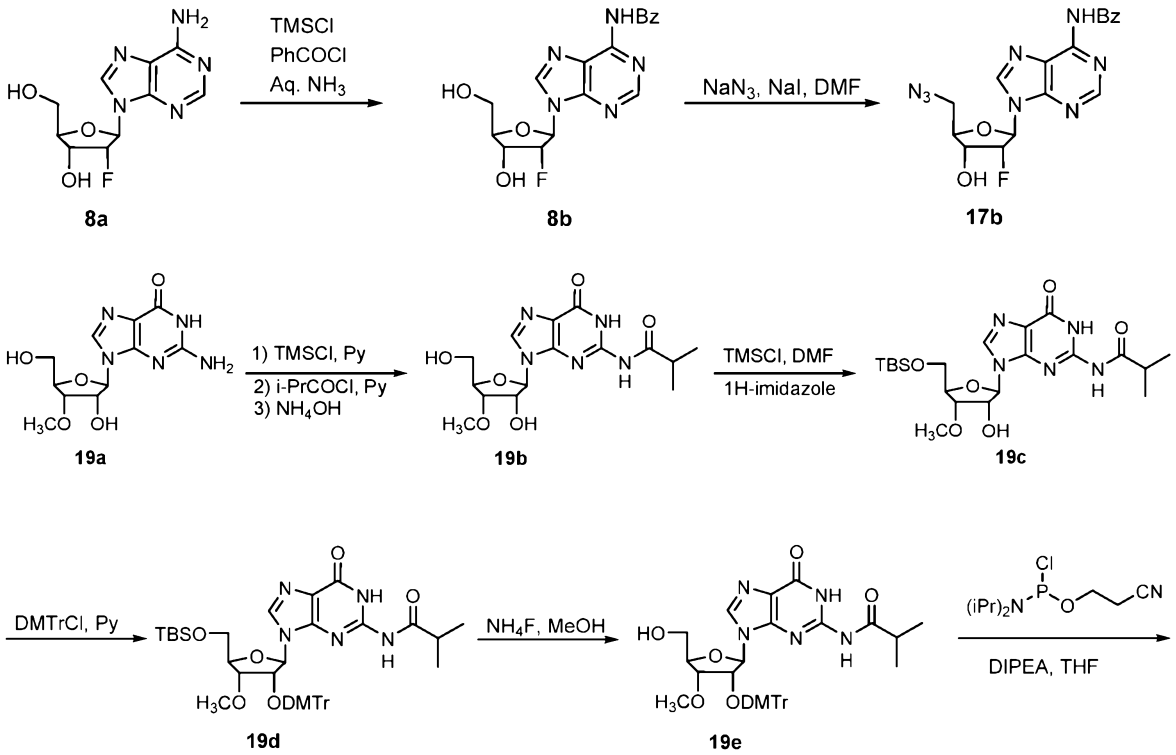
Step 9: preparation of **compound 24 sodium salt**

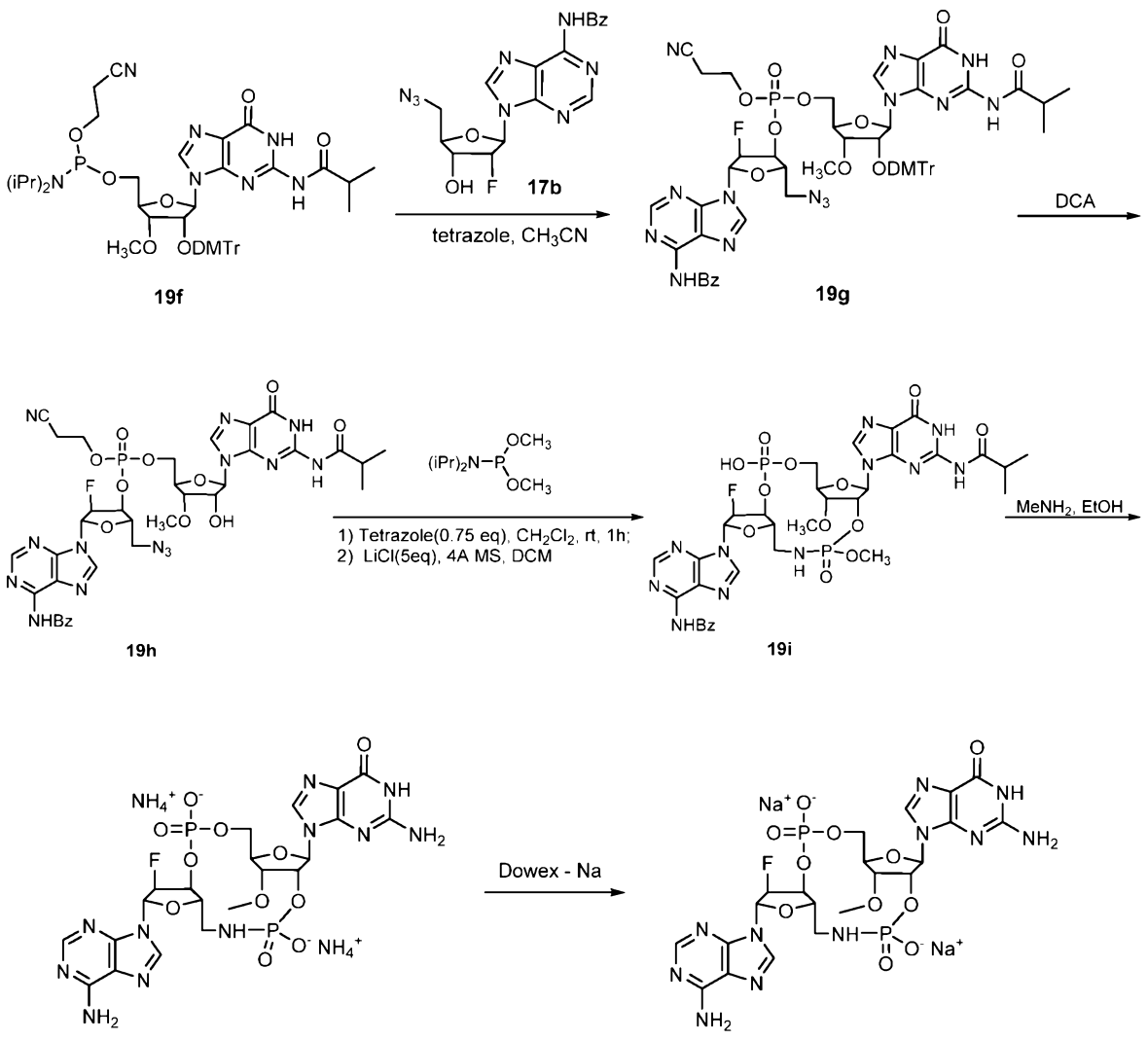
A 3 mL volume of Dowex 50W × 8, 200-400 (H form) was added to a beaker (for 2.5 mg of **compound 24 TEA salt**) and washed with deionized water (2x). To the resin was added 15 % H_2SO_4 in de-ionized water (50 mL), the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to a column with 15 % H_2SO_4 in deionized water and washed with 15 % H_2SO_4 (at least 4 CV), and then with deionized water until it was pH neutral. The resin was transferred back into the beaker, and aqueous15 % NaOH solution (50 mL) was added, and the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to the column and washed with aqueous15 % NaOH solution (at least 4 CV), and then with water until it was pH neutral (at least 4 CV). **Compound 24 TEA** salt (2.5 mg) was dissolved in deionized water (2 mL) and added to the top of the column, and eluted with deionized water. Compound was eluted in early fractions as detected by TLC (UV). The product was lyophilized to give target **compound 24 Na salt** (2.0 mg) as a white foam. ^1H NMR (400 MHz, D_2O,) δ 8.23 (s, 1H), 8.13 (s, 1H), 7.71(s, 1H), 6.04 (s, 1H), 5.95 (d, J = 8.4 Hz, 1H), 5.42 - 5.58 (m, 1H), 5.12 (d, J = 4 Hz, 0.5H), 5.01 (d, J = 4 Hz, 0.5H), 4.62-4.70 (m, 1H), 4.50-4.58 (m, 0.5 H), 4.46-4.51 (m, 0.5 H), 4.40-4.43 (m, 1H), 4.30-4.40 (m, 2H), 3.92-4.02 (m, 1H), 3.15-3.25 (m, 2H); ^{31}P NMR (162 MHz, D_2O): δ 7.95, -2.76; ^{19}F NMR (379 MHz, D_2O): δ -195.59 (quintet); ESI-MS: *m/z* 674.1 $[M-1]^-$.

Example 19



Cpd 25





Compound 19 ammonium salt

Compound 19 sodium salt

Step 1: preparation of **compound 8b**

To a solution of compound **8a** (5.0 g, 18.57 mmol) in pyridine (100 mL) was added chlorotrimethylsilane (18.16 g, 167.14 mmol) at room temperature. After 1.5 h, benzoyl chloride (7.83 g, 55.71 mmol) was added dropwise at room temperature. The final mixture was stirred at room temperature for 3 h. The mixture was quenched with water (50 mL) at 0 °C and NH_3-H_2O (50 mL) was added dropwise at 0 °C. The reaction mixture was

concentrated and purified by flash column chromatography on silica gel (DCM/MeOH= 10/1) to give compound **8b** (5.2 g) as a white solid. ^{1}H NMR (400MHz, CD_3OD and DMSO-d_6) δ 8.73 (d, J=0.8 Hz, 2H), 8.09 - 8.02 (m, 2H), 7.68 - 7.65 (m, 1H), 7.59 - 7.55 (m, 2H), 6.47 - 6.42 (m, 1H), 5.56 - 5.41 (m, 1H), 4.71 - 4.64 (m, 1H), 4.17 - 4.15 (m, 1H), 3.98 - 3.94 (m, 1H), 3.81 - 3.77 (m, 1H).

Step 2: preparation of **compound 17b**

Compound **8b** (1 g, 2.68 mmol) was co-evaporated with anhydrous toluene (2 x 20 mL) and dissolved in anhydrous DMF (16 mL). Triphenylphosphine (1.05 g, 4.02 mmol), NaN_3 (650 mg, 10.00 mmol), tetrabutylammonium iodide (197.87 mg, 0.54 mmol), carbon tetrabromide (1.33 g, 4.02 mmol) were added at room temperature. After stirring overnight at room temperature, the reaction mixture was evaporated to dryness and the resulting residue was purified by flash column chromatography on silica gel (DCM/MeOH=10/1) to give compound **17b** (890 mg) as a white solid powder.

Step 3: preparation of **compound 19b**

To a solution of compound **19a** (5.0 g, 16.82 mmol) in pyridine (72 mL) was added chlorotrimethylsilane (16.45 g, 151.38 mmol) at room temperature. After 1.5 h, isobutyryl chloride (5.38 g, 50.46 mmol) was added dropwise at room temperature. After stirring at room temperature for 3 h, the reaction mixture was quenched with water (50 mL) at 0 °C and NH_3-H_2O (50 mL) was added dropwise at 0 °C. The reaction mixture was concentrated and purified by flash column chromatography on silica gel (DCM/MeOH=10/1) to give compound **19b** (5.28 g) as a white solid. ESI-MS: *m/z* 368.1 $[M+1]^+$.

Step 4: preparation of **compound 19c**

To a solution of compound **19b** (1g, 2.72 mmol) and 1*H*-imidazole (315.04 mg, 4.63 mmol) in DMF (15 mL) was added TBSCl (656.46 mg, 4.36 mmol) at 0 °C. After stirring for 4 h at room temperature, the reaction mixture was quenched with MeOH (27 mL) and

concentrated to a residue. The residue was dissolved in DCM (35 mL), concentrated and purified by flash column chromatography on silica gel (DCM/MeOH=20/1) to afford compound **12c** (1.19 g) as a white solid. ESI-MS: *m/z* 482.2 $[M+1]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J*=5.6 Hz, 1H), 5.76 (d, *J*=4.4 Hz, 1H), 4.55 (t, *J*=4.4 Hz, 1H), 4.13 (d, *J*=3.2 Hz, 1H), 3.93 - 3.80 (m, 2H), 3.72 (d, *J*=9.6 Hz, 1H), 3.37 (s, 3H), 1.16 (d, *J*=6.8 Hz, 6H), 0.81 (s, 9H), 0.00 (d, *J*=2.4 Hz, 6H).

Step 5: preparation of **compound 19d**

To a solution of compound **19c** (1.19 g, 2.47 mmol) in Py (12 mL) was added 4,4'-(chloro(phenyl)methylene) bis(methoxybenzene) (1.67 g, 4.94 mmol) and DMAP (0.33 g, 2.72 mmol), at room temperature. After stirring at 50 °C overnight, the reaction mixture was quenched with water and extracted with dichloromethane. The organic layers were combined, was washed with water, dried (Na_2SO_4) and filtered, and the filtrate concentrated. The residue was purified by flash column chromatography on silica gel (dichloromethane: methanol = 20:1) to afford compound **19d** (1.37 g) as a white solid. ESI-MS: m/z= 784.5 $[M+1]^+$.

Step 6: preparation of **compound 19e**

To a solution of compound **19d** (1.37 g, 1.75 mmol) in THF (5 mL) was added tetrabutylammonium fluoride (7.86 mL, 7.86 mmol). After stirring at room temperature for 2 h, the reaction mixture was diluted with ethyl acetate and washed with water. The organic layers were then combined, washed with brine, filtered, and concentrated to a residue. The residue was purified by flash column chromatography on silica gel (DCM: MeOH = 20:1) to give compound **19e** (1.01 g) as a white solid. ESI-MS: *m/z*= 670.1 $[M+1]^+$.

Step 7: preparation of **compound 19f**

To a solution of compound **19e** (500 mg, 0.75 mmol) and DIPEA (289.469 mg, 2.240 mmol) in THF (1.88 mL) was added 3-((chloro(diisopropylamino)phosphanyl)oxy)

propanenitrile (530.10 mg, 2.240 mmol) at 15 °C. After stirring at room temperature for 1 h, water was added to the reaction mixture and the mixture was then extracted with dichloromethane. The organic layers were combined, washed with brine, filtered and concentrated to a residue. The residue was purified by flash column chromatography on silica gel (petroleum ether: ethyl acetate =1: 2) to give compound **19f** (411 mg). ESI-MS: *m/z*= 787.1 $[M\text{-}N(Pi)_2]^+$.

Step 8: preparation of **compound 19g**

A solution of compound **17b** (134.428 mg, 0.337 mmol) and 4Å molecular sieves (2 g) in CH_3CN (10 mL) was stirred at room temperature under an argon atmosphere for 3 min. 1*H*-tetrazole (4.499 mL, 2.025 mmol) was added. After 10 min, a solution of compound **19f** (411 mg, 0.472 mmol) in CH_3CN (3.44 mL) was added at room temperature. After stirring at 26 °C for 1 h, tert-butyl hydroperoxide (0.337 mL, 1.687 mmol) was added to the reaction mixture. After stirring at 26 °C for 1 h, the mixture was concentrated to afford compound **19g**, which was used for the next step without further purification.

Step 9: preparation of **compound 19h**

To a solution of compound **19g** (239.55 mg, 0.202 mmol) in water and dichloromethane, was added dichloroacetic acid (91.769 mg, 0.712 mmol). After stirring at room temperature for 3 h, triethylsilane (2 mL) was added to the reaction mixture. Pyridine (0.033 mL, 0.41 mmol) was then added. After stirring for 10 min at rt, the reaction mixture was concentrated and the residue was purified by flash column chromatography on silica gel (DCM: MeOH=10: 1) to afford compound **19h** (136.8 mg) as a light yellow solid. ESI-MS: *m/z*= 881.2 $[M+1]^+$.

Step 10: preparation of **compound 19i**

A solution of compound **19h** (136.8 mg, 0.155 mmol), 1*H*-tetrazole (0.259 mL, 0.116 mmol), LiCl (32.926 mg, 0.777 mmol) and 4Å molecular sieves in DCM (17.1 mL) was stirred at rt for 2 h. Dimethyl diisopropylphosphoramidite (31.513 mg, 0.163 mmol) was

added. After stirring at rt overnight, the reaction mixture was filtered, concentrated and purified by reverse phase preparative HPLC (Column: Phenomenex Gemini C18 250x50 10μm; Condition: water (10 mM NH_4HCO_3) (A) - CH_3CN (B); Begin B:16; End B: 46; Flow Rate: 22 mL/min) to afford compound **19i** (40.1 mg) as a white solid. ESI-MS: *m/z* 878.3= $[M+1]^+$.

Step 11: preparation of **compound 25**

A solution of compound **19i** (10 mg, 0.011 mmol) in methanamine in EtOH (6 mL) was stirred at 50 °C for 1 d. The reaction mixture was then concentrated and purified by reverse phase preparative HPLC (Column: DuraShell 150x25mm 5mm; Condition: water (10 mM NH_4HCO_3) (A)-CH_3CN (B); Begin B: 0; End B: 15; Flow Rate: 35 mL/min) to afford compound **25, ammonium salt** (1.5 mg) as a white solid. ^{31}P NMR (162MHz, D_2O) δ 7.69, -1.28.

