(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 May 2018 (31.05.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/098203 A1

(51) International Patent Classification:
C07H 21/02 (2006.01) *A61P 35/00* (2006.01)
A61K 31/7084 (2006.01) *A61P 31/12* (2006.01)

(21) International Application Number:
PCT/US2017/062901

(22) International Filing Date:
21 November 2017 (21.11.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/426,350 25 November 2016 (25.11.2016) US
62/502,983 08 May 2017 (08.05.2017) US
62/555,232 07 September 2017 (07.09.2017) US

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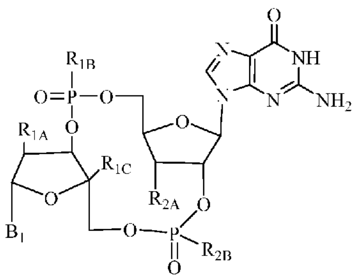
(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) **Title:** CYCLIC DINUCLEOTIDES AS STING AGONISTS



Formula (I)

(57) **Abstract:** Disclosed are compounds, compositions and methods for treating viral infections, diseases, syndromes, or disorders that are affected by the modulation of STING. Such compounds are represented by Formula (I) as follows: [Formula (I) should be inserted here] wherein R_{1A}, R_{1B}, R_{1c}, B_1, R_{2A}, and R_{2B} are defined herein.

WO 2018/098203 A1

CYCLIC DINUCLEOTIDES AS STING AGONISTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This Application claims priority to United States Provisional Patent Application No. 62/426350, filed November 25, 2016; United States Provisional Patent Application No. 62/502,983, filed May 8, 2017; and United States Provisional Patent Application No. 62/555,232, filed September 7, 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 (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.

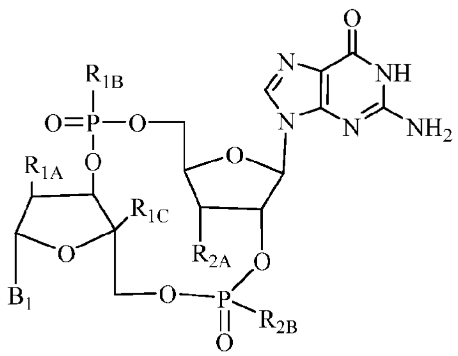
Activation of the STING pathway also contributes to an antiviral response. Loss-of-functional response, either at the cellular or organism level, demonstrates an inability to control viral load in the absence of STING. Activation of the STING pathway triggers an immune response that results in antiviral 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)



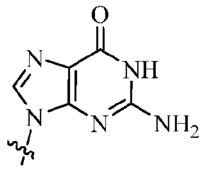
Formula (I)

wherein

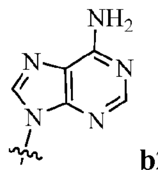
R_{1A} is hydroxy or fluoro and R_{1C} is hydrogen; or, R_{1A} is –O– and R_{1C} is CH_2 such thatR_{1A} and R_{1C} are taken together with the atoms to which they are attached to form a 5-membered ring;

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

B_1 is selected from the group consisting of rings **b1** and **b2**



b1 and



b2;

R_{2A} is selected from the group consisting of hydroxy and methoxy;

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

provided that the compound of Formula (I) is other than (1*R*,6*R*,8*R*,9*R*,10*R*,15*R*,17*R*,18*R*)-17-(2-Amino-6-oxo-6,9-dihydro-1*H*-purin-9-yl)-8-(6-amino-9*H*-purin-9-yl)-9-fluoro-3,12,18-trihydroxy-2,4,7,11,13,16-hexaoxa-3λ^5, 12λ^5-diphosphatricyclo[13.2.1.0^6,^{10}]octadecane-3,12-dione, bis-ammonium salt;

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)$_2$amino-, 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.

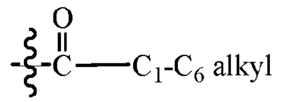
The term “aryl” refers to an unsaturated, aromatic monocyclic or bicyclic carbocyclic ring of 6 to 10 carbon members. Examples of aryl rings include phenyl and naphthalenyl.

The term “heteroaryl” refers to an aromatic monocyclic or bicyclic ring system having 5 to 10 ring members, 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.

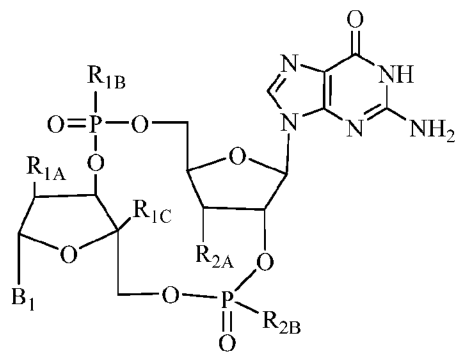
Other embodiments disclosed herein relate to a method of ameliorating and/or treating a viral infection that can include contacting a cell infected with HBV with an effective amount of one or more compounds of Formula (I), or a pharmaceutically acceptable salt form thereof, or a pharmaceutical composition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

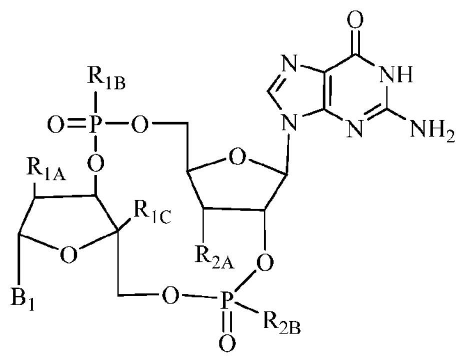
Embodiments of the present invention include a compound of Formula (I) as herein defined, or an enantiomer, diastereomer, solvate, or a pharmaceutically acceptable salt form thereof, wherein the substituents selected from one or more of the variables defined herein (e.g. R_{1A}, R_{1B}, R_{1C}, B_1, R_{2A}, R_{2B}) 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 (I)

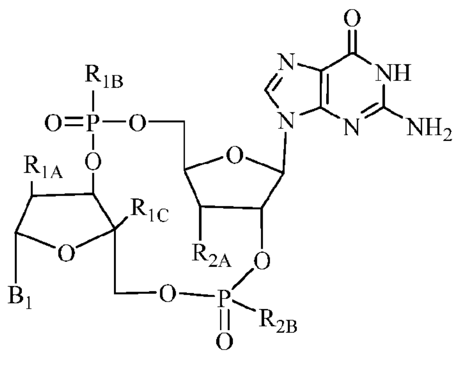
Cpd No	R_{1A}	R_{1B}	R_{1c}	B_1	R_{2A}	R_{2B}
1	OCH_3	OH	H	b2	OH	OH
2	OH	OH	H	b2	OCH_3	OH
3	OCH_3	OH	H	b2	OCH_3	OH
4	F	(**R*)-SH	H	b2	OH	(**R*)-SH
5	F	(**S*)-SH	H	b2	OH	(**S*)-SH
6	-O-	OH	CH_2 to form a ring with R_{1A}	b2	OH	OH
7	OH	(**R*)-BH_3^-	H	b2	OH	(**R*)-BH_3^-
8	OH	(**S*)-BH_3^-	H	b2	OH	(**S*)-BH_3^-
9	F	(**R*)-BH_3^-	H	b2	OCH_3	(**R*)-SH
10	F	(**S*)-BH_3^-	H	b2	OCH_3	(**R*)-SH
11	F	(**R*)-BH_3^-	H	b2	OCH_3	(**R*)-BH_3^-
12	F	(**S*)-BH_3^-	H	b2	OCH_3	(**S*)-BH_3^-
13	F	(**R*)-SH	H	b2	OCH_3	(**R*)-BH_3^-



Formula (I)

Cpd No	R_{1A}	R_{1B}	R_{1c}	B_1	R_{2A}	R_{2B}
14	F	(**S*)-SH	H	b2	OCH_3	(**S*)-BH_3^-
15	F	(**R*)-BH_3	H	b2	OCH_3	OH
16	F	(**S*)-BH_3	H	b2	OCH_3	OH
17	F	OH	H	b2	OCH_3	(**R*)-BH_3
18	F	OH	H	b2	OCH_3	(**S*)-BH_3
19	-O-	OH	CH_2 to form a ring with R_{1A}	b2	OCH_3	(*R)-BH_3
20	-O-	(*R)-SH	CH_2 to form a ring with R_{1A}	b2	OCH_3	(*R)-BH_3
21	-O-	(*R)-BH_3	CH_2 to form a ring with R_{1A}	b2	OCH_3	(*R)-BH_3
22	-O-	(*R)-BH_3	CH_2 to form a ring with R_{1A}	b2	OCH_3	OH
23	-O-	(*R)-BH_3	CH_2 to form a ring with R_{1A}	b2	OCH_3	(*R)-SH

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



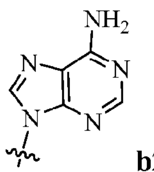
Formula (I)

wherein

R_{1A} is hydroxy or fluoro and R_{1C} is hydrogen; or, R_{1A} is –O– and R_{1C} is CH_2 such that R_{1A} and R_{1C} are taken together with the atoms to which they are attached to form a 5-membered ring;

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

B_1 is **b2**



b2;

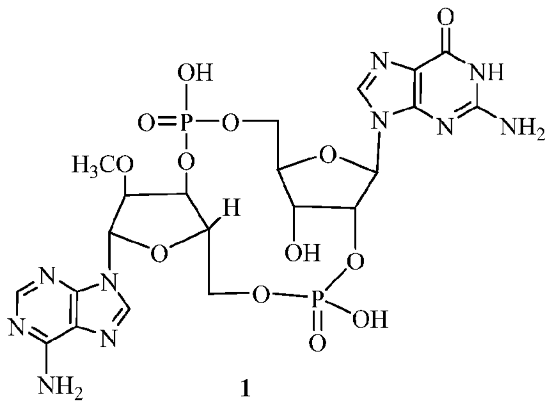
R_{2A} is selected from the group consisting of hydroxy and methoxy;

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

provided that the compound of Formula (I) is other than (1*R*,6*R*,8*R*,9*R*,10*R*,15*R*,17*R*,18*R*)-17-(2-Amino-6-oxo-6,9-dihydro-1*H*-purin-9-yl)-8-(6-amino-9*H*-purin-9-yl)-9-fluoro-3,12,18-trihydroxy-2,4,7,11,13,16-hexaoxa-3λ^5, 12λ^5-diphosphatricyclo[13.2.1.0^{6,10}]octadecane-3,12-dione, bis-ammonium salt;

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

A further embodiment of the present invention is directed to a compound of Formula (I), selected from compounds 1 to 23,



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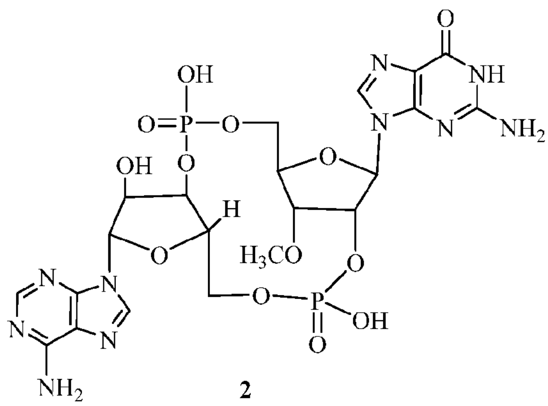

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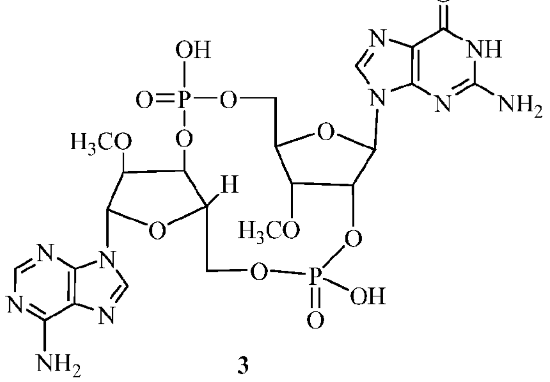

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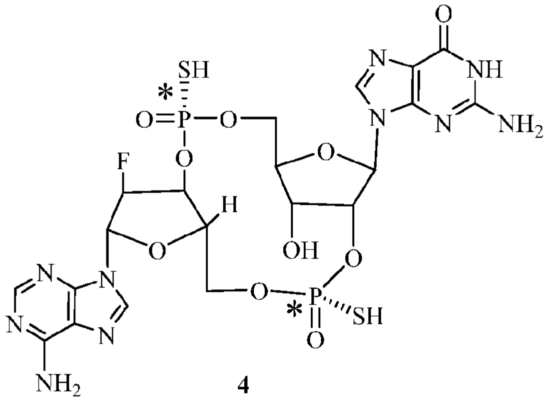