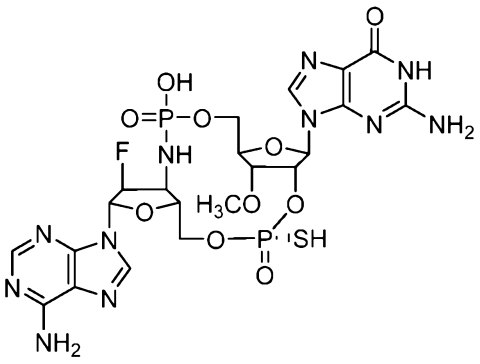
Step 12: preparation of **compound 25**

A volume of Dowex (50W x 8, 200-400, H form) (99 mL) was added to a beaker (for 74.9 mg of compound **25 ammonium salt**) and washed with deionized water (2x). To the resin was added 15% H_2SO_4 in deionized water (50 mL), the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to a column with 15% H_2SO_4 in deionized water and washed with 15% H_2SO_4 (at least 4 Column Volume (CV)), and then with deionized water until it was pH neutral. The resin was transferred back into the beaker, and a solution of 15% NaOH in deionized water (50 mL) was added, and the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to the column and washed with 15% NaOH in deionized water (at least 4 CV), and then with water until it was pH neutral (at least 4 CV). Compound **25 ammonium salt** was dissolved in deionized water (74.9 mg in 25 mL), added to the top of the column, and eluted with deionized water. Compound **25** eluted in early fractions as detected by TLC (UV). The product was lyophilized to afford **compound 25 sodium salt** (63.0 mg). ESI-MS: *m/z*= 689.9 $[M+1]^+$; ^1H NMR (400 MHz, D_2O) δ 8.08 - 8.05 (m, 1H), 7.97 (s, 1H), 7.37 - 7.36

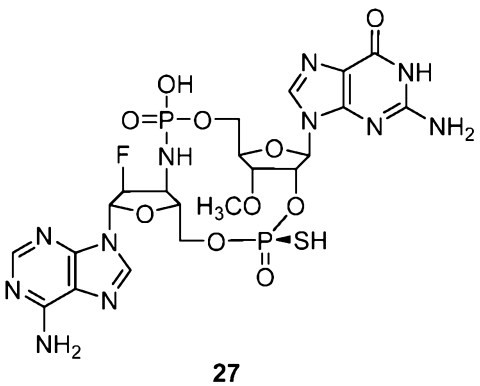
(m, 1H), 6.41 - 6.33 (m, 1H), 5.88 (d, *J*=8.0 Hz, 1H), 5.64 (d, *J*=4.4 Hz, 1H), 5.44 - 5.27 (m, 2H), 4.48 (d, *J*=2.4 Hz, 1H), 4.38 - 4.30 (m, 2H), 4.20 - 4.11 (m, 2H), 3.50 (s, 3H), 3.46 (d, *J*=13.6 Hz, 1H), 3.22 - 3.18 (m, 1H); ^{19}F NMR (376 MHz, D_2O) -196.87 (s, 1F); ^{31}P NMR (162 MHz, D_2O) 7.80 (s, 1P), -1.22 (s, 1P).

Example 20

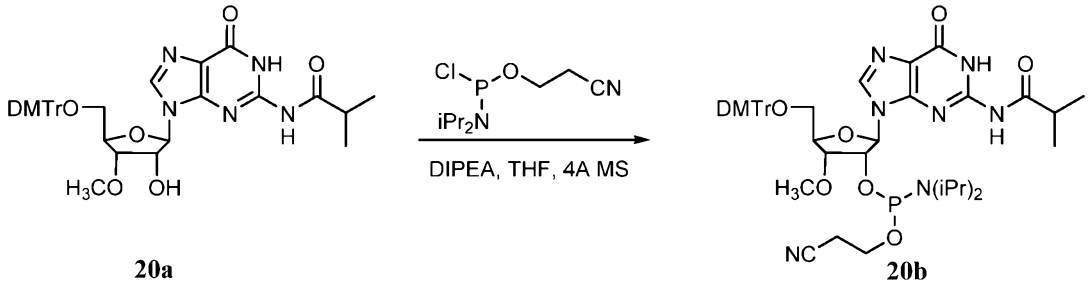
Compounds **26** and **27**



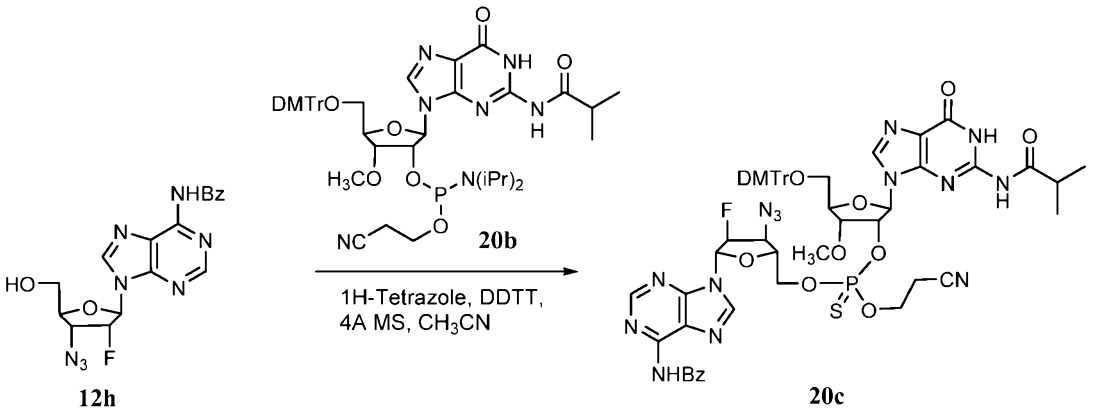
26

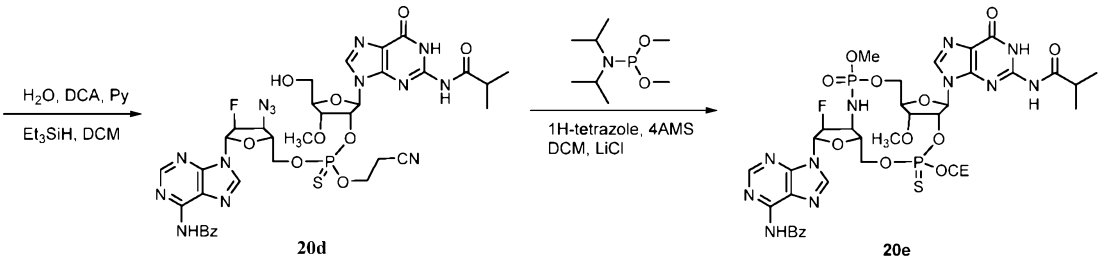


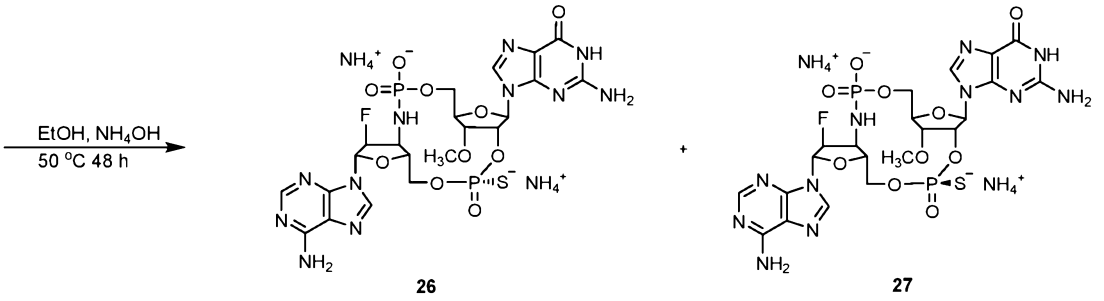
27

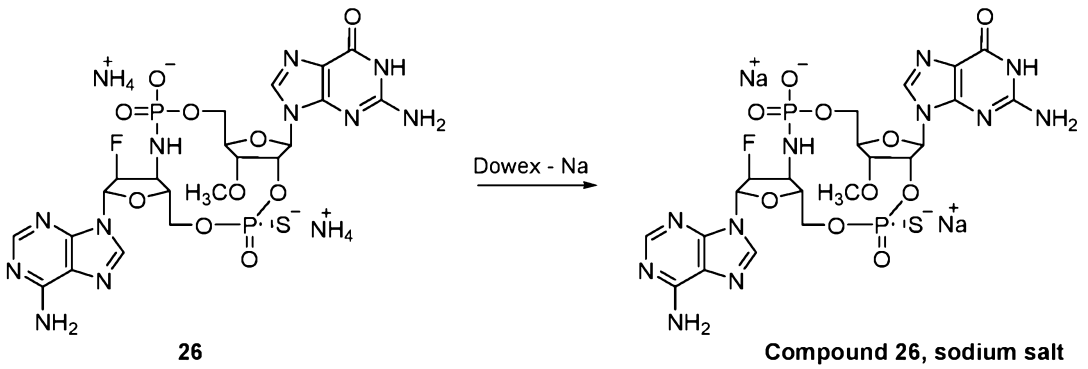


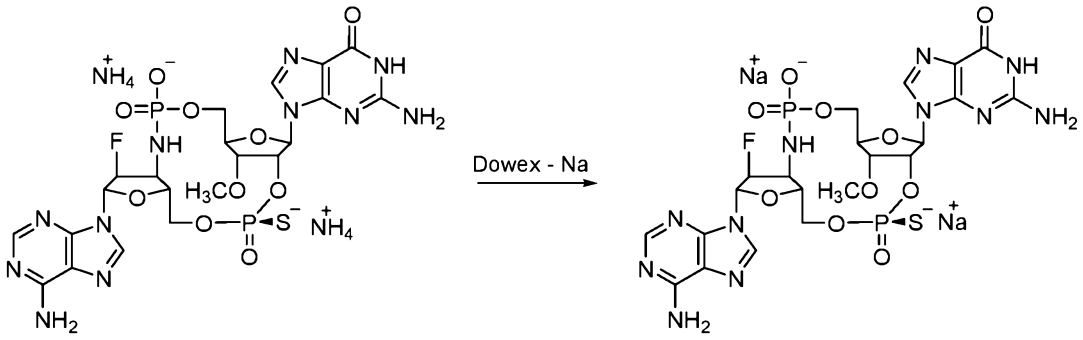
20a **20b**


12h
20b
1H-Tetrazole, DDTT,
4A MS, CH3CN
20c


H2O, DCA, Py
Et3SiH, DCM
20d
1H-tetrazole, 4AMS
DCM, LiCl
20e


EtOH, NH4OH
50 °C 48 h
26
27


Dowex - Na
26
Compound 26, sodium salt



27 Compound 27 sodium salt

Step 1: preparation **of 20b**

To a solution of **20a** (1 g, 1.49 mmol) in THF (3.8 mL) and DIPEA (0.58 g, 4.48 mmol) was added 3-((chloro(diisopropylamino)phosphino)oxy)propanenitrile (1.06 g, 4.48 mmol) at room temperature. After stirring the mixture reaction at rt for 1.5 h, water was added. Organic layer was extracted with DCM (50 mL), washed with brine and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM : MeOH=1 : 0~20:1) to give **20b** (1.35 g) as a colorless solid. ESI-MS: m/z= 787.2 [M-83]+ ; ^{31}P NMR (162 MHz, $CDCl_3$) 150.71 (s, 1P), 150.48 (s, 1P), 14.18 (s, 1P).

Step 2: preparation **of 20c**

A solution of **12h** (700 mg, 1.76 mmol) in CH_3CN (50 mL) and 4A Molecular Sieves (7 g) was stirred at room temperature under N_2 atmosphere for 3 min. 1H-Tetrazole (23.43 mL, 10.54 mmol) was then added. After stirring for 10 min, a solution of **20b** (2.06 g, 2.37 mmol) in CH_3CN (20 mL) was added. The mixture was stirred at room temperature for 1.5 h followed by addition of N,N-dimethyl-N'-(3-thioxo-3H-1,2,4-dithiazol-5-yl) formimidamide (DDTT, 1.81 g, 8.79 mmol). After stirring for 1 h at room temperature, the raction mixture was filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM: MeOH = 1: 0 to 10: 1) to give **13c** as a yellow solid (2.1 g). ESI-MS: *m/z*=1199.5 $[M+1]^+$.

Step 3: preparation of **20d**

To a solution of **20c** (2.1 g) in water (315.49 mg, 17.51 mmol) and DCM (60 mL) was added dichloroacetic acid (790.316 mg, 6.129 mmol) at room temperature. Et_3SiH (12 mL) was added and stirred at room temperature for 48 h. Pyridine (0.56 mL, 7.0 mmol) was added. After stirring for 10 min, the reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel (DCM: MeOH = 1: 0~10:1) to afford **20d** as a white solid (901 mg). ESI-MS: *m/z*=897.3 $[M+1]^+$.

Step 4: preparation of **20e**

To a solution of **20d** (500 mg, 0.56 mmol) in DCM (60 mL) was added 4A Molecular Sieves (5 g), 1H-tetrazole (0.93 mL, 0.42 mmol) , LiCl (118.18 mg, 2.79 mmol) followed by dimethyl diisopropylphosphoramidite (113.12 mg, 0.58 mmol). After stirring the reaction mixture for 72 h at room temperature, the mixture was filtered and concentrated under reduced pressure to get crude **20e** (495 mg) which was used directly into the next step without further purification. ESI-MS: *m/z*= 947.3 $[M+1]^+$.

Step 5: preparation of **cpd 26** and **cpd 27**

To a solution of **20e** (495 mg, crude) in EtOH (12 mL) was added $NH_3 \cdot H_2O$ (12 mL) at room temperature. After stirring the reaction mixture at 50°C for 3 days, the mixture was filtered and purified by reverse phase preparative HPLC (column: Agela DuraShell 150mm x 25mm x 5μM; mobile phase: water (10mM NH_4HCO_3)-ACN from 1 % to 16 %, flow rate:25 ml/min) to afford crude **26** and **27** as a white solid (220 mg). ^1H NMR (400 MHz, D_2O) δ 8.29 (br s, 1H), 8.13 (br s, 1H), 7.70 (br s, 1H), 6.25 (br d, *J* = 11.8 Hz, 1H), 5.92 - 5.70 (m, 2H), 5.24 - 4.99 (m, 1H), 4.43 - 4.26 (m, 3H), 4.20 (br d, *J* = 10.8 Hz, 2H), 4.06 (br s, 3H),3.49 (s, 2H), 3.56 - 3.45 (m, 1H); ^{19}F NMR (376 MHz, D_2O) -75.65 (s, 1F), -199.98 (br s, 1F); ^{31}P NMR (162MHz, D2O) 51.33 (br s, 1P), 6.49 (br s, 1P).

Step 6: preparation of **cpd 26** and **cpd 27**

The previous mixture of **cpd 26** and **cpd 27**was purified by reverse phase preparative HPLC (column: Phenomenex Synergi C18 150 x 30mm x 4um ; mobile phase: water(10mM NH_4HCO_3)-ACN from 0 % to 15 %, flow rate: 25 ml/min) to afford **26** ammonium salt (16.3 mg) and **27** ammonium salt (50.1 mg) as white solids.

Step 7: preparation of **26 sodium salt**

A 20 mL volume of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 16.3 mg of **26**) and washed with deionized water (2x). To the resin was added 15% H_2SO_4 in deionized water (50 mL), the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to a column with 15% H_2SO_4 in deionized water and washed with 15% H_2SO_4 (at least 4 Column Volume (CV)), and then with deionized water until it was pH neutral. The resin was transferred back into the beaker, and a solution of 15% NaOH in deionized water (50 mL) was added, and the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to the column and washed with 15% NaOH in deionized water (at least 4 CV), and then with water until it was pH neutral (at least 4 CV). Compound **26** ammonium salt was dissolved in deionized water (16.3 mg in 20 mL), added to the top of the column, and eluted with deionized water. Compound **26** was eluted in early fractions as detected by TLC (UV). The product was lyophilized to give compound **26 sodium salt** (8.5 mg). ESI-MS: *m/z*= 705.8; ^{1}H NMR (400 MHz, D_2O) δ 8.50 (s, 1H), 8.18 (s, 1H), 7.78 (s, 1H), 6.35 (d, J=13.6 Hz, 1H), 5.94 - 5.80 (m, 2H), 5.33 - 5.10 (m, 1H), 4.45 - 4.35 (m, 2H), 4.30 - 4.13 (m, 4H), 4.09 - 4.01 (m, 2H), 3.51 (s, 3H); ^{19}F NMR (377MHz, D_2O) δ -200.69 (s, 1F); ^{31}P NMR (162MHz, D_2O) δ 53.89 (s, 1P), 6.60 (s, 1P).

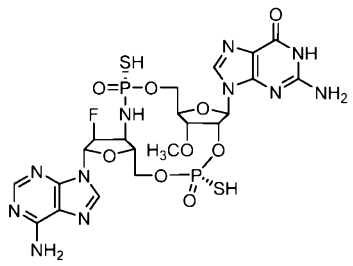
Step 8: preparation of **27 sodium salt**

A 50 mL volume of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 50.1 mg of **27**) and washed with deionized water (2x). To the resin was added 15% H_2SO_4 in deionized water (50 mL), the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to a column with 15% H_2SO_4 in deionized water and washed with

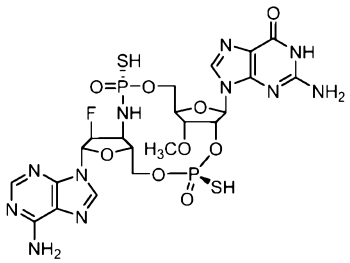
15% H_2SO_4 (at least 4 Column Volume (CV)), and then with deionized water until it was pH neutral. The resin was transferred back into the beaker, and a solution of 15% NaOH in deionized water (50 mL) was added, and the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to the column and washed with 15% NaOH in deionized water (at least 4 CV), and then with water until it was pH neutral (at least 4 CV). Compound **27** ammonium salt was dissolved in deionized water (50.1 mg in 50 mL), added to the top of the column, and eluted with deionized water. Compound **27** was eluted in early fractions as detected by TLC (UV). The product was lyophilized to give compound **27 sodium salt** (29 mg). ESI-MS: *m/z*= 705.8; ^{1}H NMR (400 MHz, D2O) δ 8.25 - 8.11 (m, 2H), 7.80 (s, 1H), 6.30 (d, J = 13.8 Hz, 1H), 5.85 - 5.73 (m, 2H), 5.33 - 5.12 (m, 1H), 4.51 - 4.42 (m, 2H), 4.33 - 4.26 (m, 2H), 4.22 - 4.11 (m, 2H), 4.09 - 4.00 (m, 2H), 3.50 (s, 3H); ^{19}F NMR (377MHz, D_2O) δ -200.70 (s, 1F); ^{31}P NMR (162MHz, D_2O) δ 51.87 (s, 1P), 6.53 (s, 1P).

Example 21

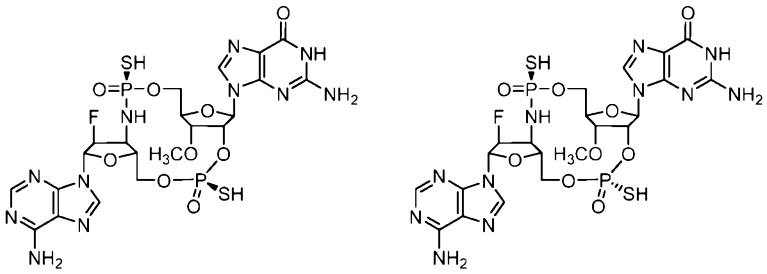
Compounds **28, 29, 30 and 31**



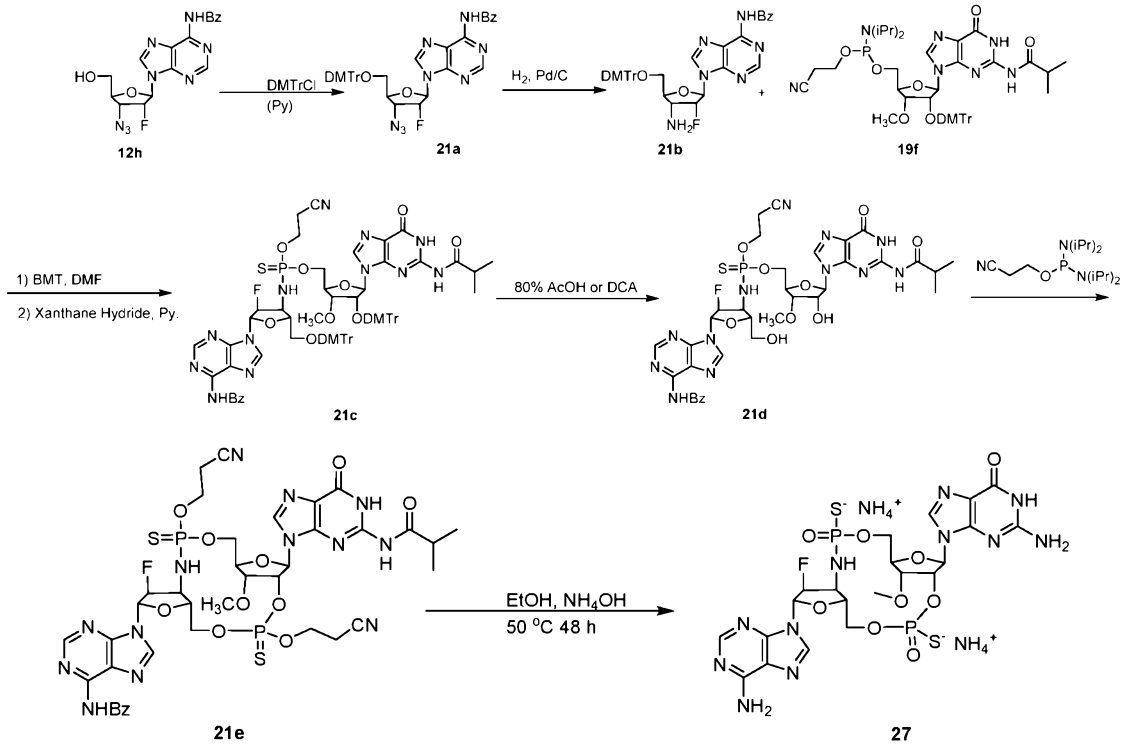
28,

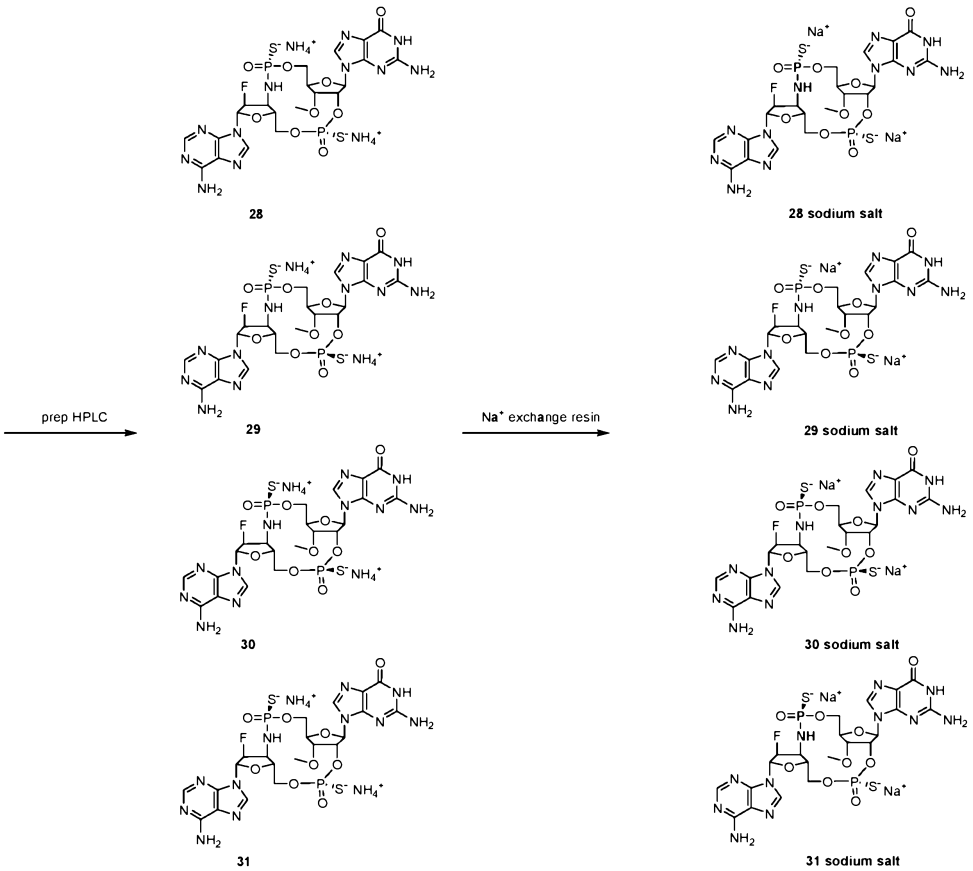


29,



30 and **31**


12h
DMTrCl
(Py)
21a
H2, Pd/C
21b
19f
1) BMT, DMF
2) Xanthane Hydride, Py.
21c
80% AcOH or DCA
21d
21e
EtOH, NH4OH
50 °C 48 h
27



Step 1: preparation of **21a**

To a solution of **12h** (500 mg,1.22 mmol) in pyridine (6 mL) was added DMTrCl (0.638 g, 1.88 mmol) at room temperature. After stirring the reaction mixture for 4 h at room temperature, it was diluted with CH_2Cl_2 (30 mL) and quenched with water. The reaction mixture was extracted with CH_2Cl_2 (20 mL), and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2: MeOH = 1: 0 to 10:1) to give **21a** as a yellow solid (933 mg). ^1H NMR (400MHz, CHLOROFORM-d) δ 9.10 (s, 1H), 8.82 - 8.75 (m, 1H), 8.78 (s, 1H), 8.66 - 8.57 (m, 1H), 8.23 (s, 1H), 8.04 (d, J=7.3 Hz, 2H), 7.72 -7.59 (m, 2H), 7.58 - 7.50 (m, 1H), 7.58 - 7.50 (m, 1H), 7.36 (d, J=7.0 Hz, 2H), 7.26 - 7.23 (m, 4H), 6.80 (d, J=8.3 Hz, 4H), 6.26 (dd,

J=1.6, 18.4 Hz,1H), 5.99 - 5.82 (m, 1H), 4.76 - 4.65 (m, 1H), 4.35 - 4.29 (m, 1H), 3.67 - 3.57 (m, 1H), 3.37 (dd, J=3.6, 11.2 Hz, 1H); ESI-MS: *m/z*=701.1 $[M+1]^+$.

Step 2: preparation of **21b**

A mixture of **21a** (933 mg, 1.105 mmol) with Pd/C (2.606 g, 2.210 mmol) as a catalyst in EtOAc (130 mL) was hydrogenated at room temperature (atmospheric pressure). After 2 h, the catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2: MeOH = 1: 0 to 10:1) to afford **21b** as a white solid (712 mg). ^1H NMR (400MHz, CHLOROFORM-d) δ 9.07 (s, 1H), 8.80 (s, 1H), 8.30 (s, 1H), 8.07 - 7.99 (m, 2H), 7.65 - 7.58 (m, 1H), 7.56 - 7.50 (m, 2H), 7.41 - 7.36 (m, 2H), 7.30 (d, J=1.2 Hz, 2H), 7.28 - 7.27 (m, 2H), 7.25 - 7.17 (m, 2H), 6.82 - 6.77 (m, 4H), 6.30 (d, J=18 Hz, 1H), 5.51 - 5.34 (m, 1H), 4.13 - 4.00 (m, 2H), 3.64 - 3.56 (m, 1H), 3.44 (dd, J=3.6, 10.8 Hz, 1H); ESI-MS: *m/z*=675.1 $[M+1]^+$.

Step 3: preparation of **21c**

A mixture of **21b** (300 mg, 0.445 mmol) and 5-Benzylmercaptotetrazole (BMT) (213.69 mg, 1.11 mmol) was azeotroped with acetonitrile three times and dissolved in anhydrous DMF (20 mL). A solution of **19f** (1.54 g, 1.78 mmol) in DMF (4 mL) was added dropwise at rt under N_2 and the reaction mixture was stirred at rt for 1 h. Xanthane hydride (133.60 mg, 0.89 mmol) and pyridine (140.68 mg, 1.78 mmol) were added to the reaction mixture. After stirring for 1 h at rt, saturated aqueous $NaHCO_3$ solution (20 mL) was added and the mixture was extracted with CH_2Cl_2 (50 mL). The organic layers were then combined and successively washed with saturated aqueous $NaHCO_3$ (30 mL), brine (50 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH = 1/0 to 10/1) to afford **21c** (510 mg) as a yellow oil. ESI-MS: *m/z*= 1174.3 $[M+1]^+$.

Step 4: preparation of **21d**

To a solution of **21c** (3.1 g) in water (476.03 mg, 26.42 mmol) and CH_2Cl_2 (100 mL) was added dichloroacetic acid (1.19 g, 9.248 mmol, 6% in DCM) at rt, followed by triethylsilane (20 mL). After stirring at rt for 24 h, pyridine (0.85 mL) was added. After stirring for 10 min, the mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2: MeOH = 1: 0 to10:1) to give **21d** as a yellow solid (1.247 g). ^{1}H NMR (400MHz, CD_3OD) δ 8.67 (d, J=2.4 Hz, 1H), 8.64 (d, J=4.2 Hz, 1H), 8.19 - 8.14 (m, 1H), 8.12 - 8.03 (m, 1H), 8.12 - 8.03 (m, 3H), 7.68 - 7.61 (m, 1H), 7.59 - 7.51 (m, 2H), 6.39 (dd, J=11.8, 18.0 Hz, 1H), 5.91 - 5.83 (m, 1H), 5.91 - 5.83 (m, 1H), 5.62 - 5.38 (m, 1H), 4.71 - 4.51 (m, 2H), 4.44-4.11 (m, 8H), 4.09 - 3.96 (m, 2H), 3.85 - 3.73 (m, 1H), 3.85 - 3.73 (m, 1H), 3.34 (s, 1H), 1.22 - 1.17 (m, 7H); ESI-MS: m/z= 1174.3 $[M+1]^+$.

Step 5: preparation of **21e**

Compound **21d** (370 mg, 0.425 mmol) was co-evaporated with a mixture of anhydrous toluene: acetonitrile (1:1, v/v, 3 × 10 mL). The resultant residue was then dissolved in CH_3CN/THF (35 mL, v/v = 7:3), followed by addition of 4Å molecular sieves (4 g) and tetrazole (7.55 mL, 0.45 M in CH_3CN). After stirring at 25 °C for 0.5 h, 3-((bis(diisopropylamino)-phosphino)oxy)propanenitrile (204.91 mg, 0.68 mmol) in CH_3CN (10 mL) was added to the above solution. The mixture was stirred at 25 °C for 2 h and additional tetrazole (1.88 mL, 0.45 M in CH_3CN) was added to above solution. After stirring the mixture at 25 °C for 0.5 h, DDTT (436.20 mg, 2.14 mmol) was added to the solution. After stirring the mixture at 25 °C for 1.5 h, the reaction mixture was filtered, concentrated under reduced pressure, and purified with a second batch by flash column chromatography on silica gel (CH_2Cl_2 : MeOH = 10:1) to give **21e** (436 mg).

Step 6: preparation of **28** to **31**

To a solution of **21e** (463 mg, 0.46 mmol) in EtOH (20 mL) was added $NH_3.H_2O$ (20 mL). After stirring the resulting solution at 50 °C for 2 days, the mixture was concentrated

under reduced pressure to afford compound **27** as a mixture of diastereoisomers. Compound **27** then was purified by reverse phase preparative HPLC (column: Agela Durashell C18 150 x 25 5μM; mobile phase: water (0.05% ammonia hydroxide v/v) - CH_3CN from 0 % to 13 %, flow rate: 35 ml/min) to afford a crude product which was re-purified by reverse phase preparative HPLC (column: Agela Durashell C18 150 x 25 5μM; mobile phase: water (0.05% ammonia hydroxide v/v)-ACN from 0 % to 10 %, flow rate: 35 ml/min) to afford compounds **28** (10.6 mg), **29** (13.6 mg), **30** (8.3 mg) and **31** (5.5 mg) as white solids (ammonium salts). LCMS for **28** to **31** - ESI-MS: *m/z*= 721.7 $[M+1]^+$.

Step 7: preparation of **28, sodium salt**

A 15 mL volume of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 10.6 mg of **15** ammonium salt) and washed with deionized water (2x). To the resin was added 15% H_2SO_4 in deionized water (50 mL), the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to a column with 15% H_2SO_4 in deionized water and washed with 15% H_2SO_4 (at least 4 Column Volume (CV)), and then with deionized water until it was pH neutral. The resin was transferred back into the beaker, and a solution of 15% NaOH in deionized water (50 mL) was added, and the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to the column and washed with 15% NaOH in deionized water (at least 4 CV), and then with water until it was pH neutral (at least 4 CV). Compound **15** ammonium salt was dissolved in deionized water (10.6 mg in 15 mL), added to the top of the column, and eluted with deionized water. The desired product was eluted in early fractions as detected by TLC (UV). The product was lyophilized to give **28 sodium salt** (9.2 mg) as a white solid. ESI-MS: *m/z*= 721.7 $[M+1]^+$; ^1H NMR (400 MHz, D_2O) 8.42 (s, 1H), 8.16 (s, 1H), 7.90 (s, 1H), 6.34 (d, *J* = 14.3 Hz, 1H), 5.83 (d, *J* = 8.8 Hz, 1H), 5.70 - 5.47 (m, 2H), 4.48 (br d, *J* = 2.3 Hz, 1H), 4.51 - 4.46 (m, 1H), 4.39 (br d, *J* = 11.8 Hz, 1H), 4.27 - 4.18 (m, 3H), 4.12 - 4.03 (m, 3H), 3.49 (s, 3H); ^{19}F NMR (376MHz, D_2O) -122.38 (br s, 1F), -199.72 (br s, 1F); ^{31}P NMR (162MHz, D_2O) 55.75 (br s, 1P), 53.63 (s, 1P).