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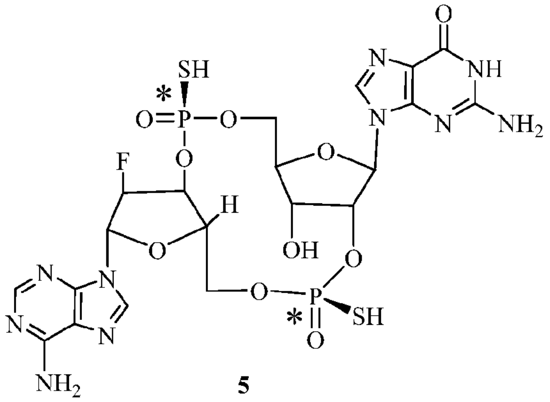

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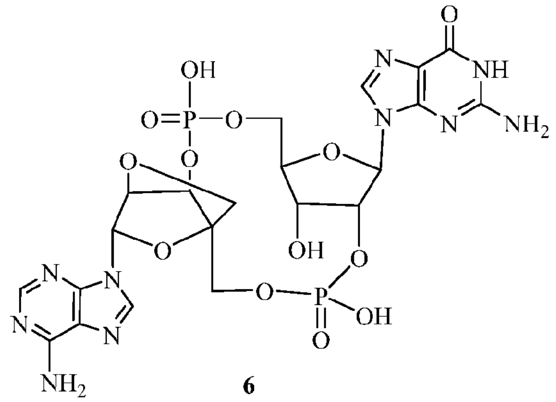

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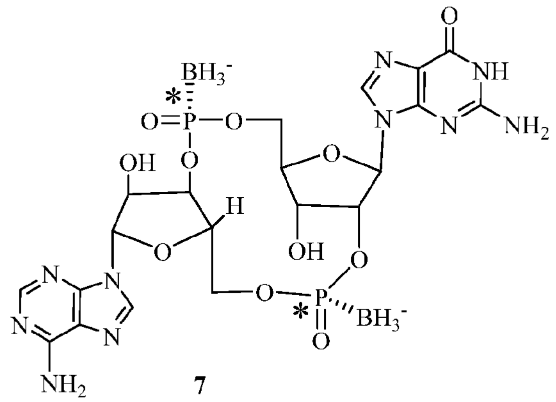

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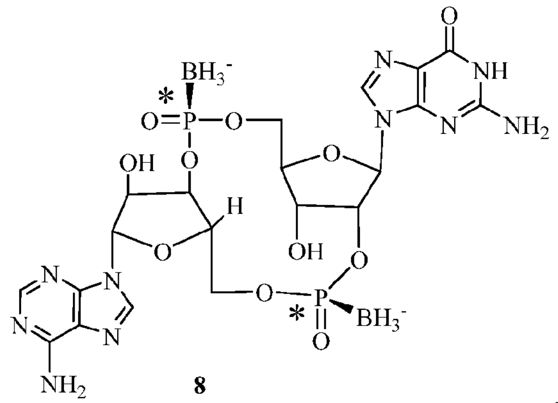

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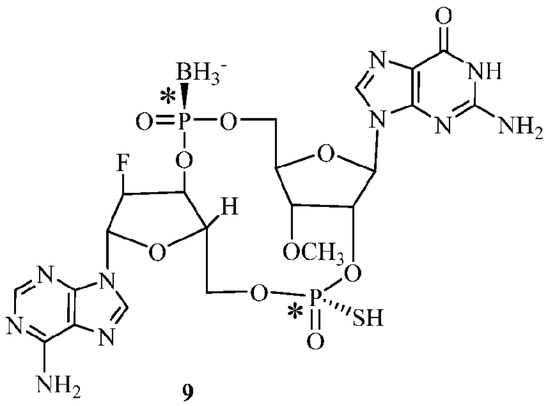

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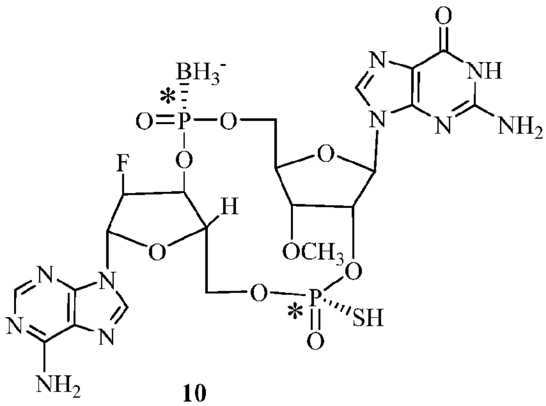

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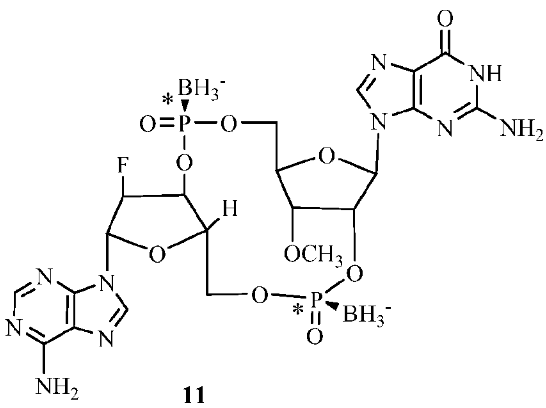

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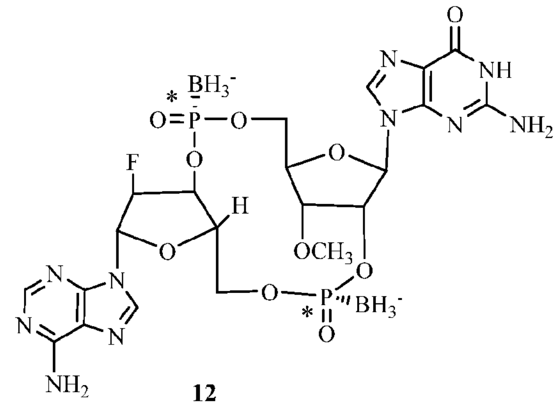

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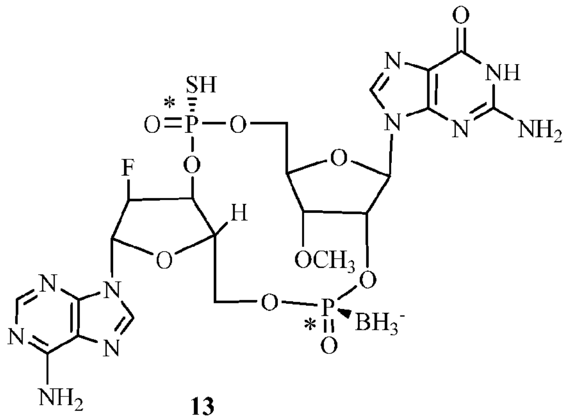

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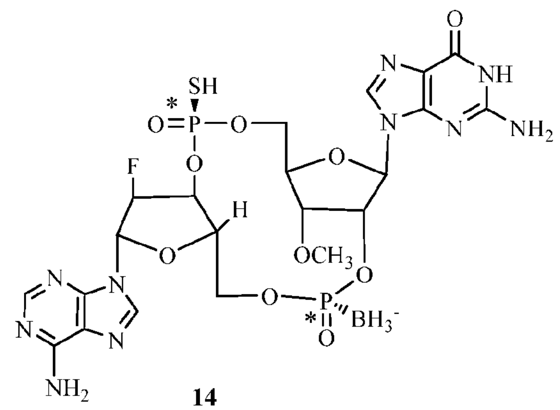

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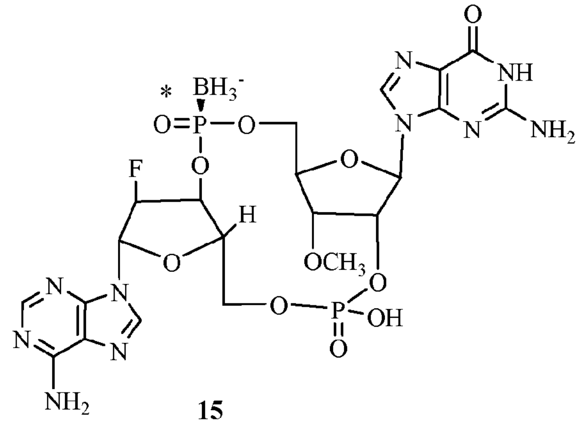

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BH_3^-
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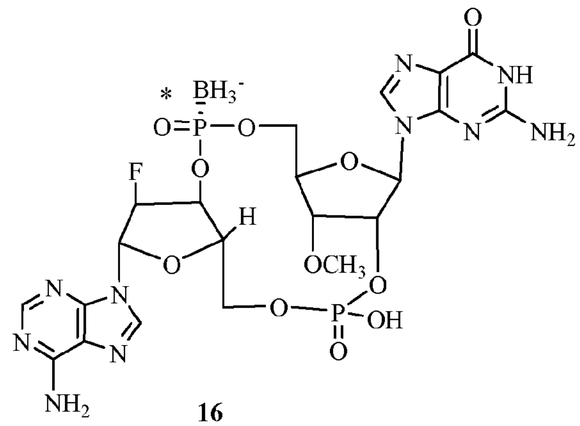

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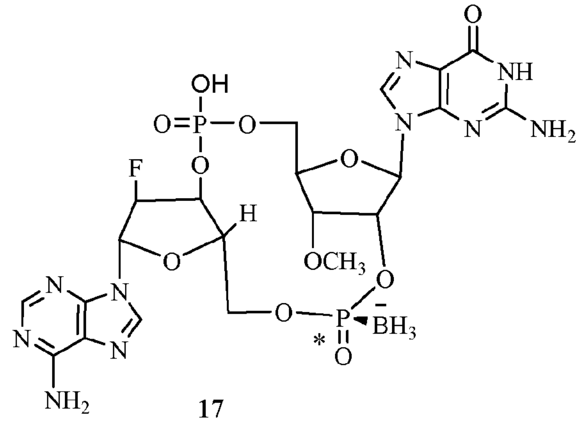
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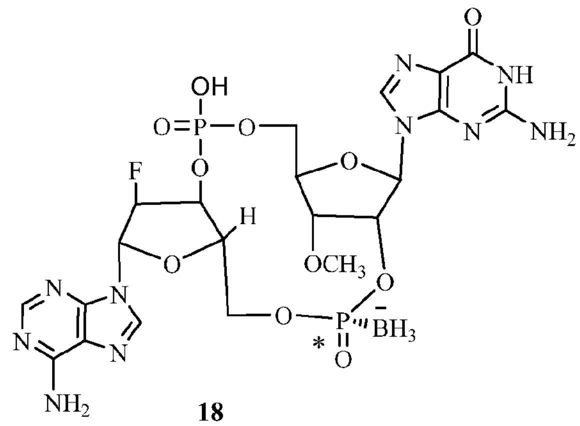
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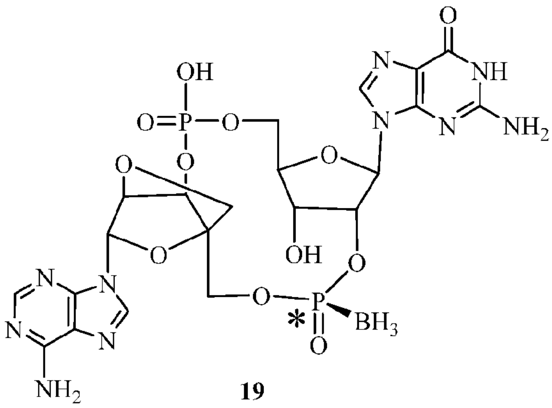

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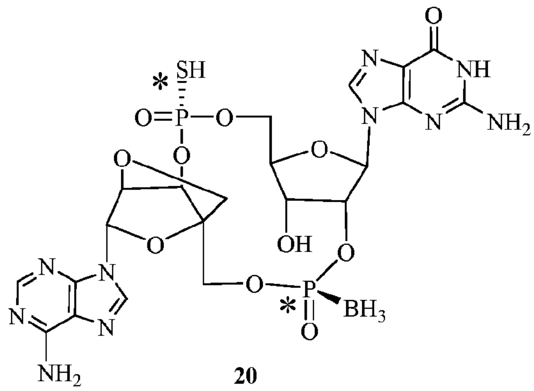

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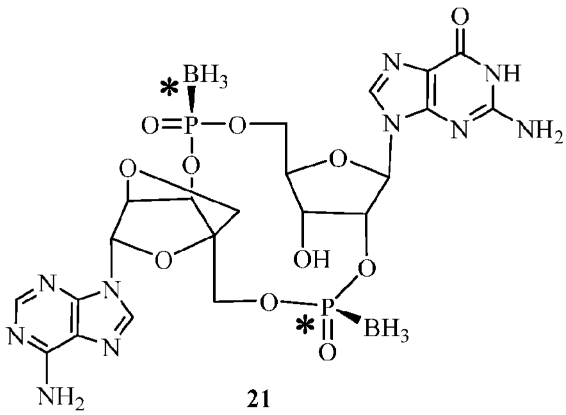