Step 8: preparation of **29, sodium salt**

Compound **29**, sodium salt (12 mg) was prepared as a white solid following the same procedure as step 7 (preparation of **28**, sodium salt); ESI-MS: *m/z*= 721.7 $[M+1]^+$; ^1H NMR (400MHz, D_2O) 8.46 (br s, 1H), 8.17 (s, 1H), 7.77 (s, 1H), 6.32 (d, *J* = 13.8 Hz, 1H), 5.90 - 5.78 (m, 2H), 5.30 - 5.11 (m, 1H), 4.47 (br s, 1H), 4.43 - 4.35 (m, 1H), 4.32 - 4.18 (m, 4H), 4.08 - 3.95 (m, 2H), 3.49 (s, 3H); ^{19}F NMR (376MHz, D_2O) -122.77 (br s, 1F), -199.68 (br s, 1F); ^{31}P NMR (162MHz, D_2O) 56.01 (s, 1P), 53.31 (br s, 1P).

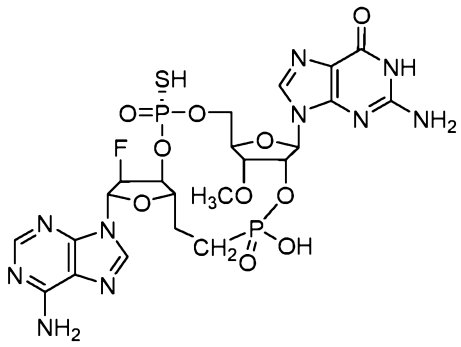
Step 9: preparation of **30, sodium salt**

Compound **30**, sodium salt (7.2 mg) was prepared as a white solid following the same procedure as step 7 (preparation of **28**, sodium salt). ESI-MS: *m/z*= 721.7 $[M+1]^+$; ^1H NMR (400MHz, D_2O) 8.17 (s, 1H), 8.08 (s, 1H), 7.97 (s, 1H), 6.34 - 6.26 (m, 1H), 5.82 (d, *J* = 8.8 Hz, 1H), 5.67 - 5.44 (m, 2H), 4.51 (br s, 1H), 4.46 - 4.38 (m, 1H), 4.34 (br d, *J* = 4.3 Hz, 1H), 4.24 (br d, *J* = 10.5 Hz, 1H), 4.16 - 3.99 (m, 4H), 3.47 (s, 3H); ^{19}F NMR (376MHz, D_2O) -122.35 (s, 1F), -199.36 (br s, 1F); ^{31}P NMR (162MHz, D_2O) 56.00 - 55.06 (m, 1P), 51.89 (s, 1P).

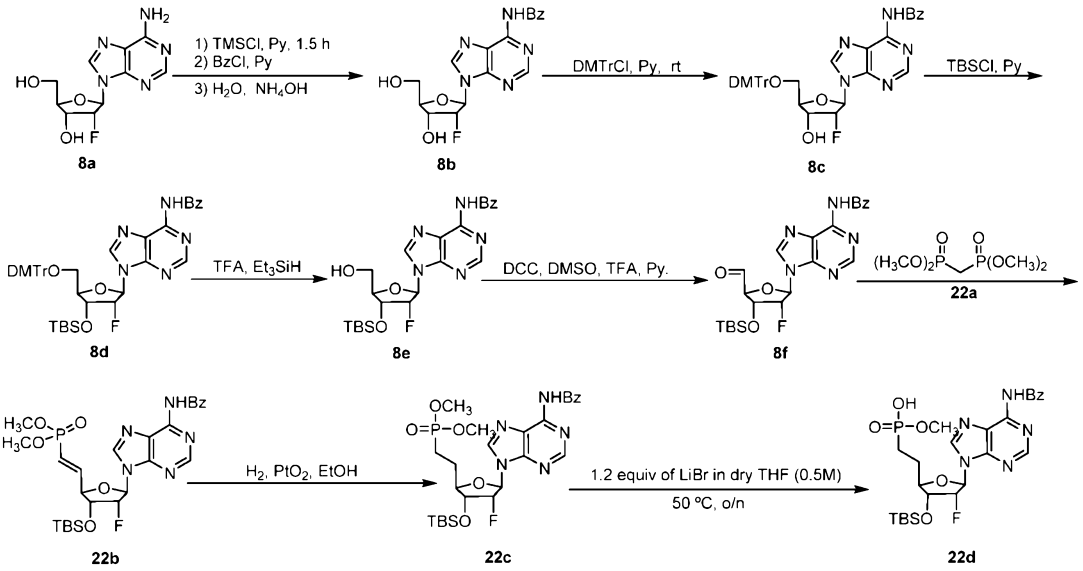
Step 10: preparation of **31, sodium salt**

Compound **31**, sodium salt (4.5 mg) was prepared as a white solid following the same procedure as step 7 (preparation of **28**, sodium salt). ESI-MS: *m/z*= 721.7 $[M+1]^+$; ^1H NMR (400 MHz, D_2O) 8.16 (d, *J* = 3.0 Hz, 2H), 7.77 (s, 1H), 6.29 (d, *J* = 13.8 Hz, 1H), 5.81 - 5.76 (m, 1H), 5.73 - 5.65 (m, 1H), 5.35 - 5.17 (m, 1H), 4.50 (br s, 1H), 4.43 (br d, *J* = 12.0 Hz, 1H), 4.35 - 4.22 (m, 4H), 4.08 (dd, *J* = 3.3, 11.8 Hz, 1H), 3.97 (dd, *J* = 2.4, 11.9 Hz, 1H), 3.46 (s, 3H); ^{19}F NMR (376 MHz, D_2O) -122.24 (s, 1F), -199.92 (s, 1F); ^{31}P NMR (162 MHz, D_2O) 55.73 (s, 1P), 51.80 (s, 1P).

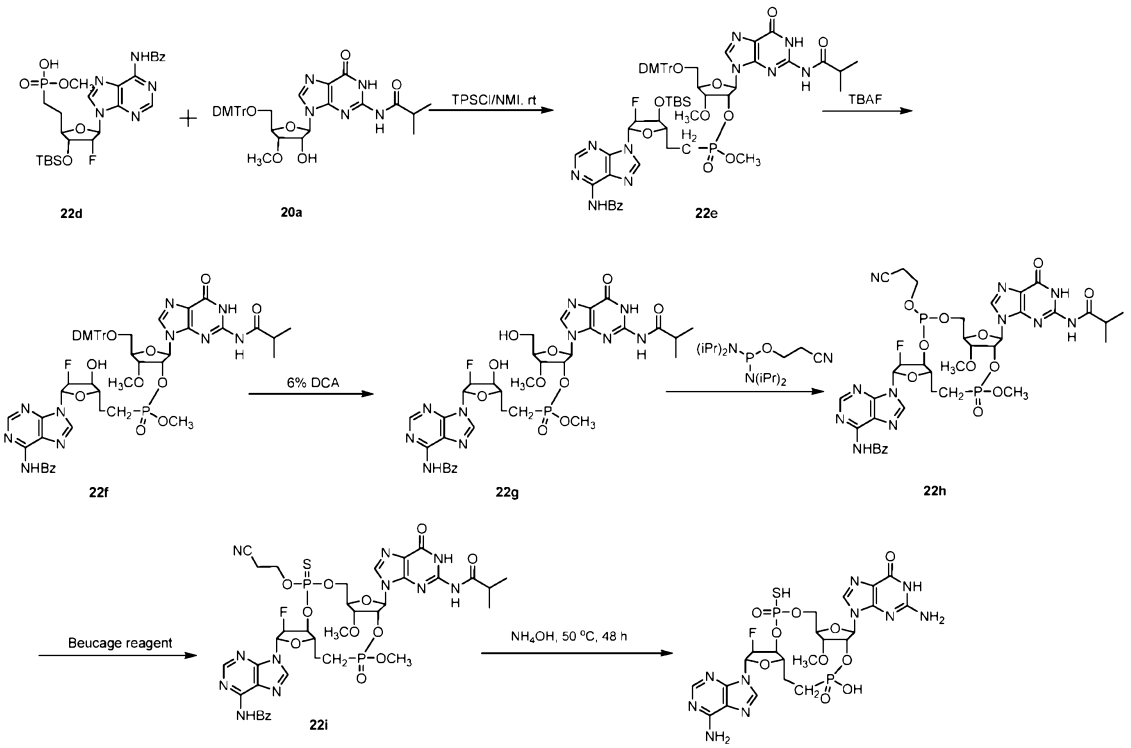
Example 22



Compound **32**



5



Compound **32**

Step 1: preparation of **Compound 8b**

To a solution of compound **8a** (30 g, 111.428 mmol) in pyridine (400 mL) was added drop-wise TMSCl (84.85 mL, 668.57 mmol) at rt. After stirring the mixture for 40 min, benzoyl chloride (46.99 g, 334.28 mmol) was added dropwise at rt. After stirring at rt overnight, the mixture was filtered and the filtrate quenched with water (120 mL) at 0 °C, followed by the addition of $NH_3.H_2O$ (120 mL), dropwise, at 0 °C. After stirring the mixture at 15 °C for 0.5 h, the mixture was concentrated under reduced pressure and the residue was diluted with ethyl acetate (600 mL). The solid was collected by filtration to afford **8b** (70 g) which was used directly for the next step without any further purification.

Step 2: preparation of **Compound 8c**

To a solution of compound **8b** (30 g, 80.35 mmol) in pyridine (250 mL) was added 4,4'-dimethoxytrityl chloride (54.45 g,160.71 mmol). After stirring at rt for 3 h, EtOAc (1 L) was added and the mixture was filtered. The organic layer was successively washed

with brine (300 mL x 3), dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH in DCM = 0% to 5%) to give compound **8c** (31.2 g) as a white solid. ESI-MS: m/z 676.3 $[M + H]^+$.

Step 3: preparation of **Compound 8d**

To a solution of compound **8c** (31.2 g,46.17 mmol) and 1H-imidazole (9.43 g, 138.52 mmol) in DMF (500 mL) was added tert-butylchlorodimethylsilane (13.92 g, 92.35 mmol) at rt under N_2. After stirring the reaction mixture at rt for 3 h, the mixture was quenched with water (1000 mL) and extracted with EtOAc (400 mL x 3). The combined organic layers were then successively dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated to afford the crude product as a yellow oil. The residue was purified by flash column chromatography on silica gel (MeOH in DCM = 0% to 5%) to give **8d** (29 g) as a yellow solid. ESI-MS: m/z 790.4 $[M + H]^+$.

Step 4: preparation of **Compound 8e**

To a solution of compound **8d** (29 g, 36.71 mmol) in DCM (160 mL) was added DCM (320 mL) followed by TFA (8 mL) and Et_3SiH (32 mL) at 0 °C. After stirring for 0.5 h at 0 °C and then at 25 °C for 4 h, the reaction mixture was concentrated under reduced pressure. The resultant residue was purified by flash column chromatography on silica gel (MeOH in DCM = 0% to 5%) to give **8e** (11.5 g, 21.60 mmol) as a white solid. ESI-MS: m/z 488.2 $[M + H]^+$.

Step 5: preparation of **Compound 8f**

To a solution of compound **8e** (11.5 g, 23.58 mmol) and 1,3-dicyclohexylcarbodiimide (19.46 g, 94.34 mmol) in DMSO (80 mL) was added pyridine (2.65 g, 33.49 mmol) and trifluoroacetic (2.01 g, 17.69 mmol) at 25 °C. After stirring the mixture at 25 °C for 17 h, the reaction mixture was diluted with EtOAc (400 mL) and water (200 mL). The organic layer was successively washed with brine (100 mL x 2), dried over anhydrous

Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give **8f** (11.45 g, 23.58 mmol) which was used for the next step without any further purification. ESI-MS: m/z 518.1 $[M + 33]^+$.

Step 6: preparation of **Compound 22b**

To a solution of compound **22a** (6.39 g, 27.52 mmol) in THF (75 mL) was added 60% NaH (1.43 g, 35.78 mmol) at 0 °C. After stirring the mixture at 0 °C for 0.5 h, a solution of compound **8f** (11.45 g, 23.58 mmol) in THF (75 mL) was added dropwise at 0 °C. After stirring 0 °C for 1 h and then at 25 °C for 2 h, the reaction mixture was diluted with aqueous saturated NH_4Cl (100 mL) and extracted with EtOAc (150 mL x 2). The organic layers were then combined and successively washed with brine (130 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH in DCM=0% to 5%) to give **22b** (6.1 g) as a yellow solid. ESI-MS: m/z 592.1 $[M + H]^+$.

Step 7: preparation of **Compound 22c**

A solution of compound **22b** (1.1 g, 1.86 mmol) and platinum (IV) oxide (0.33 g, 1.48 mmol) in EtOH (30 ml) was stirred at rt overnight under a hydrogen atmosphere (15 Psi). The mixture was then filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM/MeOH = 20/1) to give **22c** (0.8 g) as a yellow solid. ESI-MS: m/z 594.1 $[M + H]^+$.

Step 8: preparation of **Compound 22d**

LiBr solution in THF (30.32 mL, 0.5 M in THF) was added to **22c** (3 g, 5.05 mmol) at 25 °C. After stirring the mixture at 50 °C for 48 h, CH_3CN (30 mL), water (60mL) and 1 M aqueous HCl (10 mL) were added. The mixture was then lyophilized. The residue was purified by flash column chromatography on silica gel (MeOH in DCM=0% to 15%) to give **22d** (2.4 g) as a yellow solid. ESI-MS: m/z=580.1 $[M+1]^+$.

Step 9: preparation of Compound 22e

A mixture of compound **22d** (1.8 g, 3.105 mmol) and compound **20a** (2.49 g, 3.72 mmol) was azeotroped with 30 mL of dry pyridine (3x). The resulting mixture was diluted with anhydrous DCM/THF (80 mL,1:1,v/v) followed by the addition of 4Å molecular sieves (200 mg), NMI (3.06 g, 37.26 mmol) and TPSCl (2.82 g, 9.31 mmol). The resulting solution was stirred at 25 °C for 48 h under a nitrogen atmosphere. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (100 mL) and filtered. The filtrate was extracted with DCM (80 mL x 3); organic layers were then combined, dried over anhydrous $MgSO_4$, filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH in DCM=0% to 10%) to give crude product as a yellow solid (3 g). The crude product was purified by reverse phase preparative HPLC (Column: Phenomenex Gemini 250 x 50mm x 10μm; Condition: H_2O (10 mM NH_4HCO_3) (A) - CH3CN (B); Begin B:55; End B: 85; Flow Rate: 110 mL/min) to give **22e** (1.01 g) as a white solid. ESI-MS: m/z 1231.7 [M + H]+. ^1H NMR (CD_3CNd3, 400MHz) δ 11.73-12.00 (m, 1H), 9.69 (br s, 1H), 9.34 (br s, 1H), 8.53 (br s, 1H), 8.10 (s, 1H), 7.81-7.94 (m, 2H), 7.70 (d, J=9.3 Hz, 1H), 7.36-7.53 (m, 3H), 7.24 (br d, J=7.7 Hz, 2H), 7.01-7.15 (m, 7H), 6.60-6.69 (m, 4H), 5.97-6.16 (m, 1H), 5.79-5.88 (m, 1H), 5.24-5.56 (m, 2H), 4.46-4.68 (m, 1H), 3.99-4.11 (m, 2H), 3.81-3.97 (m, 1H), 3.59 (s, 6H), 3.39 (s, 1H), 3.34-3.37 (m, 1H), 3.36 (s, 1H), 3.27 (s, 1H), 3.17 (s, 3H), 2.36-2.55 (m, 1H), 0.91-1.01 (m,6H),0.77-0.82 (m, 9H), -0.12-0.08 ppm (m, 6H).

Step 10: preparation of **Compound 22f**

To a solution of compound **22e** (400 mg, 0.325 mmol) in THF (4 mL) was added 1M TBAF in THF (0.975 mL, 0.975 mmol) at 25 °C. The mixture was stirred at 25 °C for 2 h and then diluted with ethyl acetate (20 mL) and water (15 mL). The mixture was extracted with ethyl acetate (20 mL x 2). The organic layers were then combined and successively washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column

chromatography on silica gel (MeOH in DCM=0% to 5%) to afford **22f** (325 mg) as a yellow solid. ESI-MS: m/z 1117.3 $[M + H]^+$.

Step 11: preparation of **Compound 22g**

To a solution of Compound **22f** (325 mg, 0.29 mmol) in DCM (4 mL) was added water (52.37 mg, 2.93 mmol) and 6% DCA in DCM (4 mL). The mixture was stirred at 25 °C for 1 h. To the mixture was then added pyridine until the red reaction mixture turned colorless. The reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH in DCM=0% to 15%) to give **22g** (180 mg) as a white solid. ESI-MS: m/z 815.1 $[M + H]^+$.