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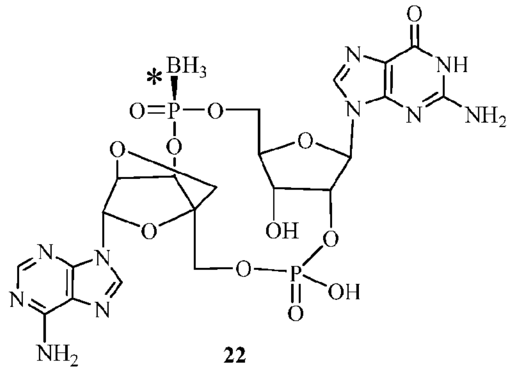

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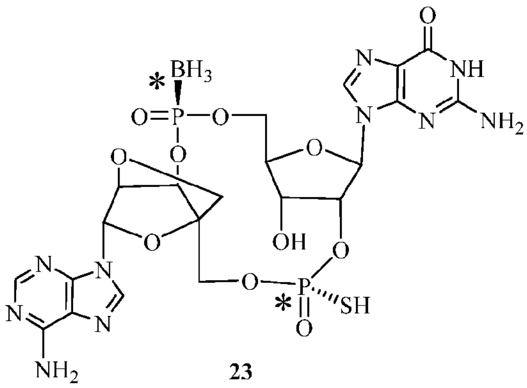
and



23

;

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/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 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 viral infection, 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:

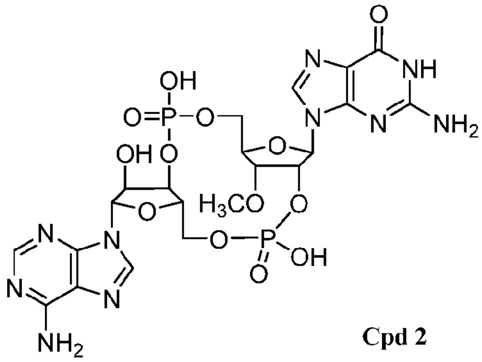
ACN	acetonitrile
AcOH	glacial acetic acid
ADDP	azodicarboxylic dipiperidide
aq.	aqueous
Bn or Bzl	benzyl
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
Boc	tert-butyloxycarbonyl
conc.	concentrated
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-dicyclohexyl-carbodiimide
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

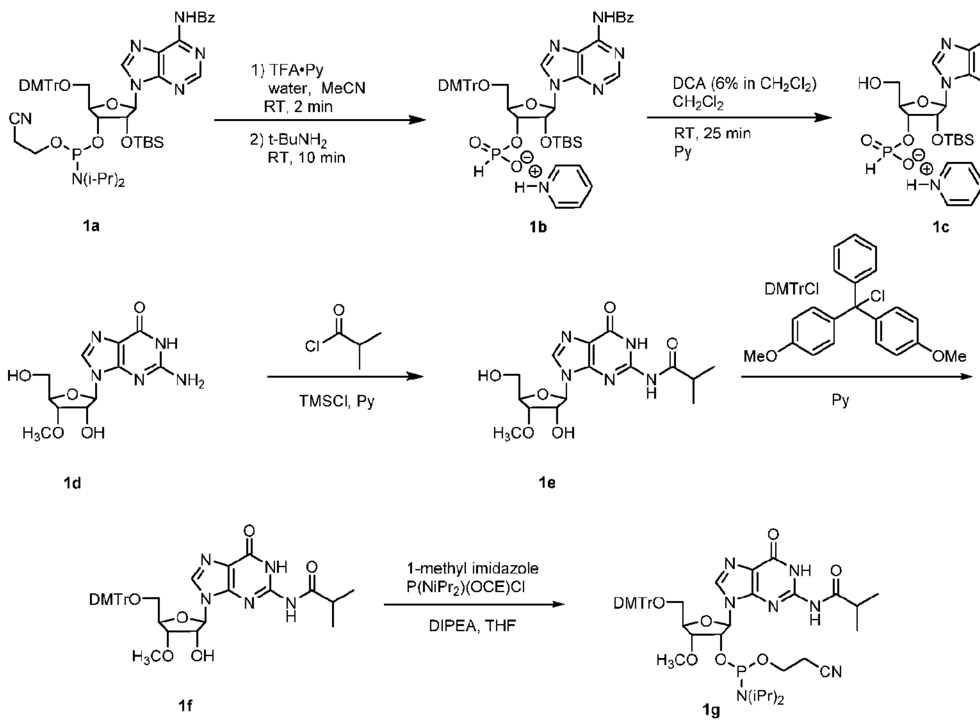
NIS	*N*-iodosuccinimide
NMM	N-methylmorpholine
NMP	*N*-methylpyrrolidone
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
PE	petrolum ether
RP	reverse-phase
rt or RT	room temperature
R_t	retention time
Sec	second or seconds
SEM-Cl	2-(trimethylsilyl)ethoxymethyl chloride
TBAF	tetrabutylammonium 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

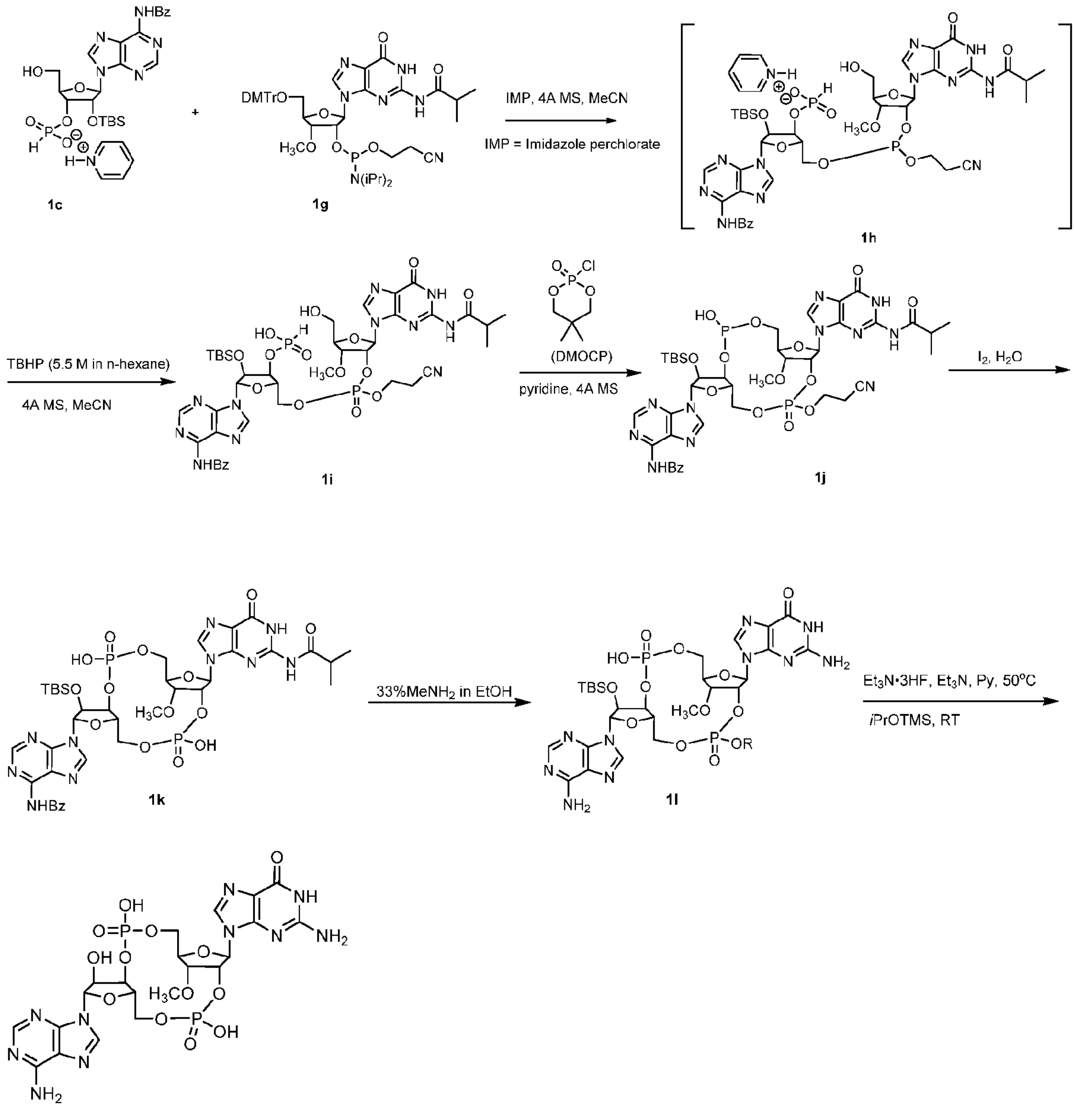
Specific Examples

Example 1

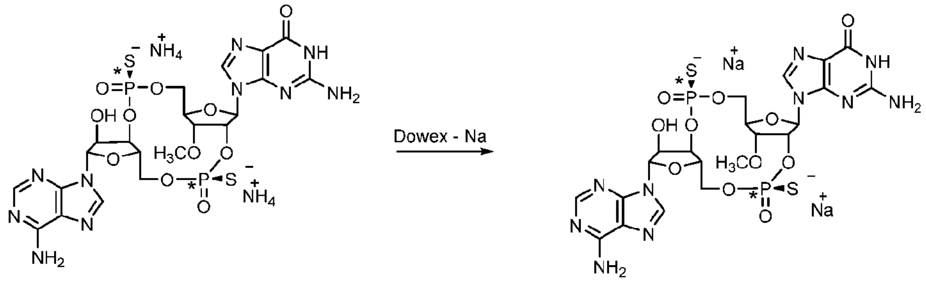


Cpd 2




IMP, 4A MS, MeCN
IMP = Imidazole perchlorate
1c
1g
1h
TBHP (5.5 M in n-hexane)
4A MS, MeCN
1i
(DMOCP)
pyridine, 4A MS
1j
I2, H2O
1k
33%MeNH2 in EtOH
1l
Et3N•3HF, Et3N, Py, 50°C
iPrOTMS, RT

Cpd 2



Compound 2 ammonium salt **Compound 2** sodium salt

Step 1: preparation of compound **1e**

To a solution of 3'-*O*-methyl-guanosine **1d** (CAS 10300-27-3, 1.0 g, 3.36 mmol) in pyridine (20 mL) was added dropwise tert-butylchlorodimethylsilane (3.2 mL, 25.2 mmol) at room temperature. After 1 h, isobutyryl chloride (1.08 g, 10.1 mmol) was added dropwise at room temperature. The final mixture was stirred at room temperature for 2 h. The mixture was quenched with water (30 mL) at 0 °C and NH_4OH (6 mL) was added dropwise at 0 °C. After 10 min, the mixture was stirred at rt for 0.5 h. The mixture was concentrated. The crude product was purified by FCC (DCM : MeOH = 10 :1) to afford **1e** (790 mg, 63.9%) as a white solid. ^1H NMR (400MHz, DMSO-d_6) 12.08 (s, 1H), 11.67 (s, 1H), 8.27 (s, 1H), 5.81 (d, J = 6.0 Hz, 1H), 5.51 (d, J = 6.0 Hz, 1H), 5.10 (t, J = 5.2 Hz, 1H), 4.59-4.57 (m, 1H), 4.01-3.99 (m, 1H), 3.86-3.84 (m, 1H), 3.65 - 3.57 (m, 2H), 3.41 (s, 3H), 3.17 (d, J = 5.2 Hz, 1H), 2.79-2.76 (m, 1H), 1.14 (s, 3H), 1.12 (s, 3H). ESI-MS: m/z= 368.0 $[M+1]^+$.

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

A solution of compound **1e** (790 mg, 2.15 mmol) and DMTrCl (0.765 g, 2.26 mmol) in pyridine (10 mL) was stirred at room temperature overnight. DMTCl (0.765 g, 2.26 mmol) was added and the reaction was stirred at room temperature for 2 h. The mixture was quenched with water (10 mL) and extracted with DCM (10 mL x 4). The combined organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated. The residue was purified by flash chromatography (DCM : MeOH = 15 : 1, R_f = 0.5) to afford compound **1f** (1.28 g, 88.9%) as a light yellow solid. ^1H NMR (400 MHz, $CDCl_3$) 11.87 (s, 1H), 7.68 - 7.66 (m, 2H), 7.57 (d, J = 7.6 Hz, 2H), 7.44 (t, J = 9.2 Hz, 4H), 7.31 - 7.29 (m, 2H), 7.22 - 7.19 (m, 1H), 6.87 -6.81 (m, 4H), 5.70 (d, J = 7.2 Hz, 1H), 5.30 -5.27 (m, 1H), 5.05 - 5.03 (m, 1H), 4.19 - 4.18 (m, 1H), 4.09 - 4.07 (m, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.58 - 3.55 (m, 1H), 3.47 (s, 3H), 3.05 - 3.03 (m, 1H), 1.47 -1.40 (m, 1H), 0.85 (d, J = 6.8 Hz, 3H), 0.55 (d, J=6.8 Hz, 3H); ESI-MS: m/z= 670.2 $[M+1]^+$.

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

To a solution of compound **1f** (1.28 g, 1.91 mmol) and DIPEA (741.0 mg, 5.73 mmol) in THF (5 mL) was added 3-((chloro(diisopropylamino)phosphino)oxy) propanenitrile (1.36 g, 5.73 mmol) at room temperature. The mixture was stirred at room temperature for 1 h. The reaction was quenched with MeOH. The mixture was extracted with EtOAc and the combined organic layers were washed with brine twice. The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated. The residue was purified by flash column chromatography (DCM : MeOH = 10:1, R_f = 0.6) to afford compound **1g** (1 g, 60.1%). ^{1}H NMR (400 MHz, CD_3CN) 7.88 (d, J = 9.0 Hz, 1H), 7.48 - 7.41 (m, 2H), 7.35 - 7.24 (m, 7H), 6.88 - 6.79 (m, 4H), 6.02 - 5.89 (m, 1H), 5.19 - 4.95 (m, 1H), 4.28 - 4.20 (m, 1H), 4.07 - 4.04 (m, 1H), 3.78 (d, J = 1.6 Hz, 7H), 3.67 - 3.48 (m, 4H), 3.44 (d, J = 15.6 Hz, 3H), 3.33 (td, J=2.8, 10.8 Hz, 1H), 2.72 - 2.65 (m, 1H), 2.61 - 2.53 (m, 1H), 2.51 (t, J = 6.0 Hz, 1H), 1.26 - 1.24 (m, 4H), 1.18 - 1.12 (m, 12H), 0.91 (d, J = 6.8 Hz, 3H); ^{31}P NMR (162 MHz, CD_3CN) 150.90 (s, 1P), 150.81 (s, 1P), 13.80 (s, 1P); ESI-MS: m/z 787.2 $[M+1]^+$.

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

To a solution of compound **1a** (4.3 g, 4.51 mmol) and water (156.8 mg, 8.7 mmol) in dry CH_3CN (16 mL) was added pyridinium trifluoroacetate (1.0 g, 5.2 mmol) at room temperature. After 1 min, t-butylamine (4 mL) was added. The resulting mixture was stirred at 15 °C for 20 min. The mixture was concentrated for 2 h to afford the crude product **1b** as a white solid (4.0 g). The crude product was used directly for the next step.

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

To a solution of compound **1b** (4.05 g, 4.35 mmol) and water (832.0 mg, 46.2 mmol) in DCM (40 mL) was added dichloroacetic acid (2.1 g, 16.3 mmol) at room temperature for 50 min. After 10 min, pyridine (730.6mg, 9.24mmol) was added. The mixture was concentrated and the residue was purified by flash column chromatography (CH_2Cl_2 : MeOH = 5 : 1, R_f = 0.4) to afford compound **1c** (2.45 g, 89.5%) as a white solid.

ESI-MS: m/z=449.9 $[M+1]^+$.

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

A solution of compound **1c** (300 mg, 0.48 mmol) and 4Å molecular sieves in dry CH_3CN (20 mL) was stirred at room temperature under N_2 for 10 min. 1*H*-imidazole perchlorate (1.5 g, 8.8 mmol) was added. After 10 min, compound **1g** (0.54 g, 0.62 mmol) in dry CH_3CN (5 mL) was added. The mixture was stirred at room temperature for 50 min. tert-Butyl hydroperoxide (0.43 mL, 2.39 mmol) was added. The resulting mixture was stirred at room temperature for 1 h and concentrated. The mixture was concentrated and the residue was purified by preparative HPLC (water (10mM NH_4HCO_3)-CH_3CN) to afford compound **1i** (168 mg, 34.1%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) 8.89 (s, 1H), 8.79 (s, 1H), 8.36 (s, 1H), 8.16 (d, J = 7.5 Hz, 2H), 7.73 - 7.67 (m, 1H), 7.62 (t, J = 7.0 Hz, 2H), 6.27 - 6.18 (m, 2H), 5.38 - 5.30 (m, 1H), 4.81 (m, 2H), 4.44 (s, 1H), 4.29 (s, 1H), 4.26 - 4.15 (m, 3H), 3.92 - 3.85 (m, 1H), 3.75 (d, J = 12.4 Hz, 1H), 3.61 - 3.57 (m, 3H), 2.74 (td, J = 6.6, 13.1 Hz, 1H), 1.21 (dd, J = 6.8, 15.2 Hz, 6H), 0.85 (s, 9H), 0.12 (s, 3H), -0.04 (s, 3H); ^{31}P NMR (162 MHz, CD_3OD) δ 3.61 (s, 1P), -1.68 (s, 1P); ESI-MS: m/z=1033.2 $[M+1]^+$.

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

To a solution of compound **1i** (160 mg, 0.16 mmol) and 4 Å molecular sieves in pyridine (40 mL) was added DMOCP (87.0 mg, 0.47 mmol) at room temperature. The mixture was stirred at room temperature for 1 h. Iodine (199.4 mg, 0.79 mmol) and water (28.3 mg, 1.57 mmol) were added. After 1 h, the mixture was filtered and then a saturated solution of Na_2SO_3 was added dropwise until the color of the filtrate changed to pale yellow. The mixture was filtered and the filtrate was concentrated. The residue was purified by preparative HPLC (water (10mM NH_4HCO_3)-CH_3CN from 23% to 53) to afford the target product **1k** (48 mg, 31.3%) as a white solid. ESI-MS: *m/z* 977.5 $[M+1]^+$.

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

Compound **1k** (40.0 mg, 0.041 mmol) was treated with a solution of methylamine in EtOH (33%, 10 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated to give crude compound **1l** (32.9 mg, 100%) which was used directly for the next step. ESI-MS: *m/z* 803.4 $[M+1]^+$.

Step 9: preparation of compound **2**

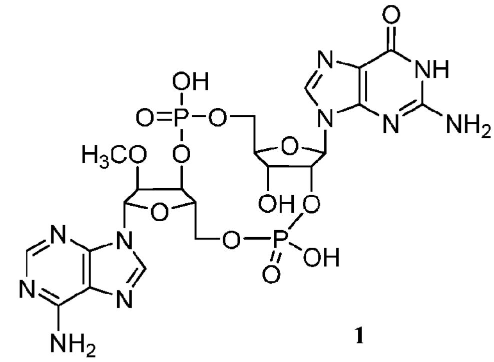
A solution of compound **1l** (32.86 mg, 0.041mmol), Et_3N (248.5 mg, 2.46 mmol) and triethylamine trihydrofluoride(198.0 mg, 1.2 mmol) in pyridine (5 mL) was stirred at 50 °C for 5 h. The mixture was diluted with THF (10 mL) and isopropoxytriethylsilane (541.5 mg, 4.1 mmol) was added at room temperature for 1.5 h. The mixture was concentrated and the residue was purified by preparative HPLC (water (0.05% NH_4OH v/v)-CH_3CN from 0% to 15 %) to afford the target product **2** as its ammonium salt (6.7 mg) as a white solid. ^1H NMR (400MHz, D_2O) δ = 8.18-8.16 (d, *J*=10 Hz, 2H), 7.74 (s, 1H), 6.06 (s, 1H), 5.79-5.77 (d, *J*=8.8 Hz, 1H), 5.62-5.56 (m, 1H), 4.99-4.94 (m, 1H), 4.45 (m, 1H), 4.39-4.34 (m, 2H), 4.15 - 4.03 (m, 4H), 3.46 (s, 3H); ^{31}P NMR (162MHz, D_2O) -1.28 (s, 1P), -2.65 (s, 1P); ESI-MS: *m/z* 689.5 $[M+1]^+$.

Step 9: preparation of (**Cpd 2, Na Salt**)

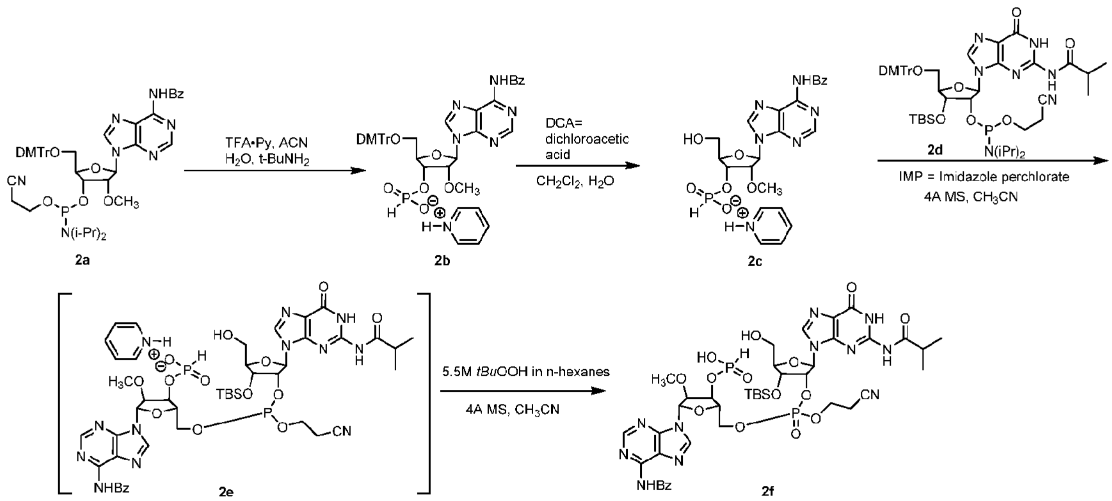
A 3 mL volume of Dowex 50W x 8, 200-400 (H form) was added to a beaker (for 6.7 mg of compound **5** ammonium salt) and washed with deionized water (2x). Then to the resin was added 15% H_2SO_4 in deionized water (50 mL), the mixture was stirred for 15 min, and decanted (1x). The resin was transferred to a column with 15% H_2SO_4 in deionized water and washed with 15% H_2SO4 (at least 4 CV), and then with deionized water until it was neutral. The resin was transferred back into the beaker, and 15% NaOH in water 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 water (at least 4 CV), and then with water until it was neutral (at least 4 CV). Compound **5** (6.7 mg) was dissolved in deionized water (6.7 mg in 1 mL) and added to the top of the column, and eluted with deionized water. The compound was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to give target compound **2 Na salt** (6.4 mg, 94.2%) as a white foam. ^1H NMR (400 MHz, D_2O) 8.16 (s, 1H), 8.13 (s, 1H), 7.74 (s, 1H), 6.04 (s, 1H), 5.78 (d, *J* = 8.8 Hz, 1H), 5.61 - 5.56 (m, 1H), 4.98 - 4.93

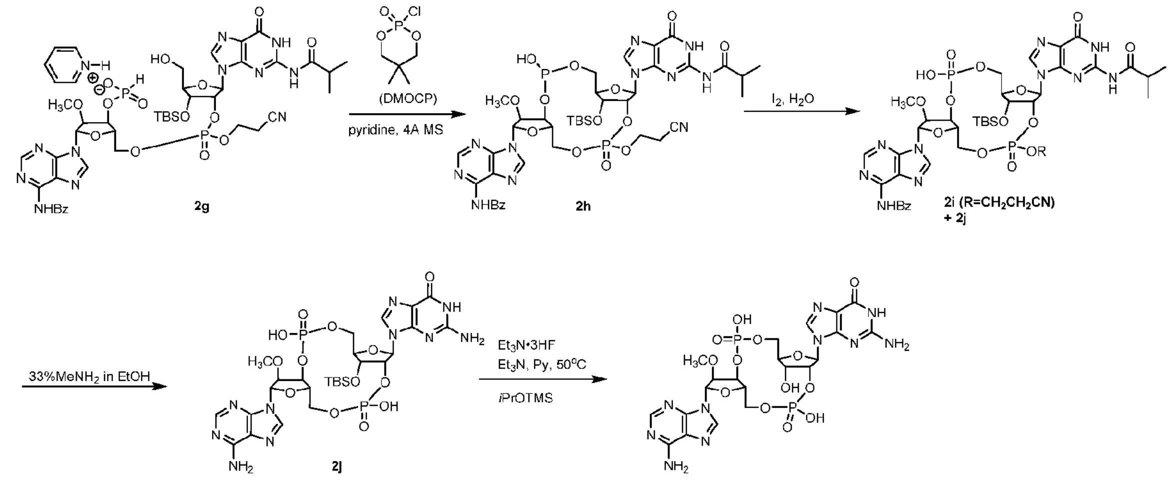
(m, 1H), 4.65 (s, 1H), 4.44 - 4.35 (m, 3H), 4.15 -4.05 (m, 4H), 3.46 (s, 3H); ^{31}P NMR (162MHz, D_2O) -1.26 , -2.64; ESI-MS: *m/z* 689.0 $[M+1]^+$.