Step 12: preparation of **Compound 22i**

To a solution of Compound **22g** (180 mg, 0.22 mmol) in CH_3CN/THF (21.6 mL, v/v=1:1) was added 4Å molecular sieve (500 mg) and tetrazole (3.96 mL, 0.45 M in CH_3CN). After stirring the reaction mixture at 25 °C for 0.5 h, 3-((bis(diisopropylamino)-phosphanyl)oxy)propanenitrile (133.18 mg, 0.44 mmol) in CH_3CN (1.8 mL) was added dropwise. After stirring the mixture at 25 °C for 2 h, additional tetrazole (1 mL, 0.45 M in CH_3CN) was added to the above solution. The mixture was stirred at 25 °C for 0.5 h to give compound **22h**. Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-Dioxide, 221.19 mg,1.105 mmol) was then added. The mixture was stirred at 25 °C for 0.5 h and then filtered. The filtrate was concentrated under reduced pressure to give **22i** (250 mg crude) ESI-MS: m/z 946.3 $[M + H]^+$.

Step 13: preparation of **Compound 32**

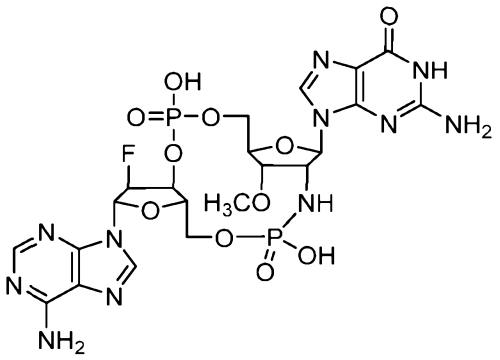
To a solution of Compound **22i** (80 mg, 0.085 mmol) in EtOH (5mL) was added $NH_3.H_2O$ (5 mL). After stirring the mixture at 50 °C for 48 h, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by reverse phase preparative HPLC (column: Synergi 4μM, Hydro RP, 250 mm × 30 mm, Mobile Phase: Buffer A: 50 mM Triethylammonium acetate in H_2O; Buffer B: 50 mM

Triethylammonium acetate in CH_3CN, gradient: 0-30% of B over 30 min, flow rate 24 mL/min) and followed by second purification by reverse phase preparative HPLC (Kinetex 5 µm, 100 A; 250 x 21.2 mm Buffer A: 0.1% Formic acid in H_2O Buffer B: 0.1% Formic acid in MeCN; Flow rate : 15 mL/min, gradient 0-30% of Buffer B in 30 min) to afford compound **32** (6.9 mg) as a white solid. ESI-MS: m/z: 703.00 [M-1]-. ESI-MS: m/z 705.10 $[M + H]^+$.

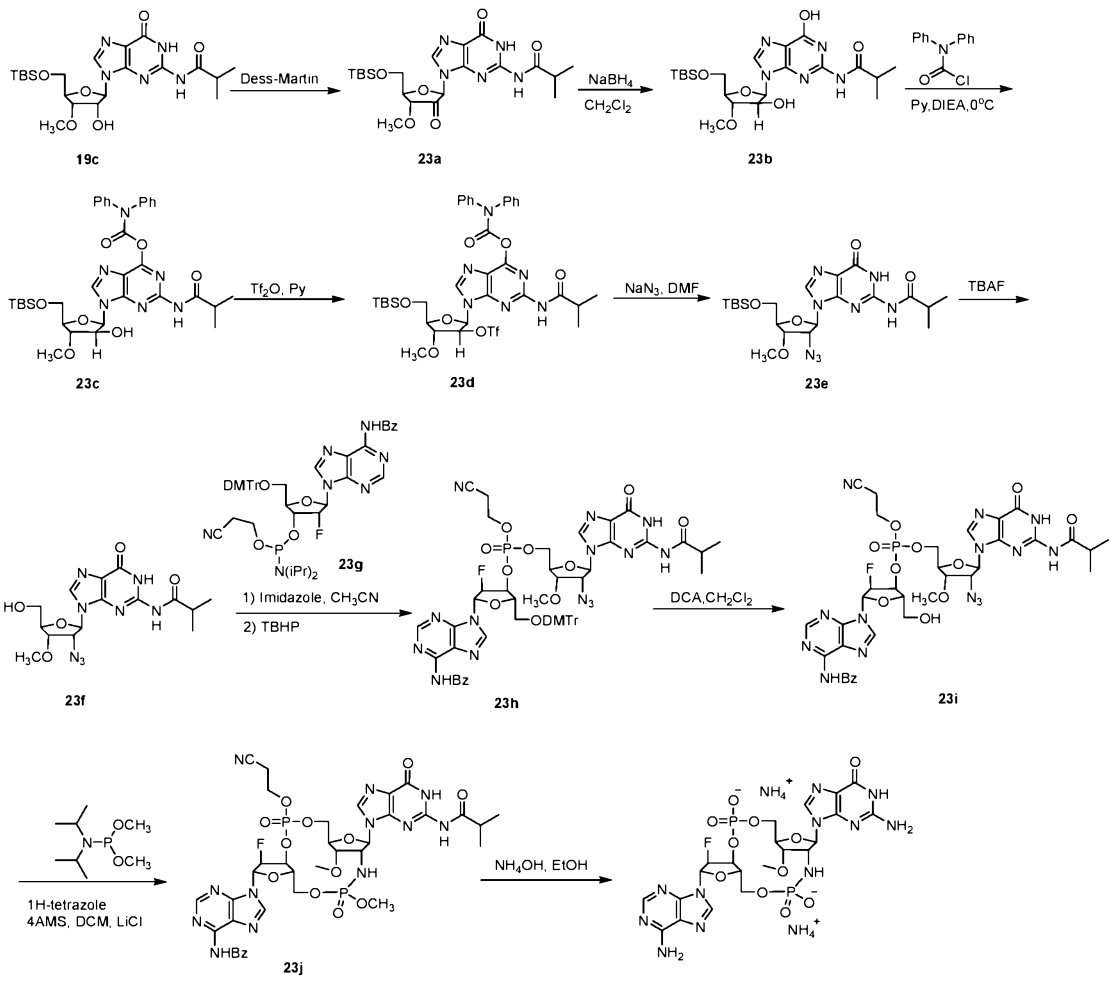
Preparation of **compound 32, sodium salt**

Dowex 50W × 8, 200-400 (5 mL, H form) was added to a beaker and washed with de-ionized water (30 mL). To the resin was added 15% H_2SO_4 in deionized water, and the mixture was gently stirred for 5 min, then decanted (30 mL). The resin was transferred to a column with 15% H_2SO_4 in deionized water and washed with 15% H_2SO_4 (at least 4 Column Volume), and then washed with deionized water until it was neutral. The resin was returned to the beaker, 15% NaOH in deionized water solution was added, and the mixture was gently stirred for 5 min, and decanted (1x). The resin was transferred to the column and washed with 15% NaOH in water (at least 4 Column Volume), and then with deionized water until it was neutral. Compound **32** triethylammonium salt (6.9 mg) was dissolved in a minimum amount of deionized water, added to the top of the column, and eluted with deionized water. Fractions were pooled and lyophilized to afford compound **32** sodium salt (6.1 mg) as a white solid (contains around 20% or impurity or other isomer with same molecular weight - Analytical data of the Major Product). ^1H NMR (400 MHz, D_2O) δ 8.08 (s, 1H), 8.00 (s, 1 H), 7.92 (s, 1H), 6.22 (d, 1H), 5.79-5.85 (m, 1 H), 5.53-5.70 (m, 1 H), 4.73-5.13 (m, 3H), 4.45- 4.50 (m, 1H), 4.21-4.35 (m, 1 H), 3.40-4.19 (m, 4H), 3.45 (s, 3H), 1.78-1.90 (m, 2H), 1.60-1.70 (m, 2H); ^{31}P NMR (162 MHz, D_2O) δ 54.66 (PS peak), 25.13 (1.0, Phosphonate); ^{19}F NMR (379 MHz, D_2O) δ -199.48 (m); ESI-MS: m/z: ESI-MS:703.00 [M-1]-. ESI-MS: m/z 705.10 $[M + H]^+$.

Example 23



Compound **33**



Compound **33**

Step 1: preparation of **23a**

To a solution of compound **19c** (1 g, 2.02 mmol) in DCM (20 mL) was added Dess-Martin Periodinane (1.45 g, 3.43 mmol) under N_2. After stirring the reaction mixture at room temperature for 16 h, saturated aqueous $NaHCO_3$ (20 mL) containing $Na_2S_2O_3$ (3 g) was added and the mixture stirred for 0.5 h. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ (20 mL). The organic layers were then combined, dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (gradient eluent:

EtOAc/petroleum ether from 0/100 to 1/0) to give **23a** (1 g) as a white solid. ESI-MS: m/z=498.1 $[M+H_2O]^+$

Step 2: preparation of **23b**

To a 0 °C pre-cooled solution of **23a** in ethanol (10 mL) was added $NaBH_4$ (0.14 g, 3.63 mmol). The reaction mixture was stirred at 0 °C for 10 min then at rt for 30 min and then diluted with EtOAc (20 mL) and brine (10 mL). The aqueous layer was extracted with EtOAc (10 mL). The organic layers were then combined, dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (gradient eluent: EtOAc/petroleum ether from 0/100 to 1/1) to give **23b** (300 mg) and recovered **23a** (200 mg) as white solids. ^1H NMR (400 MHz, DMSO-d6) δ ppm 12.07 (s, 1 H) 11.71 (s, 1 H) 8.00 (s, 1 H) 6.06 (d, J=4.85 Hz, 1 H) 5.85 (d, J=5.29 Hz, 1 H) 4.24 - 4.34 (m, 1H) 3.76 - 3.93 (m, 4 H) 3.40 (s, 3 H) 2.76 (quin, J=6.84 Hz, 1 H) 1.12 (d, J=6.84 Hz, 6 H) 0.90 (s, 9 H) 0.04 - 0.13 (m, 6 H); ESI-MS: m/z=482.1 $[M+1]^+$.

Step 3: preparation of **23c**

To a solution of compound **23b** (120 mg, 0.22 mmol) in pyridine (4 mL) and DIEA (87.96 mg, 0.68 mmol) was added diphenylcarbamyl chloride (105.11 mg,0.45 mmol). After stirring at 25 °C for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (gradient eluent: EtOAc/petroleum ether from 0/100 to 1/0) to give **23c** (110 mg) as a white solid. ^1H NMR (400 MHz, $CDCl_3$-d) δ ppm 8.32 (s, 1 H) 7.96 (s, 1 H) 7.31 - 7.51 (m, 8 H) 7.25 (br s, 2 H) 6.21 (d, J=3.01 Hz, 1 H) 4.90 (d, J=9.54 Hz, 1 H) 4.37 (br d, J=9.03 Hz, 1 H) 4.14 (d, J=2.26 Hz, 1 H) 3.98 (s, 1 H) 3.94 (d, J=3.01 Hz, 1 H) 3.92 - 3.99 (m, 1 H) 3.82 (dd, J=11.17, 2.38 Hz,1 H) 3.50 (s, 3 H) 2.93 (br s, 1 H) 1.27 (d, J=6.78 Hz, 6 H) 0.93 (s, 9 H) 0.14 (s, 6 H); ESI-MS: m/z=699.6 $[M+Na]^+$.

Step 4: preparation of **23d**

To a solution of compound **23c** (200 mg, 0.28 mmol) in pyridine (5 mL) was added Tf_2O (606.11 mg, 2.14 mmol) at 0 °C. After stirring at 0-5 °C for 3 h, the reaction mixture was diluted with DCM (10 mL) and washed with brine (5 mL x 1). The phases were separated and the aqueous layer extracted with DCM (10 mL x 2). The organic layers were then combined, dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (gradient eluent: EtOAc/petroleum ether from 0/100 to 1/1) to give **23d** (250 mg) as white solid. ESI-MS: m/z=831.2 $[M+Na]^+$

Step 5: preparation of **23e**

To a solution of compound **23d** (610 mg, 0.75 mmol) in DMF (15 mL) was added sodium azide (814 mg, 7.06 mmol). After stirring at 25 °C for 16 hours, the mixture was diluted with DCM (100 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL). Organic layers were then combined, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The oily residue was purified by flash column chromatography on silica gel (gradient eluent: EtOAc/petroleum ether from 0/100 to 1/00 to give **23e** (500 mg) as a white solid. ^1H NMR (400 MHz, DMSO-d6) δ ppm 12.12 (s, 1 H) 11.68 (s, 1 H) 8.21 (s, 1 H) 5.91 (d, J=4.63 Hz, 1 H) 4.87 (t, J=4.96 Hz, 1 H) 4.26 (br t, J=5.07 Hz, 1 H) 4.08 (br d, J=4.41 Hz, 1 H) 3.86 (br dd, J=11.47, 4.19 Hz, 1 H) 3.71 - 3.81 (m, 1 H) 3.46 (s, 3 H) 2.77 - 2.82 (m, 1 H) 1.12 (d, J=6.84 Hz, 6H) 0.87 (s, 9 H) 0.06 (s, 6 H); ESI-MS: m/z=507.2 $[M+1]^+$.

Step 6: preparation of **23f**

To a solution of compound **23e** (450 mg, 0.84 mmol) in THF (10 mL) was added TBAF (1.52 mL, 1.52 mmol, 1 M in THF) at 0 °C. After stirring at room temperature for 4 h, the mixture was concentrated under reduced pressure to give an oil. The oil was dissolved in DMCM (50 mL) and washed with brine (20 mL). The phases were separated and the aqueous phase extracted with CDM (20 mL x 2). The organic layers were then

combined, dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (gradient eluent: DCM/MeOH from 1/0 to 100/7) to give **23f** (220 mg) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$-d) δ ppm 12.13 (br s, 1 H) 8.57 (br s, 1 H) 7.81 (s, 1 H) 5.84 (d, J=7.72 Hz, 1 H) 5.08 (br s, 1 H) 4.59 (dd, J=7.50, 5.29 Hz, 1 H) 4.33 (d, J=1.76 Hz, 1 H) 4.20 (dd, J=5.29, 1.98 Hz, 1 H) 4.03 (dd, J=12.46, 2.09 Hz, 1 H) 3.76 (br s, 1 H) 3.56 (s, 3 H) 2.61 - 2.76 (m,1 H) 1.27 - 1.35 (m, 6 H); ESI-MS: m/z=415.0 $[M+Na]^+$

Step 7: preparation of **23h**

A solution of compound **23f** (220 mg, 0.56 mmol) and 4Å molecular sieves (3 g) in CH_3CN (18 mL) was stirred at room temperature under a N_2 atmosphere for 3 min. 1H-tetrazole (7.48 mL, 3.36 mmol, 0.45M in CH_3CN) was added dropwise. After stirring for 10 min, a solution of compound **23g** in CH_3CN (4 mL) was added dropwise. After stirring the mixture at room temperature for 1 h, *tert*-butylhydroperoxide (0.56 mL, 2.80 mmol, 5M in decane) was added. After stirring the mixture at room temperature for 1 h, the mixture was filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (gradient eluent: DCM:MeOH = 1: 0 to 10: 1) to give **23h** (950 mg, crude product) as a white solid which was used directly for the next step without any further purification. ESI-MS: m/z=1183.5 $[M+1]^+$

Step 8: preparation of **23i**

To a solution of compound **23h** (950 mg, crude product) in DCM (15 mL) and water (68.83 mg, 3.82 mmol) was added dichloroacetic acid (172.57 mg,1.33 mmol) at room temperature followed by the addition of triethylsilane (3.5 mL). After stirring at room temperature for 2 h, pyridine (121 mg, 1.53 mmol) was added and the mixture was stirred at room temperature for 10 min, then concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (gradient eluent: DCM:MeOH = 1: 0 to 10: 1) to give **23i** (570 mg) as a white solid. ^{1}H NMR (400 MHz, CD_3OD-d4) δ ppm 8.57 - 8.74 (m, 2 H) 8.04 - 8.16 (m, 3 H) 7.66 (d, J=6.61 Hz, 1 H) 7.53 - 7.61 (m, 2 H)

6.38 - 6.53 (m, 1H) 5.90 - 6.06 (m, 1 H) 4.53 (br d, J=5.51 Hz, 2 H) 4.40 - 4.46 (m, 1 H) 4.31 - 4.40 (m, 4 H) 3.92 (br d, J=13.23 Hz, 1 H) 3.73 - 3.83 (m, 1 H) 3.58(d, J=11.69 Hz, 3 H) 2.70 - 2.79 (m, 1 H) 1.21 (dd, J=6.84, 2.43 Hz, 6 H); ESI-MS: m/z=881.4 $[M+1]^+$

Step 9: preparation of **23j**

To a solution of compound **23i** (470 mg,0.39 mmol), 1H-tetrazole (0.66 mL,0.3 mmol, 0.45M in CH_3CN), LiCl (84.23 mg,1.98 mmol) and 4Å molecular sieves (2 g) in DCM (60 mL) was added dimethyl N,N-diisopropylphosphoramidite (94.50 mg, 0.48 mmol). The reaction mixture was stirred for 72 h at room temperature, filtered and concentrated under reduced pressure to give **23j** (400 mg, crude product) as a white solid which was used directly for the next step without any further purification. ESI-MS: m/z=931.1 $[M+1]^+$

Step 10: preparation of **compound 33**

To a solution of compound **23j** (400 mg, crude product) in EtOH (15 mL) was added $NH_3.H_2O$ (15 mL) at room temperature. After stirring at 50 °C for 3 days, the reaction mixture was filtered and the filtrate concentrated under reduced pressure to dryness. The residue was purified by reverse phase preparative HPLC (column: Synergi 4μM, Hydro RP, 250 mm × 30 mm, Mobile Phase: Buffer A: 50 mM Triethylammonium acetate in H_2O; Buffer B: 50 mM Triethylammonium acetate in CH_3CN, gradient: 0-30% of B over 30 min, flow rate 24 mL/min) to give compound **33** (24.5 mg). Compound **33** was further purified by reverse phase preparative HPLC (Synergi 4μM, Hydro RP, 250 mm × 30 mm, Buffer A: 0.1% Formic acid in H_2O Buffer B: 0.1% Formic acid in MeCN; Flow rate: 15 mL/min, gradient 0-30% of Buffer B in 30 min) to afford compound **33** triethylammonium salt (16.3 mg).

Sodium Salt exchange of compound 33:

Dowex 50W × 8, 200-400 (5 mL, H form) was added to a beaker and washed with de-ionized water (30 mL). To the resin was added 15% H_2SO_4 in deionized water, the mixture was gently stirred for 5 min, and decanted (30 mL). The resin was transferred to a

column with 15% H_2SO_4 in deionized water and washed with 15% H_2SO_4 (at least 4 Column Volume), and then with deionized water until the resin was neutral. The resin was transferred back into the beaker, 15% NaOH in deionized water solution was added, and the mixture was gently stirred for 5 min, then decanted. The resin was transferred to the column and washed with 15% NaOH in water (at least 4 Column Volume), and then with deionized water until it was neutral. Compound **33** triethylammonium salt (16.3 mg) was dissolved in a minimum amount of deionized water, added to the top of the column, and eluted with deionized water. Fractions were pooled and lyophilized to afford compound **33** sodium salt (15.4 mg) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ ppm 8.21 (s, 1H), 8.15 (s, 1H), 7.66 (s, 1H), 6.22 (d, 1H), 5.49 (d, 1H), 5.20-5.36 (d, 1H), 4.88 (d, 1H),4.30-4.37 (m, 3H), 4.06-4.12 (m, 2H), 3.80-3.90 (m, 2H), 3.42 (s, 3H); ^{31}P NMR (162 MHz, D2O): δ 4.23 (amidate), -1.32 (phosphate); ^{19}F NMR (379 MHz, D2O): δ -201.958 (m); ESI-MS: m/z: ESI-MS: m/z: 687.70 [M-1]-. ESI-MS: m/z 690.10 [M + H] $^+$.

Biological Examples

In Vitro Assays

STING SPA binding assay

The human STING SPA binding assay measures displacement of tritium labeled 2',3'cGAMP (cyclic (guanosine-(2' –> 5')-monophosphate-adenosine-(3' –> 5')-monophosphate) to biotinylated STING protein. A soluble version of recombinant STING was expressed in E.coli that lacks the four transmembrane domains and contains residues 139-379 of Q86WV6 with an R at position 232 (H232R). Based on the allele frequency of 58% of the population, H232R is considered to be a wild type (Yi, et. al., "Single Nucleotide Polymorphisms of Human STING can affect innate immune response to cyclic dinucleotides" PLOS ONE. 2013, 8(10), e77846). The STING construct has an N-terminal HIS tag, followed by a TEV protease cleavage site and an AVI tag to allow directed biotinylation by BirA biotin ligase (Beckett et al., A minimal peptide substrate in biotin

holoenzyme synthetase-catalyzed biotinylation. (1999) Protein Science 8, 921-929). The HIS tag is cleaved after purification and prior to biotinylation.

The assay was run in 1536-well plates in a total volume of 8 µL per well by adding 8 nM [^{3}H]-2'3'-cGAMP and 40 nM biotin-STING protein in assay buffer [25mM HEPES (Corning 25-060-C1) pH 7.5, 150 mM NaCl (Sigma S5150), 0.5 mg/mL BSA (Gibco 15260-037), 0.001% Tween-20 (Sigma P7949), molecular grade water (Corning 46-000-CM)]. Test compounds (80 nL) were added with an acoustic dispenser (EDC Biosystems) in 100% DMSO for a final assay concentration of 1% DMSO. Plates were centrifuged for 1 min and incubated for 60 min at room temperature. Finally, (2 µL) polystyrene streptavidin SPA beads (PerkinElmer RPNQ0306) were added and plates were sealed and centrifuged for 1 min at room temperature. Plates were dark adapted for 2 h and read on a ViewLux (Perkin Elmer) for 12 min per plate. A saturation binding curve for [^{3}H]-2'3'-cGAMP showed a K_D of 3.6 ± 0.3 nM for binding to STING, comparable to reported values for the natural ligand (Zhang et al., Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING.

Other natural ligands including cyclic-di-GMP also returned values in this assay within the expected range. Reference compound is cGAMP and results are reported as percent inhibition and IC_{50} values. Binding to mouse STING used a construct similar to the one described above containing residues 138-378 of Q3TBT3.

Full length human STING binding assay

Human STING from residues 1-379 of Q86WV6 with an R at position 232 (H232R) with an N-terminal 6HIS tag followed by a FLAG tag, a TEV protease cleavage site and an AVI tag for biotinylation was recombinantly expressed in HEK293-EXPI cells. Purified membranes were prepared from these cells and STING expression was confirmed and quantified by immunoblot. STING containing membranes were combined with test compound in a Greiner 384-well assay plate and incubated at room temperature for one hour in the same assay buffer used for the STING SPA binding assay. Next, [^{3}H]-2'3'-cGAMP was added and plates were incubated for 30 min at room temperature. Reactions

were transferred to a prewashed Pall 5073 filter plate and each well was washed 3 times with 50 µL assay buffer. Filter plates were dried at 50 °C for 1 h. To each well, 10 µL of Microscint scintillation fluid was added and plates were sealed and read on a TopCount (Perkin Elmer) for 1 min per well.

STING SPR binding assay

Compounds were analyzed on an S200 biacore SPR instrument (GE Healthcare). E.coli produced truncated STING protein was immobilized on a series S streptavidin chip via biotin capture (GE Healthcare #BR100531) with. Compounds were screened at 1:2 dilutions from 100 uM to 0.195 uM in run buffer (10mM HEPES, pH 7.4, 150mM NaCl, 0.005% P20, 1mM TECEP). Steady state affinity and kinetic evaluations were carried out using 1:1 binding model (STING was treated as a dimer). Run parameters were as follows: 60 sec on, 300 sec off for the IFM compounds, cyclic-di-GMP (60sec on/60sec off), thiol isomer 1 (60 sec on/300 sec off) and cGAMP (60sec on/1200sec off) with a flow rate of 50µL/min and data collection at 40 Hz at 25 °C.

STING human cell reporter assay

Agonism of the human STING pathway is assessed in THP1-ISG cells (Invivogen, cat #thp-isg) derived from human THP1 monocyte cell line by stable integration of an interferon regulatory factor (IRF)-inducible SEAP reporter construct. THP1-Blue ISG cells express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of an ISG54 minimal promoter in conjunction with five interferon (IFN)-stimulated response elements. As a result, THP1-Blue ISG cells allow the monitoring of IRF activation by determining the activity of SEAP. The levels of IRF-induced SEAP in the cell culture supernatant are readily assessed with alkaline phosphatase detection medium, a SEAP detection reagent. These cells are resistant to Zeocin. 2'3'cGAMP was used as a positive control in this assay. To run the assay, 60,000 cells were dispensed in 30 µL/well of a white, opaque bottom tissue culture treated 384-well plate.

Test compounds were added in a volume of 10 µL (1% DMSO final concentration). Compounds are initially prepared in 100% DMSO, spotted on an intermediate dilution plate and then diluted in media prior to transfer. The assay was incubated for 24 h at 37 °C, 5% CO_2 then plates were centrifuged at 1200 rpm (120x g) for 5 min. After final incubation, 90 µL of alkaline phosphatase detection medium-substrate was added to each well of a new 384-well clear plate and 10 µL of the cell supernatant was transferred from the assay plate to the new alkaline phosphatase detection medium-plate using a Biomek FX and mixed 4 times. Plates were incubated at RT for 20 min then absorbance at 655 nm was determined on the Tecan Safire2.

STING mouse cell reporter assay

Agonism of the mouse STING pathway is assessed in RAW Lucia cells (Invivogen,cat # rawl-isg) derived from mouse RAW-264.7 macrophage cell line by stable integration of an interferon-inducible Lucia luciferase reporter construct. RAW Lucia cells express a secreted luciferase reporter gene under the control of an ISG54 minimal promoter in conjunction with five interferon (IFN)-stimulated response elements. As a result, RAW Lucia cells allow the monitoring of IRF activation by determining the activity of luciferase. The levels of IRF-induced luciferase in the cell culture supernatant are readily assessed with QUANTI-Luc™, a luciferase detection reagent. These cells are resistant to Zeocin. 2'3'cGAMP is used as a positive control in this assay. To run the assay, 100,000 cells were dispensed in 90µL/well of a clear, flat bottom tissue culture treated 96-well plate. Test compounds were added in a volume of 10µL. The assay was incubated for 24 and 48 hours at 37ºC, 5% CO2. After incubation, 20µL of the cell supernatant from the assay plate was transferred to a new 96-well white plate and 50uL of QUANTI-Luc substrate was added. The plate was incubated, shaking, at RT for 5 minutes then luminescence was read on an EnVision 2104 with 0.1s integration time.

Human interferon-β induction assay

THP1-Blue ISG cells are used to measure the secretion of IFN-β into the culture supernatant following STING pathway activation. To run the assay, anti-IFN-β capture antibodies were coated on 96 well MultiArray plates (Mesoscale Discovery). After a one hour incubation, plates were washed and 50 µL supernatant from the STING human cell reporter assay plates or IFN-β standards were mixed with 20 µL Sulfotag-conjugated detection antibody in the coated plates. Plates were incubated, shaking for 2 h, washed, and read buffer was applied. Electrochemiluminescence was measured on the SectorImager.

STING cell signaling pathway assessment

Agonism of the STING pathway was measured in THP1 BLUE ISG cells by western blot of phospho-STING(S366), phospho-TBK1(S172) and phospho-IRF3(S396). Briefly, 5 million cells in 90 µL nucleofection buffer were mixed with 10 µL test compounds. These mixtures were electroporated using program V-001 on an Amaxa Nucleofector (Lonza). Cells were transferred into 12 well plates with fresh media and allowed to recover for one hour at 37 °C, 5% CO_2. Cells were then washed in cold HBSS and lysed in RIPA buffer. Samples were total protein normalized and either diluted in ProteinSimple sample buffer or LDS loading buffer. Samples were heat denatured at 95°C for 5 min, then PeggySue (ProteinSimple) was used to measure phospho- and total STING and IRF3 while the NuPAGE (Invitrogen) system was used to measure TBK1. Data was analyzed using Compass or Licor Odyssey software, respectively.

STING in vivo activity

For all studies, female Balb/c mice were obtained from Charles River Labs (Wilmington, MA) and used when they were 6-8 weeks of age and weighed approximately 20 g. All animals were allowed to acclimate and recover from any shipping-related stress for a minimum of 5 days prior to experimental use. Reverse osmosis chlorinated water and irradiated food (Laboratory Autoclavable Rodent Diet 5010, Lab Diet) were provided ad

libitum, and the animals were maintained on a 12 h light and dark cycle. Cages and bedding were autoclaved before use and changed weekly. All experiments were carried out in accordance with The Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Janssen R & D, Spring House, PA. Each experimental group contained 8 mice. In vivo efficacy in a mouse CT26 tumor model was determined by implanting 500,000 CT26 colon carcinoma tumor cells subcutaneously into Balb/c mice and allowing tumors to establish to 100-300 mm^3. Compounds were injected intratumorally formulated in phosphate buffered saline in a volume of 0.1mL per injection. Mice were administered 0.05 mg every three days for a total of three doses. Efficacy was measured as the percent tumor growth inhibition (TGI) calculated by the reduction in size of the Treated tumor volume (T) over the Control tumor volume (C) according to the following formula: ((C-T)/(C))*100 when all control animals were still on study. Cures were defined as the number of animals with no measurable tumor detected 10 tumor volume doubling times (TVDT) after the last dose was administered. The resultant data are presented in Table 3.

Table 3.

Cpd No.	hSTING SPA IC50 (μM)	human cell reporter EC50 (μM)	SPR human STING KD (μM)	mSTING SPA IC50 (μM)	human IFN-β (ranking value)	In vivo activity (%TGI)	In vivo activity (cures)
1	7.31	0.13	0.12	0.2		ND	ND
2	56.7	0.85	0.12			ND	ND
5	1.83	6.7			652	ND	ND
6	0.061	0.86			2977	ND	ND
7	< 0.01	0.22			4263	110.5	5
8	0.27	2.05			2521	ND	ND

Cpd No.	hSTING SPA IC50 (μM)	human cell reporter EC50 (μM)	SPR human STING KD (μM)	mSTING SPA IC50 (μM)	human IFN-β (ranking value)	In vivo activity (%TGI)	In vivo activity (cures)
9	0.96	1.30	-	-	1125	ND	ND
10	6.69	4.66	-	-	1148	ND	ND
11	2.46	5.34	-	-		ND	ND
12	0.27	0.56	-	-	2637	ND	ND
13	0.042	0.11	-	-	2866	ND	ND
17	0.038	0.8	0.00485	< 0.01	317	ND	ND
18	< 0.01	3.73	-	-	628	ND	ND
24	>100	9.5	-	-	-	ND	ND
25	0.085	2.88	-	-	1228	ND	ND
26	< 0.01	1.97	-	-	943	ND	ND
27	0.024	3.36	-	-	793	ND	ND
28	0.061	5.8	-	-	-	ND	ND
29	< 0.01	5.67	-	-	-	ND	ND
30	0.011	1.76	-	-	-	ND	ND
31	< 0.01	6.41	-	-	-	ND	ND
32	0.034	-	-	-	-	ND	ND
33	< 0.01	-	-	-	-	ND	ND

ND: Not Done

In vivo activity determination

Activity may be assessed in animal models by implanting MC38 cells into C57BL/6 mice or CT26 cells into the right flank of Balb/c mice and allowing the tumors to establish to a size of approximately 100-200 mm^3. Tumors may be injected with vehicle (PBS or HBSS) or test compounds intratumorally in a volume of 100 uL per injection. Each treatment group may have 7-8 mice and the treatments may be administered every three days for a total of 3 doses (q3dx3). Tumor size may be measured with a caliper and estimated tumor weight may be calculated using the following formula: tumor weight = $w^2(l)/2$ where w = width and l = length in millimeters. Efficacy may be determined both by percent tumor growth inhibition (%TGI) and also by number of "cures" in each group. %TGI may be calculated as the percent reduction in size of the treated tumor volume (T) over the control tumor volume (C) according to the following formula ((C-T)/(C-starting size))*100 when all control animals were still on study. Cures may be defined as the number of animals with no measurable tumor detected 10 tumor volume doubling times (TVDT) after the last dose was administered.

Biological Example 2

STING primary human PBMC cytokine induction assay

Agonism of the human STING pathway is assessed in primary human peripheral blood mononuclear cells (PBMC) derived from human whole blood. 1 pint (approximately 420 ml) of fresh donor blood (AllCells Inc., Alameda, CA) is layered over Lymphocyte Separation Medium (1.077-1.080 g/ml, Corning, Manassas, VA), then centrifuged at 500g for 20 min at RT without applying break. The PBMC collected at the interface between serum and Lymphocyte Separation Medium are harvested, washed, then counted. PBMC are composed of subtypes of lymphocytes and monocytes, such as B cells, T cells, etc., and these subtypes have been characterized in the literature to express different levels of the STING protein. In response to STING agonists, such

as 2'3'-cGAMP, these cells become activated and are induced to express a variety of proinflammatory and antiviral cytokines. Also, upon stimulation with STING agonists, these cells upregulate activation markers. The levels of cytokine induction can be measured by a variety of methods including ELISA, Luminex and MSD. The levels of activation marker upregulation can be measured by flow cytometry.

To run the assay, 1,000,000 cells were dispensed into 225 µL/well of flat-bottom, tissue culture treated, 96-well plates. Test compounds were added in a volume of 25 µL at 10x concentration. Some compounds were solubilized in 100% DMSO and the final concentration of DMSO in the cultures receiving these compounds was 1%. The assay was incubated for 48 h at 37 °C, 5% CO_2. 200 µl of supernatants were harvested without disturbing cells on the bottom of the plate, then frozen at -20 °C until time of Luminex measurement. Luminex assays were performed using G-CSF, IFNα2, IFNγ, IL-1b, IL-6, IL-10, IL-12 (p40), IL-12 (p70), TNFa from MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel - Immunology Multiplex Assay kit and IFNβ1 analyte from MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel IV kit (EMD Millipore, Billerica, MA), following the manufacturer's protocol. Cytokine induction was measured using a Luminex FlexMAP 3D® instrument (Luminex Corporation, Radnor, PA). Analysis of collected Luminex data was performed using MILLIPLEX Analyst software (EMD Millipore).