Example 2

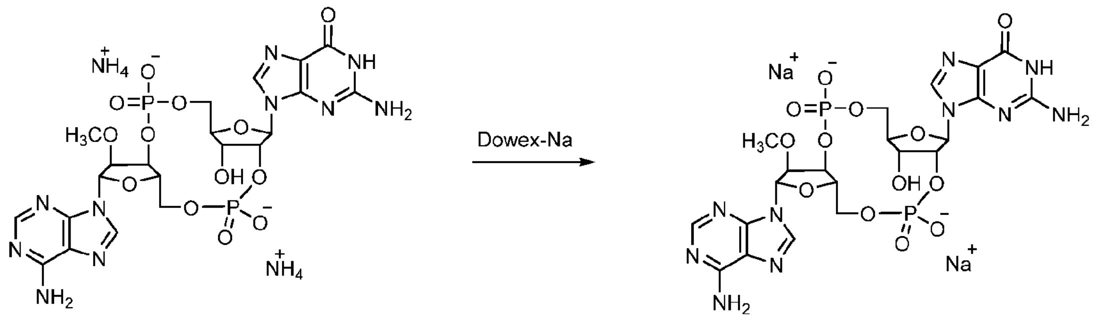


1





Compound 1, ammonium salt



Compound 1, ammonium salt **Compound 1**, sodium salt

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

To a solution of DMT-2'-OMe-Bz-adenosine-CE phosphoramidite **2a** (1.0 g, 1.13 mmol) and water (40.6 mg, 2.25 mmol) in dry CH_3CN (4 mL) was added pyridinium trifluoroacetate (261.0 mg, 1.35 mmol) at 15 °C. To the reaction mixture was added t-butylamine (4 mL). The mixture was concentrated to afford 1 g of the crude compound **2b** as a white solid, which was co-evaporated with DCM (3x) and used directly for the next step. ESI-MS: *m/z*=450.0 $[M+1]^+$. (DMT = 4,4'-dimethoxytrityl).

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

To a solution of compound **2b** (930.0 mg, 1.12 mmol) and water (0.2 g, 11.2 mmol) in CH_2Cl_2 (10 mL) was added dichloroacetic acid (0.51 g, 4.0 mmol) at 15 °C for 0.5 h. After 10 min, pyridine was added. The mixture was concentrated and the residue was purified by flash column chromatography (CH_2Cl_2 : MeOH =5: 1, R_f = 0.5) to afford compound **2c** (400 mg, 0.76 mmol, 67.6% yield) as a white solid. ^{31}P NMR (400 MHz, DMSO-d_6) δ 0.05; ESI-MS: *m/z* = 450.0 (M+1).

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

A solution of compound **2c** (860.0 mg, 1.63 mmol) and 4Å molecular sieves (1 g) in dry CH_3CN (16 mL) was stirred at 15 °C under N_2 for 10 min. 1*H*-Imidazole perchlorate (5.16 g, 30.26 mmol) was added. After 10 min, DMT-3'-O-TBDMS-G(iBu)-CE phosphoramidite **2d** (2.05 g, 8.11 mmol) in dry CH_3CN (4 mL) was added. The mixture was stirred at room temperature for 50 min. A solution of tert-butyl hydroperoxide (TBHP, 1.48 mL, 8.14 mmol, 5.5 M in hexane) was added. The resulting mixture was stirred at 15 °C for 1 h. The mixture was concentrated and the residue was purified by preparative HPLC (water (10 mM NH_4HCO_3)-CH_3CN) to afford compound **2f** (600 mg, 0.58 mmol, 35.7% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.61 (d, *J* = 5.2 Hz, 1H), 8.41 - 8.30 (m, 1H), 8.24 - 8.17 (m, 1H), 8.04 - 7.90 (m, 2H), 7.61 - 7.49 (m, 2H), 7.48 - 7.40 (m, 2H), 6.11 - 6.03 (m, 1H), 6.02 - 5.98 (m, 1H), 5.36 - 5.12 (m, 1H), 4.55 - 4.43 (m, 2H), 4.41 - 4.32 (m, 1H), 4.30 - 4.19 (m, 2H), 4.13 - 4.02 (m, 1H), 3.99 - 3.85 (m, 2H), 3.75 - 3.65 (m, 1H), 3.63 - 3.53 (m, 1H), 3.37 (s, 3H), 2.70 - 2.69 (m, 1H), 2.60 - 2.52 (m, 2H), 1.10 - 1.03 (m, 6H), 0.82-0.77 (m, 9H), 0.04 - 0.00 (m, 6H); ^{31}P NMR (162 MHz, CD_3OD) δ 3.17, 3.13, -2.58, -2.69; ESI-MS: m/z=517.1 $[M/2+1]^+$ and 1032.3 $[M+1]^+$.

Step 4: preparation of compound **2i** + compound **2j**

To a solution of compound **2g** (280.0 mg, 0.27 mmol) and 4Å molecular sieves (1 g) in pyridine (60 mL) was added 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxa- phosphinane (DMOCP, 150.2 mg, 0.81 mmol) at 16 °C. The mixture was stirred at 16 °C for 1 h. Iodine (344.3 mg, 1.36

mmol) and water (48.9 mg, 2.71 mmol) were added. After 1 h, the reaction was quenched with a saturated solution of Na_2SO_3. The mixture was filtered and the filtrate was concentrated. The residue was purified by preparative HPLC (water (10 mM NH_4HCO_3)-CH_3CN) to afford a mixture of compound **2i** and compound **2j** (170 mg, 0.17 mmol, 60.8% yield) as a white solid. ESI-MS: *m/z*=1030.4 $[M+H]^+$.

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

A mixture of compound **2i** and compound **2j** (170 mg, 0.17 mmol) was treated with a solution of methylamine in EtOH (15 mL, 33%) and the resulting solution was stirred at 15 °C for 1 h. The crude product **2j** (134.3 mg) was used directly for the next step.

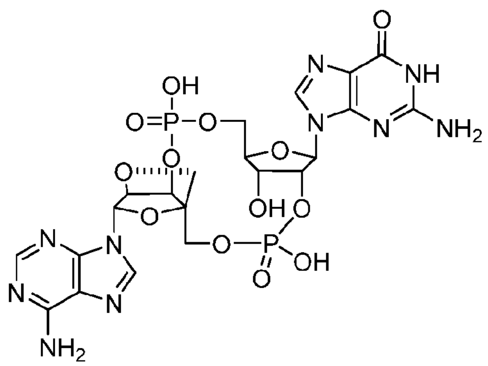
Step 6: preparation of compound **1**

A solution of compound **2j** (134.3 mg, crude), Et_3N (1.0 g, 10.0 mmol) and triethylamine trihydrofluoride (Et_3N-3HF, 807.4 mg, 5.00 mmol) in pyridine (10 mL) was stirred at 50 °C for 5 h. The mixture was diluted with THF (10 mL) and isopropoxy trimethylsilane (2.2 g, 16.7 mmol) was added. After stirring at 15 °C for 1 h, the mixture was concentrated at 15 °C and the residue was purified by preparative HPLC (water (0.05% NH_4OH v/v)-CH_3CN) to afford compound **1** as its ammonium salt (19.5 mg, 0.028 mmol) as a white solid upon lyophilization. ^1H NMR (400 MHz, D_2O) δ 8.26 (s, 1H), 8.15 (s, 1H), 7.78 (s, 1H), 6.116 (s, 1H), 5.87 (d, J = 8.4 Hz, 1H), 5.66 (s, 1H), 4.95 (s, 1H), 4.48 (d, J = 4.4 Hz, 1H), 4.24 (m, 5H), 3.97 (d, J = 11.7 Hz, 1H), 3.83 (d, J = 12.0 Hz, 1H), 3.69 (s, 3H); ^{31}P NMR (162 MHz, D_2O) δ -1.57, -3.38; ESI-MS: m/z = 688.9 $[M+H]^+$.

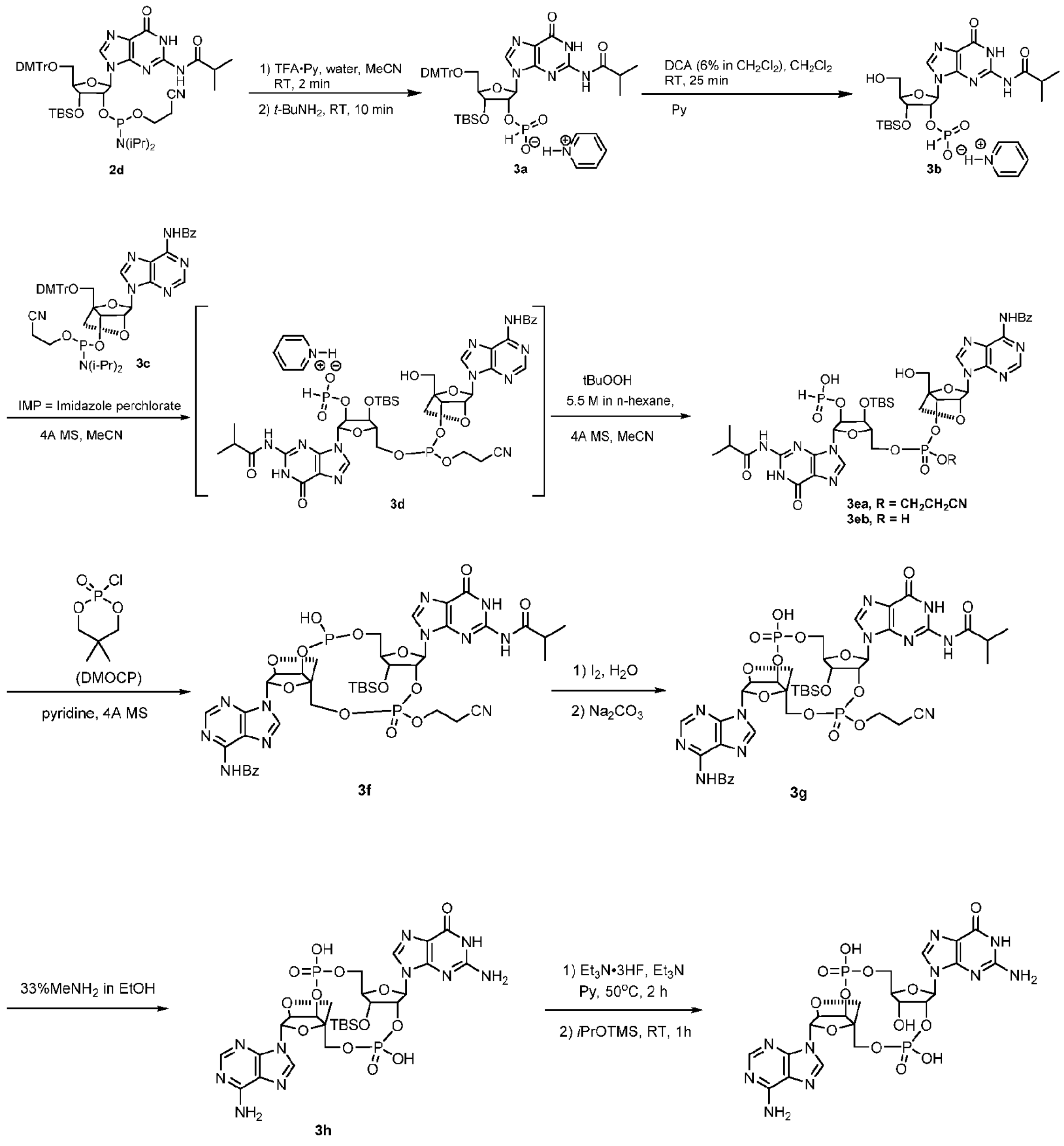
Preparation of **compound 1**, sodium salt

Dowex 50W × 8, 200-400 (25 mL, H form) was added to a beaker and washed with deionized water (60 mL). Then to the resin was added 15% H_2SO_4 in deionized water, and the mixture was gently stirred for 5 min, and decanted (50 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 CV), and then with deionized water until it was neutral. The resin was transferred back into the beaker, 15% NaOH in deionized water solution was added, and mixture was gently stirred for 5 min, and decanted (1x). The resin was transferred to the column and washed with 15% NaOH in H_2O (at least 4 CV), and then with deionized water until it was neutral. Compound **1**, ammonium salt (16 mg) was dissolved in a minimum amount of deionized water, added to the top of the column, and eluted with deionized water. Appropriate fractions of CDN based on UV were pooled together and lyophilized to afford the sodium salt form of compound **1** (13.5 mg). ^{1}H NMR (400 MHz, D_2O) δ 8.17 (s, 1H), 8.14 (s, 1H), 7.73 (s, 1H), 6.14 (s, 1H), 5.83 (d, J = 8.8 Hz, 1H), 5.58-5.52 (m, 1H), 5.01 (s, 1H), 4.98-4.90 (m, 1H), 4.04-4.51 (m, 5H), 4.04 (d, J = 11.7 Hz, 1H), 3.78 (d, J = 12.0 Hz, 1H), 3.69 (s, 3H); 31P NMR (162 MHz, D_2O) δ -1.63, -2.29; ESI-MS: m/z = 689 [M+H]+.

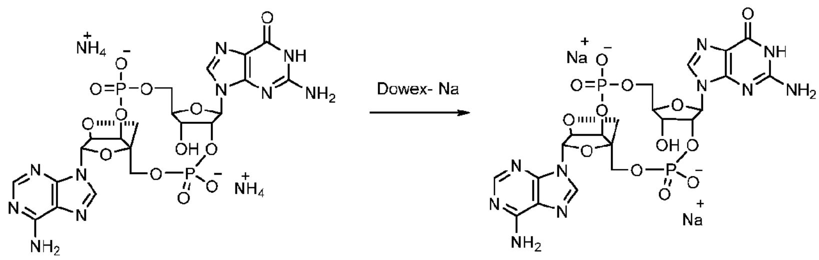
Example 3



Cpd 6



Cpd **6** ammonium salt



Cpd **6** ammonium salt   Cpd **6** sodium salt

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

To a solution DMT-3'-O-TBDMS-G(iBu)-CE phosphoramidite compound **2d** (1 g, 1.03 mmol) and water (37.1 mg, 2.06 mmol) in CH_3CN (4 mL) was added pyridinium trifluoroacetate (238.9 mg, 1.2 mmol) at room temperature. To the reaction mixture was added tert-butylamine (4 mL). The resulting mixture was stirred at room temperature for 20 min. The mixture was concentrated to afford compound **3a** (941.1 mg) as a white solid, which was co-evaporated with DCM (3x) and used directly for the next step.

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

To a solution of compound **3a** (941.1 mg, 1.03 mmol) and water (0.19 g, 10.0 mmol) in CH_2Cl_2 (30 mL) was added a solution of dichloroacetic acid (0.47 g, 3.62 mmol, 6% in DCM) at room temperature for 0.5 h. Pyridine (0.163 g, 2.06 mmol) was added. After 10 min, the mixture was concentrated and the residue was purified by flash column chromatography (DCM : MeOH = 5 : 1, R_f = 0.5) to afford **3c** (515 mg, 0.84 mmol) as a white solid. ESI-MS *m/z* 532.1 $[M+1]^+$.

Step 3: preparation of compound **3ea** + compound **3eb**

A solution of compound **3b** (500 mg, 0.82 mmol) and 4Å MS (0.5 g) in dry CH_3CN (10 mL) was stirred at room temperature under N_2 for 3 min. 1*H*-Imidazole perchlorate (IMP, 2.54 g, 15.1 mmol) was added. After 10 min, LNA-dA(Bz)-CE phosphoramidite, compound **3c** (943 mg, 1.06 mmol) in CH_3CN (5 mL) was added. The mixture was stirred at room temperature for

50 min. A solution of tert-butyl hydroperoxide (TBHP, 5.5 M in hexane, 0.74 mL, 4.09 mmol) was added. The resulting mixture was stirred at room temperature for 1 h. The mixture was concentrated and the residue was purified by preparative HPLC (water (10mM NH_4HCO_3)-ACN) to afford a mixture of compound **3ea** and compound **3eb** (135.7 mg, 0.132 mmol) as a white solid. The product mixture was used directly for the next step. ESI-MS *m/z* 1030.1 $[M+1]^+$.

Step 4: preparation of compound **3g**

To a solution of compound **3ea** and compound **3eb** (135.7 mg, 0.132 mmol) and 4Å molecular sieves (0.5 g) in pyridine (30 mL) was added DMOCP (72.6 mg, 0.39 mmol) at 29 °C. The mixture was stirred at 29 °C for 1 h. Iodine (166.3 mg, 0.66 mmol) and water (23.6 mg, 1.31 mmol) were added. After 1 h, the reaction was quenched with a saturated solution of Na_2SO_3. The mixture was filtered and the filtrate was concentrated. The residue was purified by preparative HPLC (water (10mM NH_4HCO_3)-CH_3CN from 35% to 65%) to afford compound **3g** (22 mg, 0.021 mmol) as a white solid. ESI-MS *m/z* 514.5 $[M/2+1]^+$ and 1029.3 $[M+1]^+$.

Step 5: preparation of compound **3h**

Compound **3g** (22 mg, 0.021 mmol) was treated with a solution of methylamine in EtOH (33%, 10 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated to give crude compound **3h**, which was co-evaporated with pyridine (3x) and used directly into the next step.

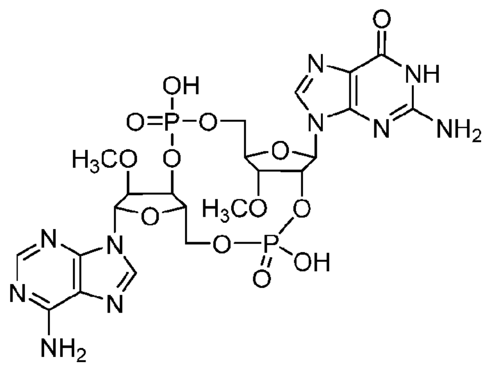
Step 6: preparation of compound **6** ammonium salt

A solution of compound **3h**, Et_3N (176.7 mg, 1.75 mmol) and triethylamine trihydrofluoride (Et_3N-3HF, 140.7 mg, 0.87 mmol) in pyridine (10 mL) was stirred at 50 °C for 5 h. The mixture was diluted with THF (10 mL) and isopropoxytrimethylsilane (384.9 mg, 2.91 mmol) was added at 15 °C for 1 h. The mixture was concentrated at room temperature and the residue was purified by preparative HPLC (water (0.05% NH_4OH v/v)-CH_3CN from 0% to 15 %) to afford compound **6** as its ammonium salt (6.1 mg, 0.009 mmol) as a white solid upon lyophilization. ^1H NMR (400 MHz, D_2O) 8.30 (s, 1H), 8.11 (s, 1H), 7.83 (brs, 1H), 6.19 (s, 1H), 5.96 (d, J = 6.8 Hz, 1H), 4.98 (s, 1H), 4.57 (s, 1H), 4.36 - 4.30 (m, 3H), 4.16 (d, J = 6.0 Hz, 3H), 4.04 (d, J = 7.6 Hz, 1H), 3.86 (d, J = 11.6 Hz, 2H); ^{31}P NMR (162 MHz, D_2O) -1.73, -3.40; ESI-MS *m/z* 687.0 $[M+1]^+$.

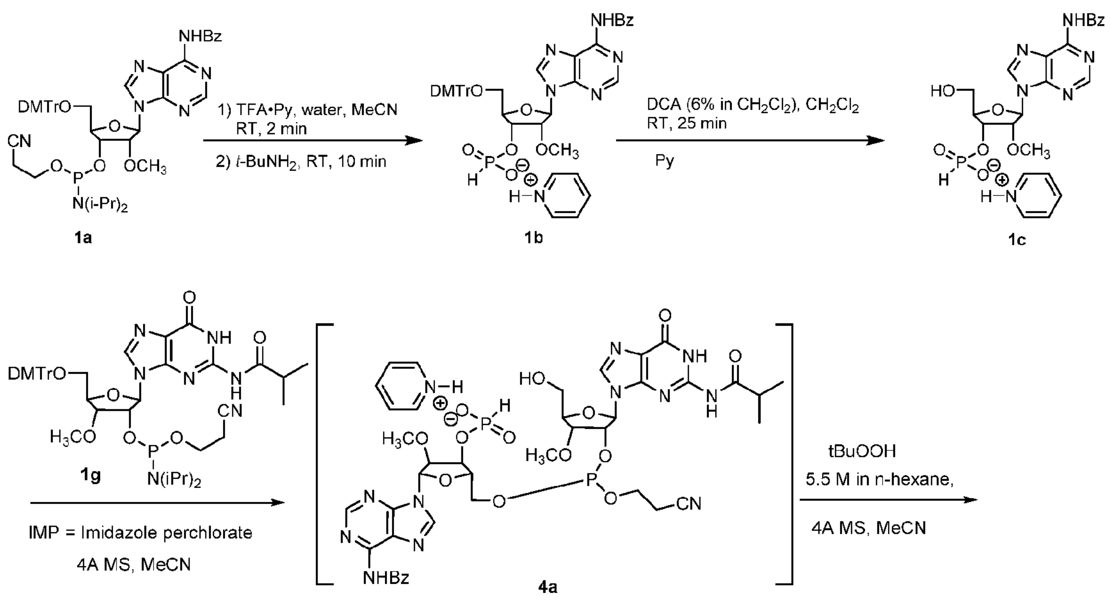
Preparation of **compound 6** sodium salt

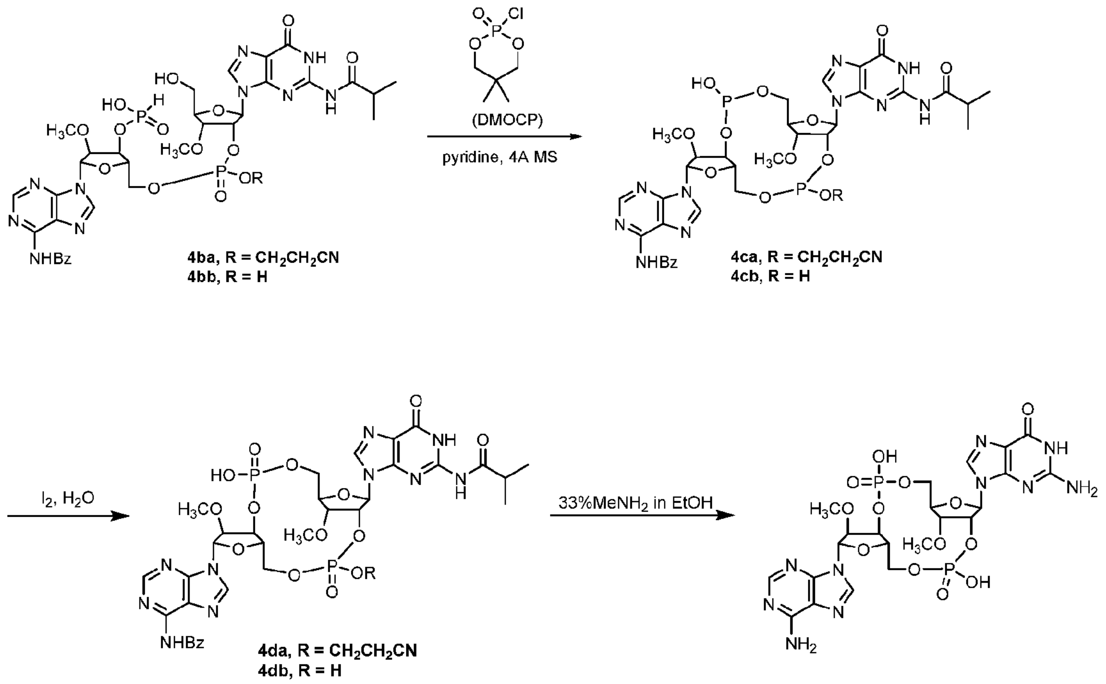
Dowex 50W × 8, 200-400 (2mL, H form) was added to a beaker and washed with deionized water (15 mL). Then to the resin was added 15% H_2SO_4 in deionized water, the mixture was gently stirred for 5 min, and decanted (10 mL). The resin was transferred to a column with 15% H_2SO_4 in deionized water and washed with 15% H_2SO_4 (at least 4 CV), and then with deionized water until it was neutral. The resin was transferred back into the beaker, 15% NaOH in deionized water solution was added, and 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 CV), and then with deionized water until it was neutral. Compound **6**, ammonium salt (3.5 mg) was dissolved in a minimum amount of deionized water, added to the top of the column, and eluted with deionized water. Appropriate fractions of CDN based on UV were pooled together and lyophilized to afford the sodium salt of compound **6** (3.02 mg). ^{1}H NMR (400 MHz, D_2O): δ 8.11 (s, 1H), 7.88 (s, 1H), 7.74 (s, 1H), 6.02 (s, 1H), 5.85 (d, J = 8.4 Hz, 1H), 5.65-5.58 (s, 1H), 4.91 (d, J = 3.2 Hz, 1H), 4.83 (s, 1H), 4.50 (d, J = 4.8 Hz, 1H), 4.35-4.28 (m, 1H), 4.26-4.19 (m, 2H), 4.13-4.01 (m, 3H), 3.90 (d, J = 8 Hz, 1H), 3.76 (d, J = 12.4 Hz, 2H); ^{31}P-NMR (162 MHz, D_2O) δ -1.64, -1.91.