Suppression of HBV virus in PHH cells using conditioned media from STING activated primary human PBMC

Primary human hepatocytes can be infected with hepatitis B virus and during an established infection, will produce viral proteins such as HBsAg and HBeAg that can be detected by ELISA. Therapeutic treatment with compounds such as entecavir can suppress HBV reproduction, which can be measured by decreased viral protein production. (# of cells) $4x10^5$ cells/well primary human hepatocytes (BioReclamation, Westbury, NY) were dispensed into 500 µL/well of flat-bottom, tissue culture treated, 24-well plates. 24 h later, cells were infected with 30-75 moi of HBV. On the next day, the PHH were washed 3x and fresh maintenance media was added to the cells. Concurrently, PBMC were isolated as described previously. To stimulate the

PBMC, 10,000,000 cells were dispensed into 400 µL/well of flat-bottom, tissue culture treated, 24-well plates. Test compounds were added in a volume of 100 µL, then the cultures were incubated for 48 h at 37 °C, 5% CO_2. Supernatants were harvested. Cells were measured for activation marker upregulation using flow cytometery. Briefly, cells were stained with fluorescently labeled antibodies directed to CD56, CD19, CD3, CD8a, CD14, CD69, CD54, CD161, CD4 and CD80. Samples were analyzed on an Attune NxT flow cytometer (Thermo Fisher, Carlsbad, CA)

From the stimulated PBMC cultures, a portion of supernatant was reserved for cytokine detection by Luminex, as described previously. The rest of the supernatant was divided in half, and one aliquot was stored at 4°C for use on d8 of the assay. The other aliquot of supernatant was diluted 1:1 with 2X PHH media, then added to the d4 infected PHH cells. After 96 h, the spent media was changed and supernatant was added at a dilution of 1:1 with 2X PHH media. At this point an interim measurement of HBsAg was performed using an HBsAg ELISA kit (Wantai Bio-pharm, Beijing, China). Following 96 h, the media was collected and HBsAg was measured.

Table 4: Fold induction of cytokines in PBMC cultures stimulated with CDN compounds. Fold induction is calculated by measuring the concentrations of the cytokine induced after 48 h by approximately 20 µM of compound, then dividing by base line levels of cytokine production of cells incubated with PBS. The data is the average of multiple donors over three experiments. nt = not tested.

Table 4.

Cpd No.	IL-6	IL-10	IFN-γ	IL-1b	IFN-α	TNFa	IL-12p40	IL-12p70	G-CSF	IFN-β
1	3.0	11.3	57.2	7.5	4.4	17.8	1.5	122.2	1.1	19.7

Table 5: Fold induction of cytokines in PBMC cultures stimulated with higher concentrations of CDN compounds. Fold induction is calculated by measuring the concentrations of the cytokine induced after 48 h the indicated concentration of compound, then

dividing by base line levels of cytokine production of cells incubated with PBS. The data is the average of multiple donors over three experiments. nt = not tested.

Table 5.

Cpd No.	Top Conc (μM)	IL-6	IL-10	IFNγ	IL-1β	IFNα2	TNFα	IL12 p 40	IL12 p70	G-CSF	IFNβ1
1	40	436.0	6.2	535.9	92.9	13.2	87.0	1.1	9.0	8.3	23.4
17	40	499.1	8.5	883.8	141.8	30.6	178.0	2.0	3.9	6.7	42.7
18	40	759.7	20.2	1507.3	171.5	51.5	95.8	1.8	7.4	31.2	6.7
25	40	6546.8	61.3	6181.6	2946.5	71.5	1551.5	12.0	327.9	804.5	110.6

Table 6. Conditioned media from PBMCs stimulated with CDN can suppress viral load of HBV infected PHH cells. PBMCs were stimulated with the indicated CDN at 20, 4, 0.8 μM for 48 h. Supernatants were mixed with fresh media at a ratio of 1:1, then added to HBV infected PHH cells. HBsAg production was measured 8 days later. The data is an average of two independent donors.

Table 6.

Cpd No.	EC50 (μM)
1	4.02E-04

Table 7. CDN activate PBMC. PBMCs were stimulated with 20 μM of CDN for 48 h. Cells were assessed by flow cytometry for upregulation of CD54 on monocytes. The fold increase in Mean Fluoresence Intensity was calculated relative to the levels on resting cells. The data is an average of two independent donors.

Table 7.

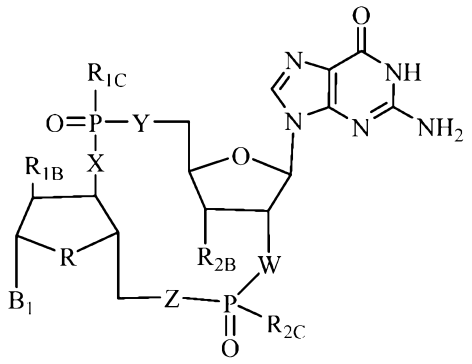
Cpd No.	**MFI**
1	5.6
4-2'3'-cGAMP	4.5

Cpd No.	MFI
PBS	1.0

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptations and/or modifications as come within the scope of the following claims and their equivalents

Claims:

1. A compound of Formula (I)



Formula (I)

wherein

R is CH_2 or O;

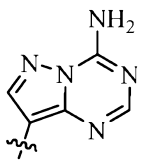
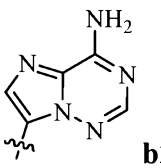
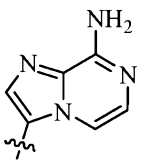
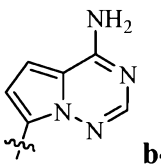
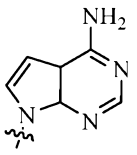
R_{1B} is hydrogen, hydroxy, or fluoro;

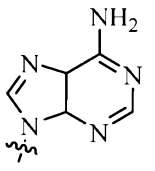
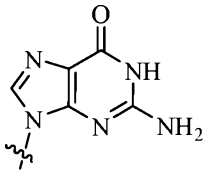
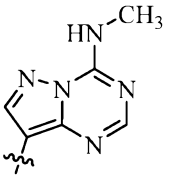
R_{1C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

R_{2B} is hydrogen, hydroxy, methoxy, or fluoro;

R_{2C} is selected from the group consisting of hydroxy, thiol, and BH_3^- ;

B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, **b7** and **b8**

 **b1**,  **b2**,  **b3**,  **b4**,  **b5**,

 **b6**,  **b7**, and  **b8**;

W is -O- or -NH-;

X is -O- or -NH-;

Y is $-CH_2-$, -O- or -NH-;

Z is $-CH_2-$, -O- or -NH-;

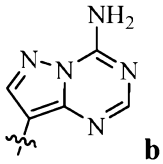
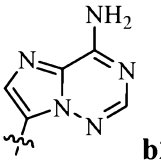
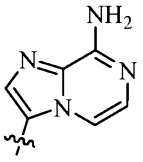
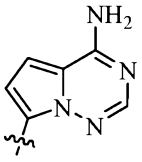
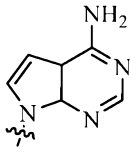
such that only one of X and Y is NH, and only one of W and Z is NH, in any instance;

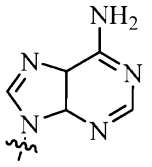
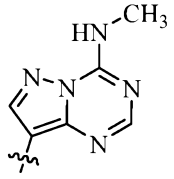
and, such that when R is CH_2 and B_1 is selected from **b6** or **b7**, then R_{2B} is other than fluoro or hydroxy;

furthermore, provided that a compound of Formula (I) is other than a compound wherein R, W, X, Y, and Z, are each O; R_{1B} and R_{2B} are each hydroxy; B_1 is **b1**; and R_{1B} and R_{2B} are each hydroxy;

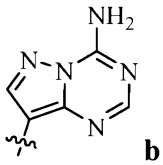
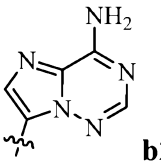
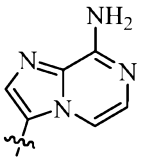
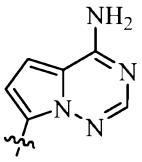
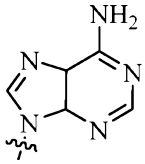
or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

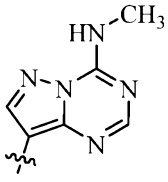
2. The compound of claim 1 wherein B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, and **b8**

 **b1,**  **b2,**  **b3,**  **b4,**  **b5,**

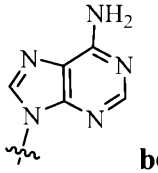
 **b6,** and  **b8** .

3. The compound of claim 1 wherein W is O; and B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b6**, and **b8**

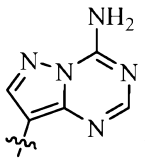
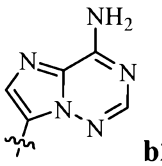
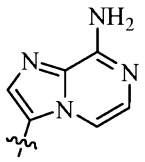
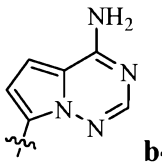
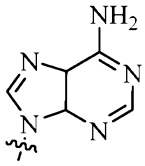
 **b1,**  **b2,**  **b3,**  **b4,**  **b6,**

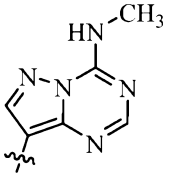
 and **b8** .

4. The compound of claim 3 wherein B_1 is **b6**

 **b6** .

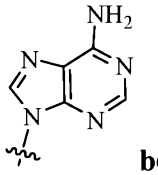
5. The compound of claim 1 wherein W is NH; and B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b6**, and **b8**

 **b1,**  **b2,**  **b3,**  **b4,**  **b6,**

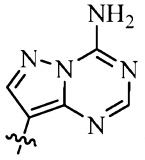
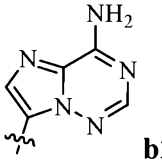
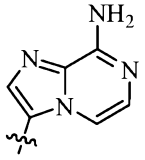
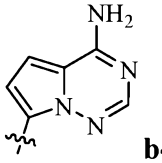
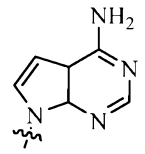


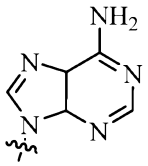
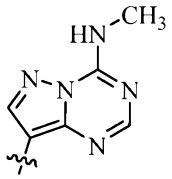
and **b8** .

6. The compound of claim 5 wherein B_1 is **b6**

 **b6** .

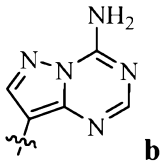
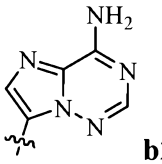
7. The compound of claim 1 wherein R is CH_2; and B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, and **b8**

 **b1,**  **b2,**  **b3,**  **b4,**  **b5,**

 **b6,** and  **b8**.

8. The compound of claim 7 wherein W and X are -O- ; such that when B_1 is **b6**, then R_{2B} is other than fluoro or hydroxy.

9. The compound of claim 1 wherein R is O; and B_1 is selected from the group consisting of rings **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, and **b8**


b1,

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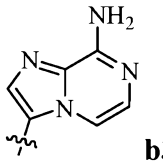
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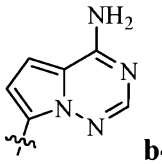
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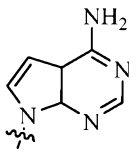
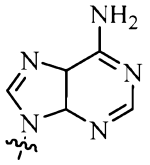
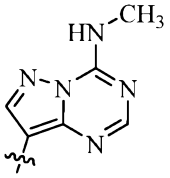
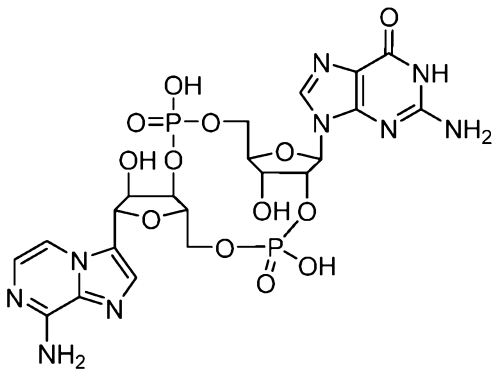

b5,

b6, and

b8.

10. A compound of claim 1 selected from the group consisting of compounds **1** to **33**,



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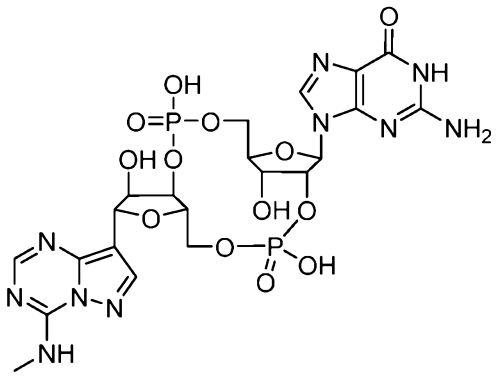

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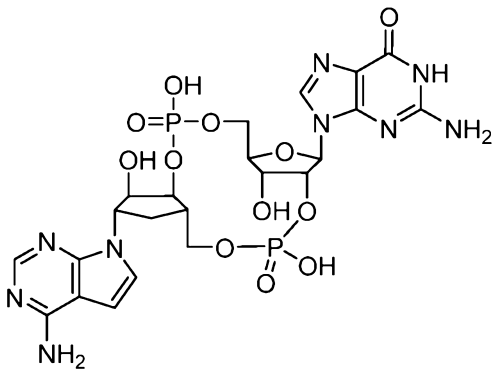

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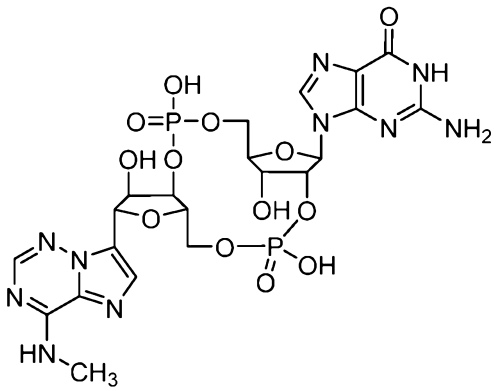

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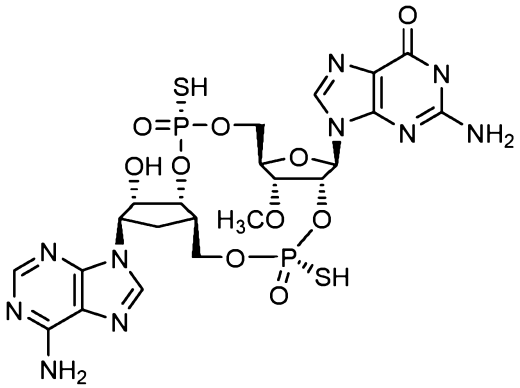

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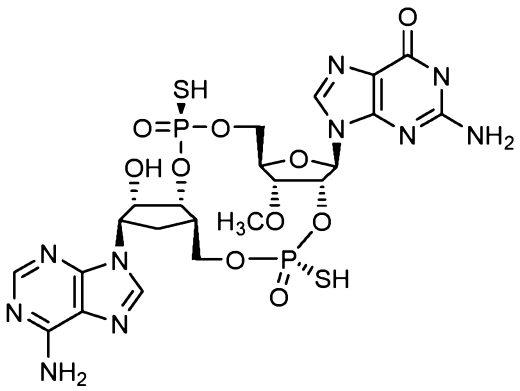

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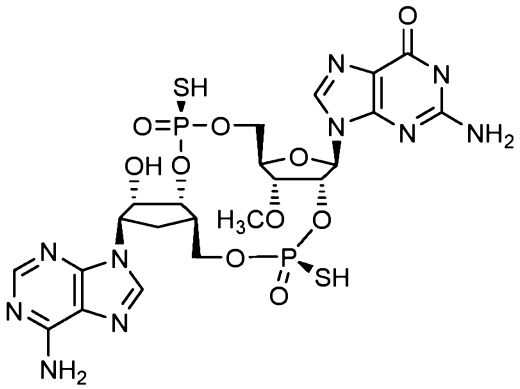

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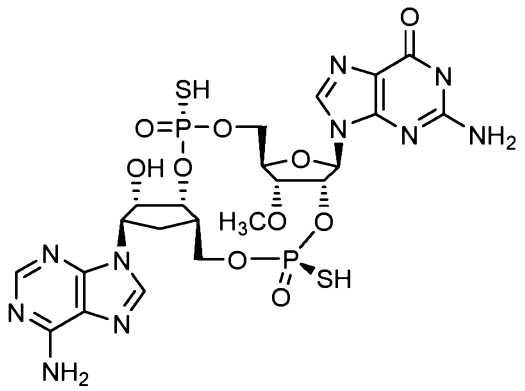