Example 4

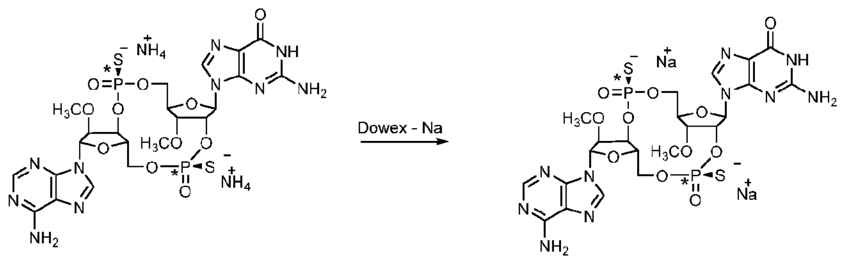


Cpd 3





Cpd 3



Compound 3 ammonium salt **Compound 3** sodium salt

Step 1: preparation of compound **4ba** and compound **4bb**

A solution of compound **1c** (300 mg, 0.57 mmol) and 4Å molecular sieves (0.5 g) in dry CH_3CN (10 mL) was stirred at 29 °C under N_2 for 3 min. 1*H*-Imidazole perchlorate (1.76 g, 10.5 mmol) was added. After 10 min, a solution of compound **1g** (500 mg, 0.58 mmol) in dry CH_3CN (10 mL) was added. The mixture was stirred at room temperature for 50 min and tert-butyl hydroperoxide (TBHP, 0.52 mL, 2.84 mmol) was added. The resulting mixture was stirred at 29 °C for 1 h. The mixture was concentrated and the residue was purified by preparative HPLC (water (10 mM NH_4HCO_3)-CH_3CN) to afford a mixture of compounds **4ba** and **4bb** (100 mg, 0.114 mmol) as a white solid. ^{31}P NMR (162MHz, DMSO) -0.66, -2.44; ESI-MS: *m/z* 932.3 (M+1).

Step 2: preparation of compound **4da** and compound**4db**

To a suspension of compound **4ba** and compound **4bb** (100.0 mg, 0.11 mmol) and 4Å molecular sieves (0.5 g) in pyridine (30 mL) was added DMOCP (59.4 mg, 0.32 mmol) at 28 °C. The mixture was stirred at 28 °C for 1 h. Iodine (136.2 mg, 0.54 mmol) and water (19.3 mg, 1.1 mmol) were added. After 1 h, the reaction was quenched with a saturated solution of Na_2SO_3. The mixture was filtered and the filtrate was concentrated. The residue was purified by preparative HPLC (water (10 mM NH_4HCO_3)-ACN from 1% to 28%) to afford a mixture of compounds **4da** and **4db** (50 mg, 0.057 mmol) as a white solid. The product was used directly for the next step.

Step 3: preparation of compound **3**, **ammonium salt**

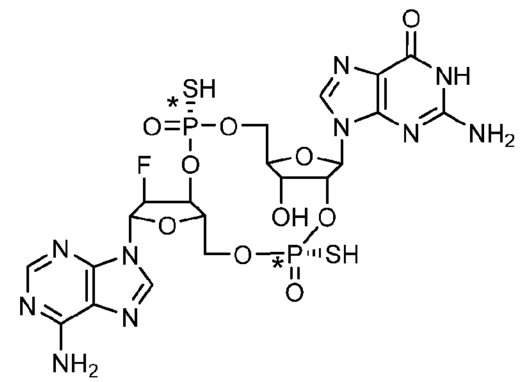
A mixture of compounds **4da** and **4db** (50 mg, 0.057 mmol) was treated with a solution of methylamine in EtOH (33%, 20 mL) and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated to give crude compound **3**, which was purified by preparative HPLC (water (0.05% NH_4OH v/v)-CH_3CN from 0% to 10%) to give compound **3** ammonium salt as a white solid (16 mg, 0.023 mmol). ^{31}P NMR (162 MHz, D_2O) -1.53, -3.41.

The product was further purified by preparative HPLC (water (0.05% NH_4OH v/v)-CH_3CN from 0% to 10%) to afford compound **3** as its ammonium salt, as a white solid.

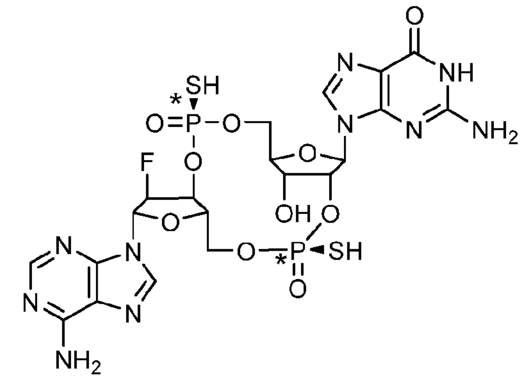
Step 4: preparation of compound **3**, **sodium salt**

Compound **3** ammonium salt was dried under high vacuum to give a white solid (12 mg). Dowex 50W x 8, 200-400 (H form, 3mL) was added to a beaker (for 12 mg of compound **6**) and washed with deionized water (2x). Then to the resin was added 15% H_2SO_4 in deionized water (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 deionized water and washed with 15% H_2SO_4 (at least 4 CV), and then with deionized water until it was neutral. The resin was transferred back into the beaker, and 15% NaOH in deionized water 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 deionized water (at least 4 CV), and then with deionized water until it was neutral (at least 4 CV). Compound **3** was dissolved in deionized water (12 mg in 1 mL), added to the top of the column, and eluted with deionized water. The converted sodium salt was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to give compound **3**, sodium salt (7.4 mg, 0.010 mmol). ^{1}H NMR (400 MHz, D_2O) δ ppm 8.17 (s, 1H), 8.14 (s, 1H), 7.74 (s, 1H), 6.14 (s, 1H), 5.79 (d, J = 8.8 Hz, 1H), 5.65 - 5.59 (m, 1H), 5.02 - 5.00 (m, 1H), 4.44 (s, 1H), 4.36 - 4.29 (m, 3H), 4.15 - 4.11 (m, 3H), 4.04 - 4.01 (m, 1H), 3.66 (s, 3H), 3.46 (s, 3H); ^{31}**P** NMR (162 MHz, D_2O) -1.53 , -2.62; ESI-MS *m/z* 702.5 (M+1).

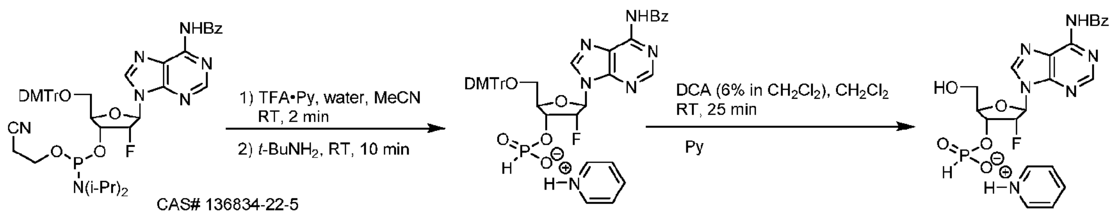
<u>Example 5</u>



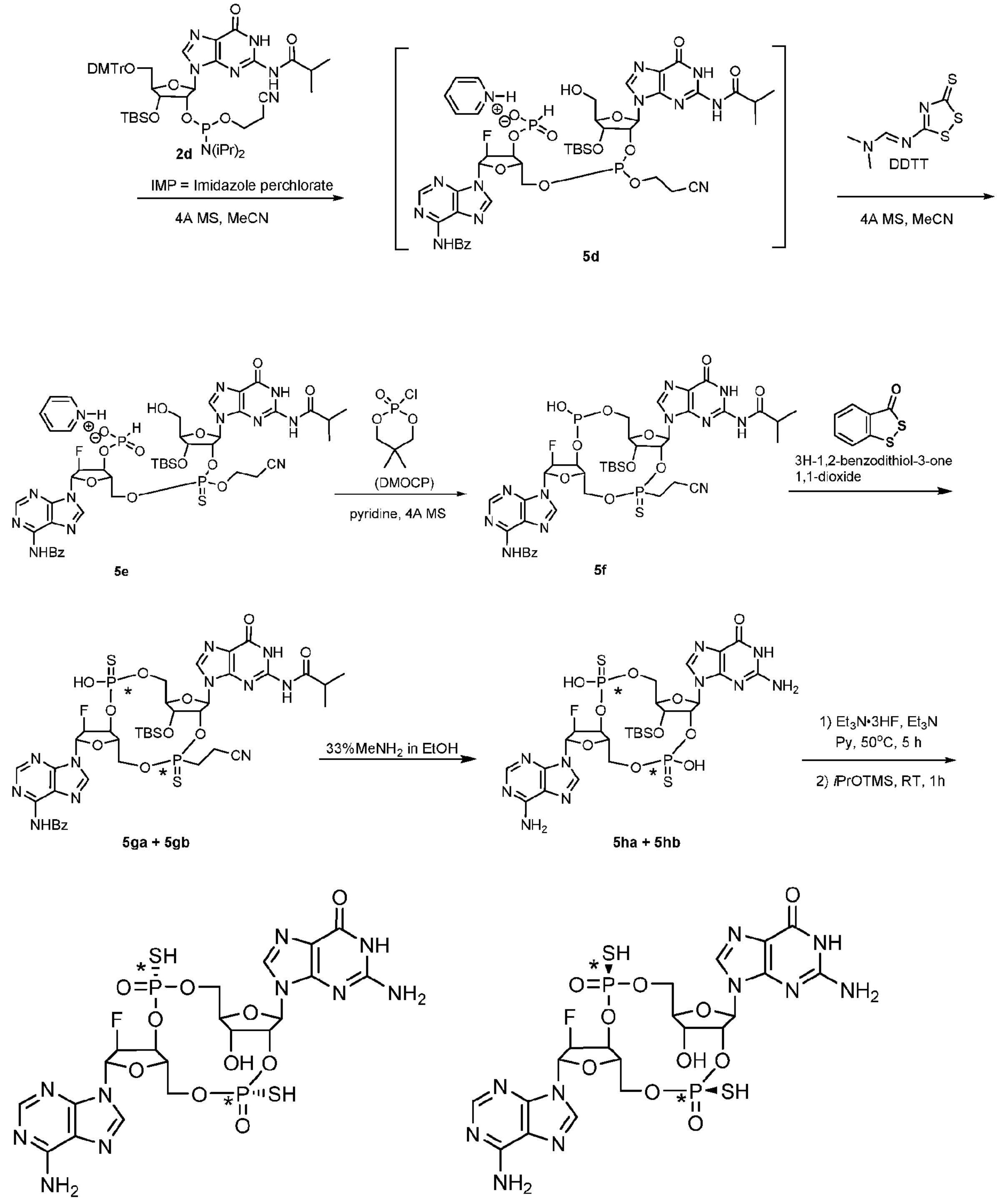
Cpd **4**



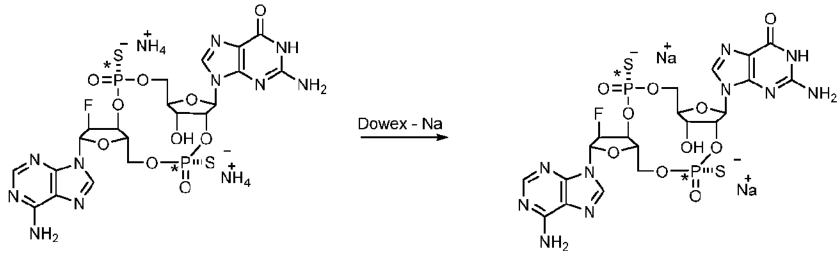
Cpd **5**



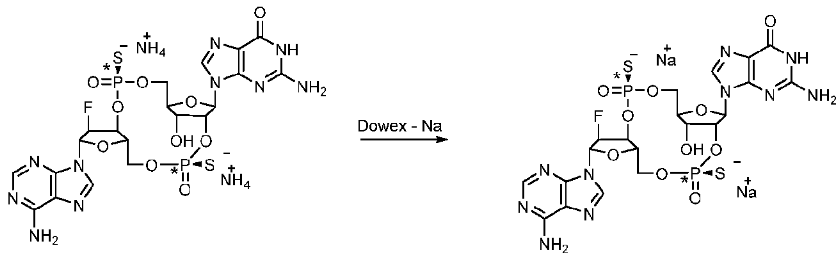
5a **5b** **5c**


DMTrO
TBSO
2d
N(iPr)2
CN
IMP = Imidazole perchlorate
4A MS, MeCN
5d
NHBz
TBSO
DDTT
4A MS, MeCN
5e
(DMOCP)
pyridine, 4A MS
5f
3H-1,2-benzodithiol-3-one 1,1-dioxide
5ga + 5gb
33%MeNH2 in EtOH
5ha + 5hb
1) Et3N•3HF, Et3N Py, 50°C, 5 h
2) iPrOTMS, RT, 1h

Cpd 4 **Cpd 5**



Compound 4 ammonium salt **Compound 4** sodium salt



Compound 5 ammonium salt **Compound 5** sodium salt

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

To a solution of DMT-2'-F-dA(Bz)-CE phosphoramidite **5a** (2.20 g, 2.51 mmol) in CH_3CN (12.0 mL) was added water (90.5 mg, 5.02 mmol, 2.0 eq) and pyridinium trifluoroacetate (582.1 mg, 3.01 mmol, 1.2 eq). The mixture was stirred at 25 °C for 5 min. Then tert-butylamine (12.0 mL) was added and the reaction mixture was stirred at 25 °C for 15 min. The mixture was concentrated under reduced pressure to give a foam, which was dissolved in CH_3CN (10.0 mL) and concentrated again to afford compound **5b** (1.69 g, 2.29 mmol, 91.0% yield) as a white foam. ESI-MS: *m/z* 740.2 $[M + H]^+$.