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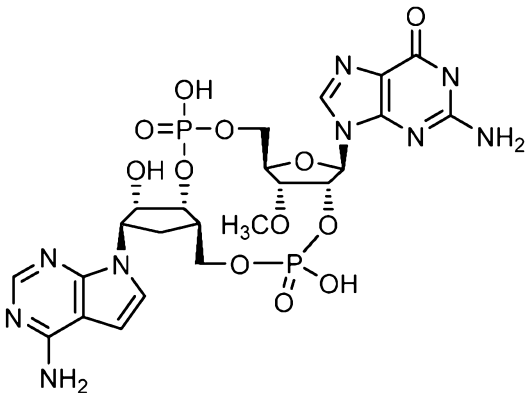

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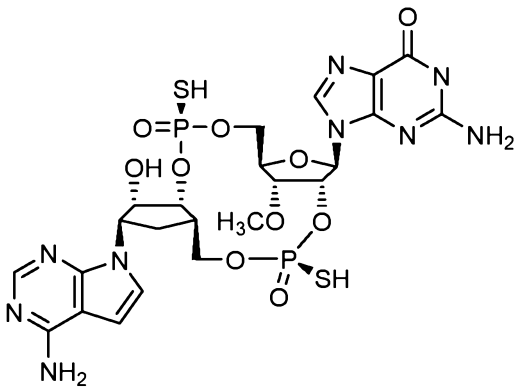

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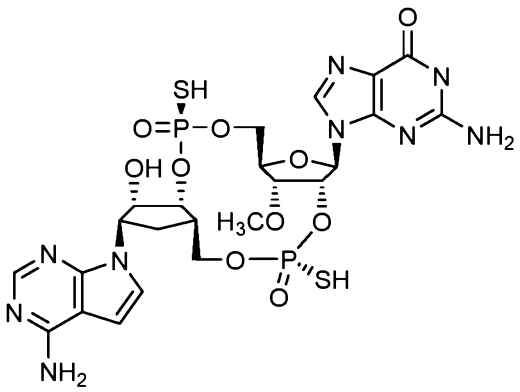

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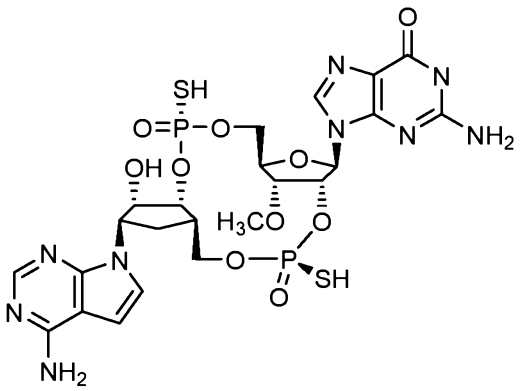

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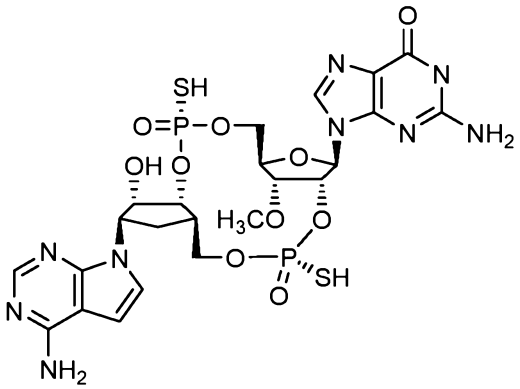

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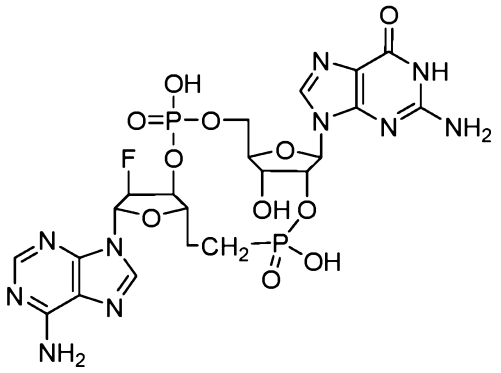

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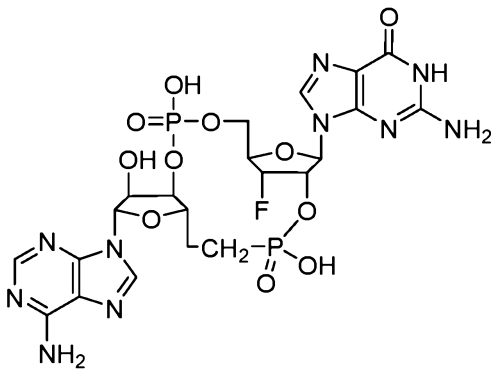

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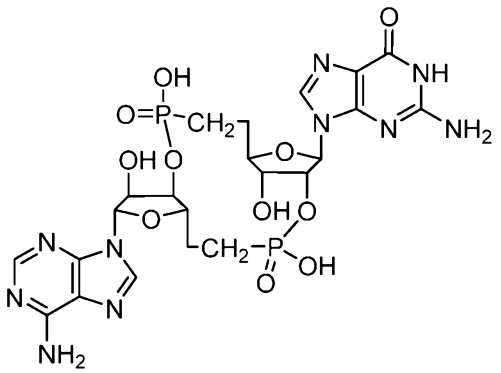

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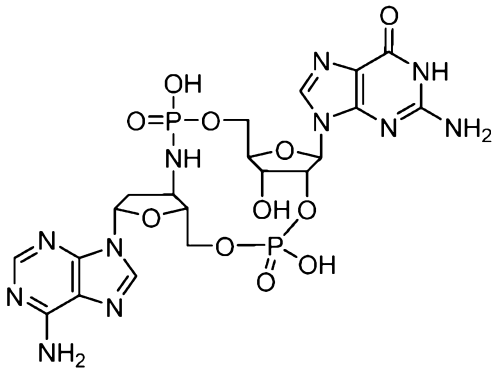

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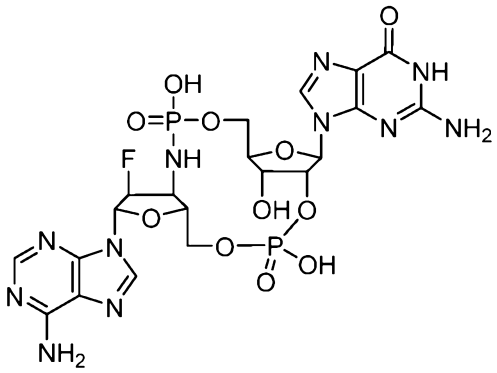

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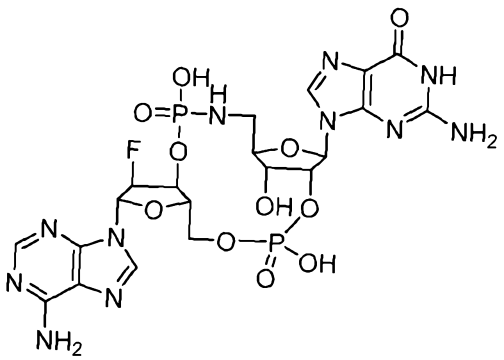

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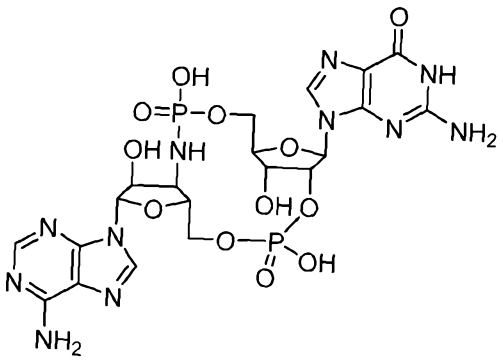

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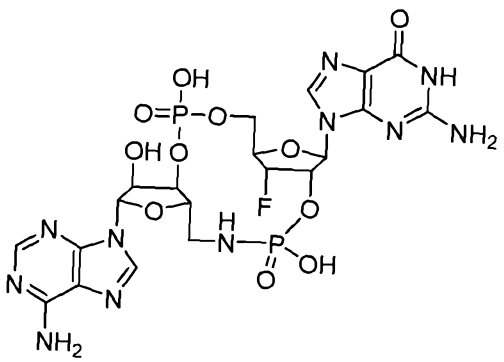

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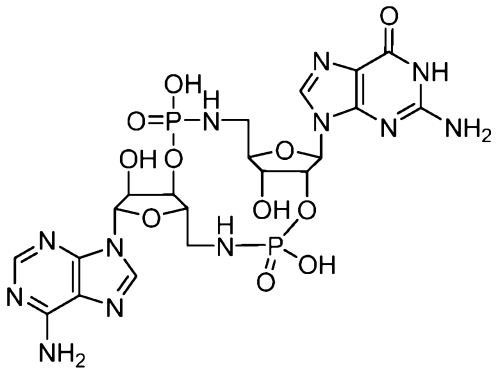

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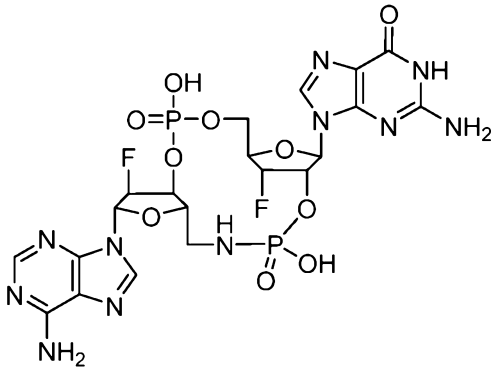

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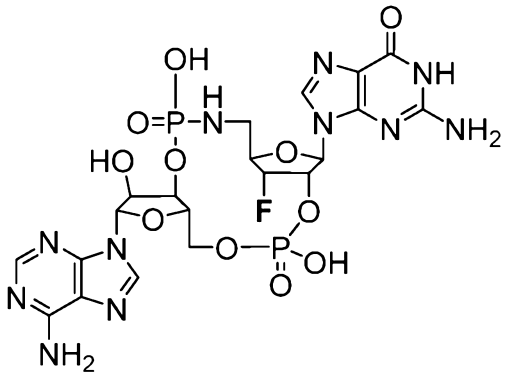

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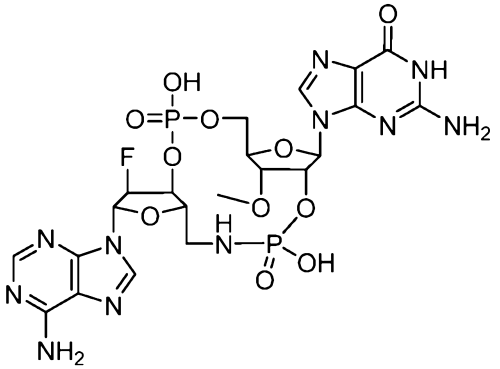

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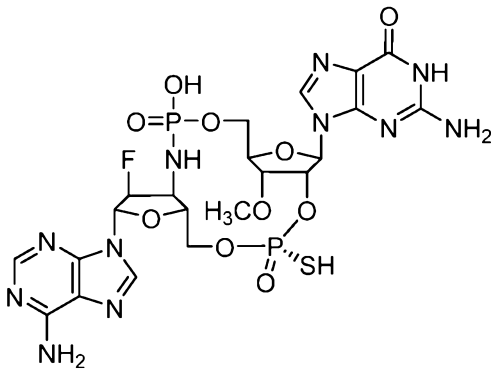

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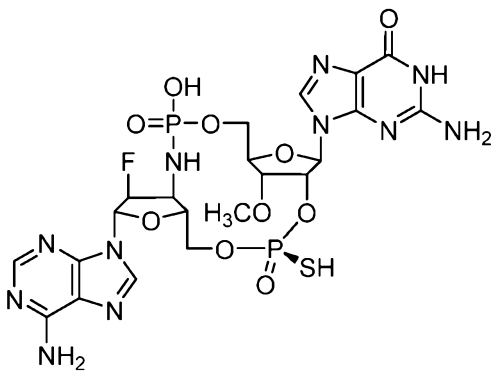

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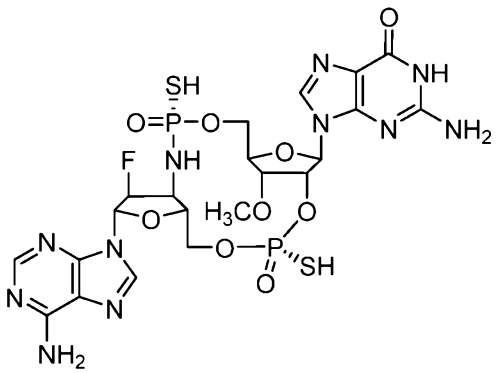

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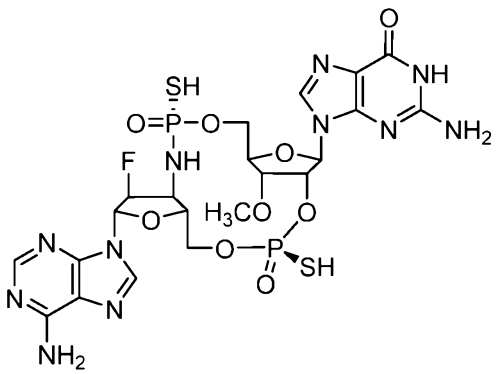

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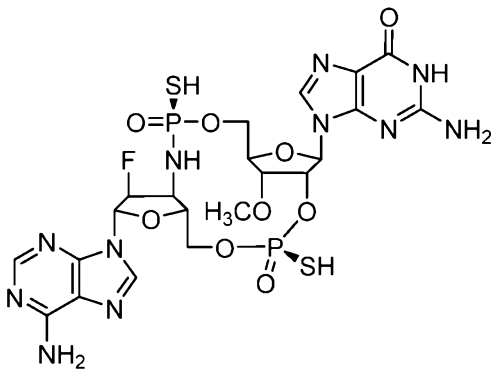

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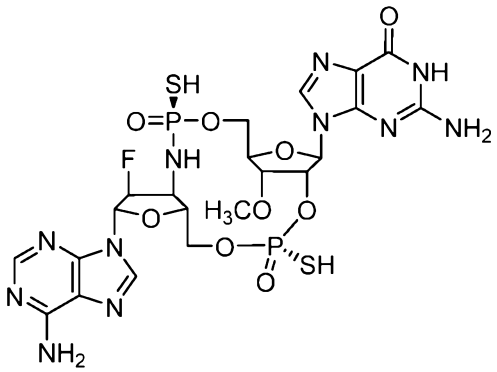

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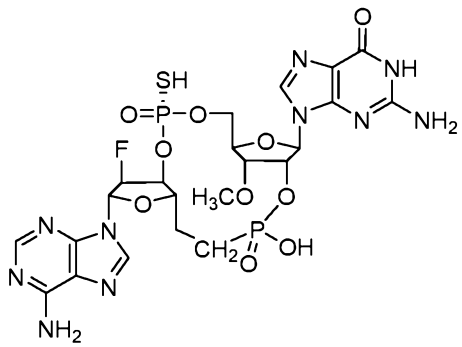

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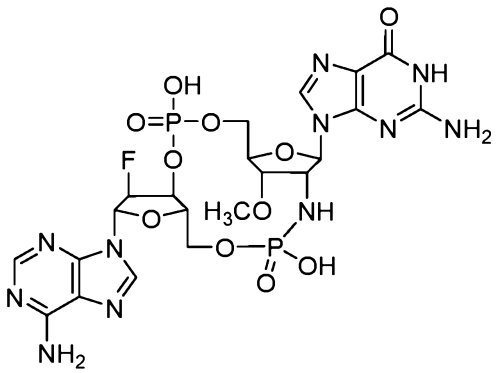

33;

or a pharmaceutically acceptable salt form thereof.

11. A pharmaceutical composition comprising a compound of claims 1 to 10 and at least one of a pharmaceutically acceptable carrier, a pharmaceutically acceptable excipient, and a pharmaceutically acceptable diluent.

12. The pharmaceutical composition of claim 11, wherein the composition is a solid oral dosage form.

13. The pharmaceutical composition of claim 11, wherein the composition is a syrup, an elixir or a suspension.

14. A method of treating a disease, syndrome, or condition modulated by STING, comprising administering to a subject in need thereof a therapeutically effective amount of the compound of claim 1.

15. A method of treating a disease, syndrome, or condition, wherein said disease, syndrome, or condition is affected by the agonism of STING, comprising administering to a subject in need thereof a therapeutically effective amount of the compound of claim 1.

16. The method of claim 15 wherein said disease, syndrome, or condition is cancer.

17. The method of claim 16 wherein said cancer is selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, and fibrosarcoma.

18. The method of claim 15, wherein said disease, syndrome, or condition is a viral infection.

19. The method of claim 18, wherein the viral infection is hepatitis B.

20. A method of treating a disease, syndrome, or condition selected from the group consisting of viral infection, melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, and fibrosarcoma, comprising administering to a subject in need thereof a therapeutically effective amount of the composition of claim 8.

21. The method of claim 20, wherein the viral infection is hepatitis B.

22. The use of a compound as defined in claim 1 for the preparation of a medicament for treating a disease, syndrome, or condition selected from the group consisting of viral infection, melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, and fibrosarcoma, in a subject in need thereof.

23. The use of a compound as defined in claim 1, for use in a method for treating a disease, syndrome, or condition selected from the group consisting of viral infection, melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, and fibrosarcoma, in a subject in need thereof.