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

To a solution of compound **5b** (1.69 g, 2.29 mmol) in CH_2Cl_2 (24.0 mL) was added water (411.8 mg, 21.9 mmol, 10.0 eq) and a solution of 2, 2-dichloroacetic acid (6% in DCM, 24 mL) slowly. The mixture was stirred at 25 °C for 0.5 h. The reaction was quenched with pyridine (2 mL) and the reaction mixture was concentrated to afford a residue, which was purified by column chromatography on silica gel (DCM/MeOH = 10/1 to 5/1) to afford compound **5c** (856 mg, 1.62 mmol, 70.9% yield) as a white foam.

Step 3: preparation of compound **5e**

To a solution of compound **5c** (380 mg, 0.70 mmol) in CH_3CN (12.0 mL) was added 4Å molecular sieves (0.5 g), the resulting mixture was stirred at 25 °C for 10 min. 1*H*-Imidazole perchlorate (IMP, 356.7 mg, 2.1 mmol, 3.0 eq) was added and the mixture stirred for an additional 10 min before DMT-3'-O-TBDMS-G(iBu)-CE phosphoramidite **2d** ((*J. Am. Chem. Soc.* 2001,123, 8165-8176), 811.6 mg, 0.84 mmol, 1.2 eq) was added. The mixture was stirred at 25 °C for 1 h to afford a solution of compound **5d** (a solution in CH_3CN), then N,N-dimethyl-N'-(5-sulfanylidene-1,2,4-dithiazol-3-yl)methanimidamide (DDTT, 715.7 mg, 3.49 mmol, 5 eq.) was added to above reaction mixture at 25 °C and stirred for 1 h at the same temperature. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to afford a residue, which was purified by preparative HPLC (H_2O-CH_3CN) to afford compound **5e** (130.0 mg, 0.125 mmol, 18.0% yield over two steps) as a white solid. ESI-MS: *m/z* 1036.1 $[M + H]^+$.

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

To a solution of compound **5e** (130.0 mg, 0.125 mmol) in pyridine (24 mL) was added 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide (DMOCP, 69.5 mg, 0.38 mmol, 3.0 eq) at 25 °C, the mixture was stirred at 25 °C for 1 h to afford a solution of compound **5f** (127.7 mg, 0.125 mmol, 100% yield, a solution in pyridine) which was used for the next step without further purification.

Step 5: preparation of compound **5ga** + compound **5gb**

To a solution of compound **5f** (127.7 mg, 0.125 mmol) in pyridine was added 3H-benzo[c][1,2]dithiol-3-one (211.1 mg, 1.26 mmol, 10 eq) at 25 °C, and the resulting mixture was stirred at 25 °C for 1 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to afford a residue, which was purified by preparative HPLC (water (0.225% formic acid)-CH_3CN) to afford compound **5ga** (22.0 mg, 0.021 mmol, 18.1% yield over two steps) as a white solid and compound **5gb** (55.0 mg, 0.052 mmol, 45.2% yield over two steps) as a white solid. ESI-MS: *m/z* 1050.2 $[M + H]^+$, 525.8 $[M/2 + H]^+$ (compound **5ga**). ESI-MS: *m/z* 1050.2 $[M + H]^+$, 525.6 $[M/2 + H]^+$ (compound **5gb**).

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

Compound **5gb** (55.0 mg, 0.052 mmol, 1.00 eq) was treated with a solution of methylamine (3.00 mL, 35% in EtOH) and the resulting mixture was stirred at 25 °C for 12 h. The reaction mixture was concentrated under reduced pressure to afford compound **5hb** (39.0 mg, 0.047 mmol, 90.5% yield) which was used for the next step without further purification. ESI-MS: *m/z* 823.1 $[M + H]^+$.

Step 7: preparation of compound **4**, **ammonium salt**

To a solution of compound **5hb** (44.0 mg, 0.053 mmol) in pyridine (13.0 mL) was added Et_3N (324.7 mg, 3.2 mmol, 60 eq) and triethylamine trihydrofluoride (258.6 mg, 1.6 mmol, 30 eq) at 25 °C, and the mixture was stirred at 50 °C for 5 h, then isopropoxytrimethylsilane (707.4 mg, 5.3 mmol, 100 eq) was added at 15 °C and stirred for 1 h. The mixture was concentrated at 15 °C and the residue was purified by preparative HPLC (water (0.05% NH_4OH v/v)-CH_3CN) to afford compound **4** as its ammonium salt (6.0 mg, 0.008 mmol, 15.8% yield) as a white solid. ^1H NMR (400 MHz, D_2O) δ 8.33 (s, 1H), 8.25 (s, 1H), 7.85 (s, 1H), 6.45 (d, *J* = 14.3 Hz, 1H), 5.92 (d, *J* = 8.5 Hz, 1H), 5.77 (s, 1H), 5.51 (s, 0.5H), 5.38 (s, 0.5H), 5.23 (d, *J* = 21.5 Hz, 1H), 4.53-4.42 (m, 5H), 4.10 (d, *J* = 11.3 Hz, 1H), 3.99 (d, *J* = 12.8 Hz, 1H); ^{19}F NMR (376 MHz, D_2O) □ -122.94 (br, s, 1F); ^{31}P NMR (162 MHz, D_2O) □ 55.99 (brs, 1P), 51.19 (brs, 1P); ESI-MS: *m/z* 708.9 $[M + H]^+$.

Step 6a: preparation of compound **5ha**

Compound **5ga** (13.0 mg, 0.012 mmol, 1.00 eq) was treated with a solution of methylamine (1.00 mL, 35% in EtOH) and the solution was stirred at 25 °C for 12 h. The reaction mixture was concentrated under reduced pressure to afford compound **5ha** (9.0 mg, 0.011 mmol, 88.4% yield) which was used for the next step without further purification. ESI-MS: *m/z* 823.3 $[M + H]^+$.

Step 7a: preparation of compound **5**, **ammonium salt**

To a solution of compound **5ha** (39.0 mg, 0.047 mmol) in pyridine (7.0 mL) was added Et_3N (287.8 mg, 2.84 mmol, 60 eq) and triethylamine trihydrofluoride (229.2 mg, 1.42 mmol, 30 eq) at 25 °C, the mixture was stirred at 50 °C for 5 h, then isopropoxytrimethylsilane (627.0 mg, 4.74 mmol, 100 eq) was added and at 15 °C and stirred for 1 h. The mixture was concentrated at 15 °C and the residue was purified by preparative HPLC (water (0.05% NH_4OH v/v)-CH_3CN) to afford compound **5** as its ammonium salt (6.60 mg, 0.009 mmol, 19.6% yield) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 8.54 (s, 1H), 8.25 (s, 1H), 7.84 (s, 1H), 6.46 (d, J = 13.8 Hz, 1H), 5.94 (d, J = 8.3 Hz, 1H), 5.81-5.75 (m, 1H), 5.54 (d, J = 2.8 Hz, 0.5H), 5.41 (d, J = 3.0 Hz, 0.5H), 5.30-5.23 (m, 1H), 4.54-4.40 (m, 5H), 4.09-4.04 (m, 2H); ^{19}F NMR (376 MHz, D_2O) δ -201.92 (brs, 1F); ^{31}P NMR (162 MHz, D_2O) δ 55.97 (s, 1P), 53.90 (brs, 1P); MS: m/z 708.9 $[M + H]^+$.

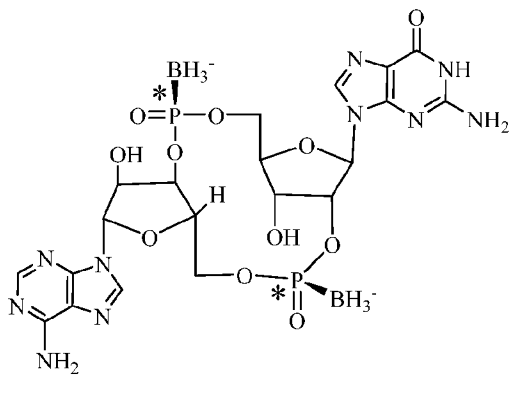
Step 8: preparation of **compound 4, sodium salt**

Dowex 50W × 8, 200-400 (3 mL, H form) was added to a beaker and washed with deionized water (15 mL). Then to the resin was added 15% H_2SO_4 in deionized water, the mixture was gently stirred for 5 min, and decanted (10 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 CV), and then with deionized water until it was neutral. The resin was transferred back into the beaker, 15% NaOH in deionized water solution was added, and 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 CV), and then with deionized water until it was neutral. Compound **4,** ammonium salt (6.9 mg) was dissolved in a minimal amount of deionized water, added to the top of the column, and eluted with deionized water. Appropriate fractions of CDN based on UV were pooled together and lyophilized to afford the sodium salt form of compound **4** (6.45 mg). ^{1}H NMR (400 MHz, D_2O) δ 8.41 (s, 1H), 8.12 (s, 1H), 7.73 (s, 1H), 6.35 (d, J = 13.6 Hz, 1H), 5.83 (d, J = 8.4 Hz, 1H), 5.72-5.68 (m, 1H), 5.43 (d, J = 2.8 Hz, 0.5 H), 5.30 (d, J = 2.8 Hz, 0.5 H), 5.18-5.10 (m, 1H), 4.65-4.29 (m, 5H), 4.05-3.93 (m, 2H); ^{19}F NMR (376 MHz, D_2O) δ -201.76; ^{31}P NMR (162 MHz, D_2O) □ 55.88, 53.91.

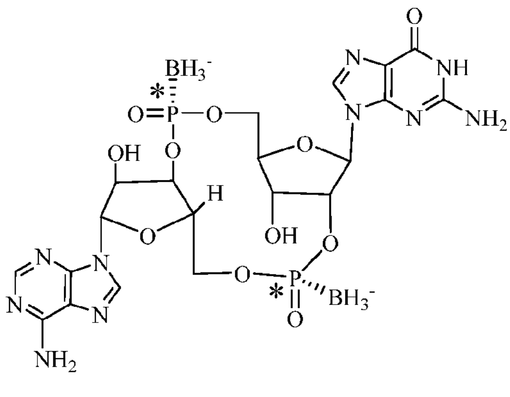
Step 9: preparation of **compound 5**, **sodium salt**

Dowex 50W × 8, 200-400 (3 mL, H form) was added to a beaker and washed with deionized water (15 mL). Then to the resin was added 15% H_2SO_4 in deionized water, the mixture was gently stirred for 5 min, and decanted (10 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 CV), and then with deionized water until it was neutral. The resin was transferred back into the beaker, 15% NaOH in deionized water solution was added, and 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 CV), and then with deionized water until it was neutral. Compound **5**, ammonium salt (6.0 mg) was dissolved in minimal amount of deionized water, added to the top of the column, and eluted with deionized water. Appropriate fractions of CDN based on UV were pooled together and lyophilized to afford the sodium salt form of compound **5** (5.12 mg). ^{1}H NMR (400 MHz, D_2O) □ 8.15 (s, 1H), 8.12 (s, 1H), 7.72 (s, 1H), 6.34 (d, J = 14.4 Hz, 1H), 5.81 (d, J = 8 Hz, 1H), 5.65-5.58 (m, 1H), 5.45 (d, J = 3.2 Hz, 0.5H), 5.32 (d, J = 3.6 Hz, 0.5H), 5.20-5.05 (m, 1H), 4.65-4.29 (m, 5H), 3.90-4.04 (m, 2H); ^{19}F NMR (376 MHz, D_2O) δ -201.92; ^{31}P NMR (162 MHz, D_2O) □□55.74, 53.23; MS: *m/z* 709.00 [M + H]+ .

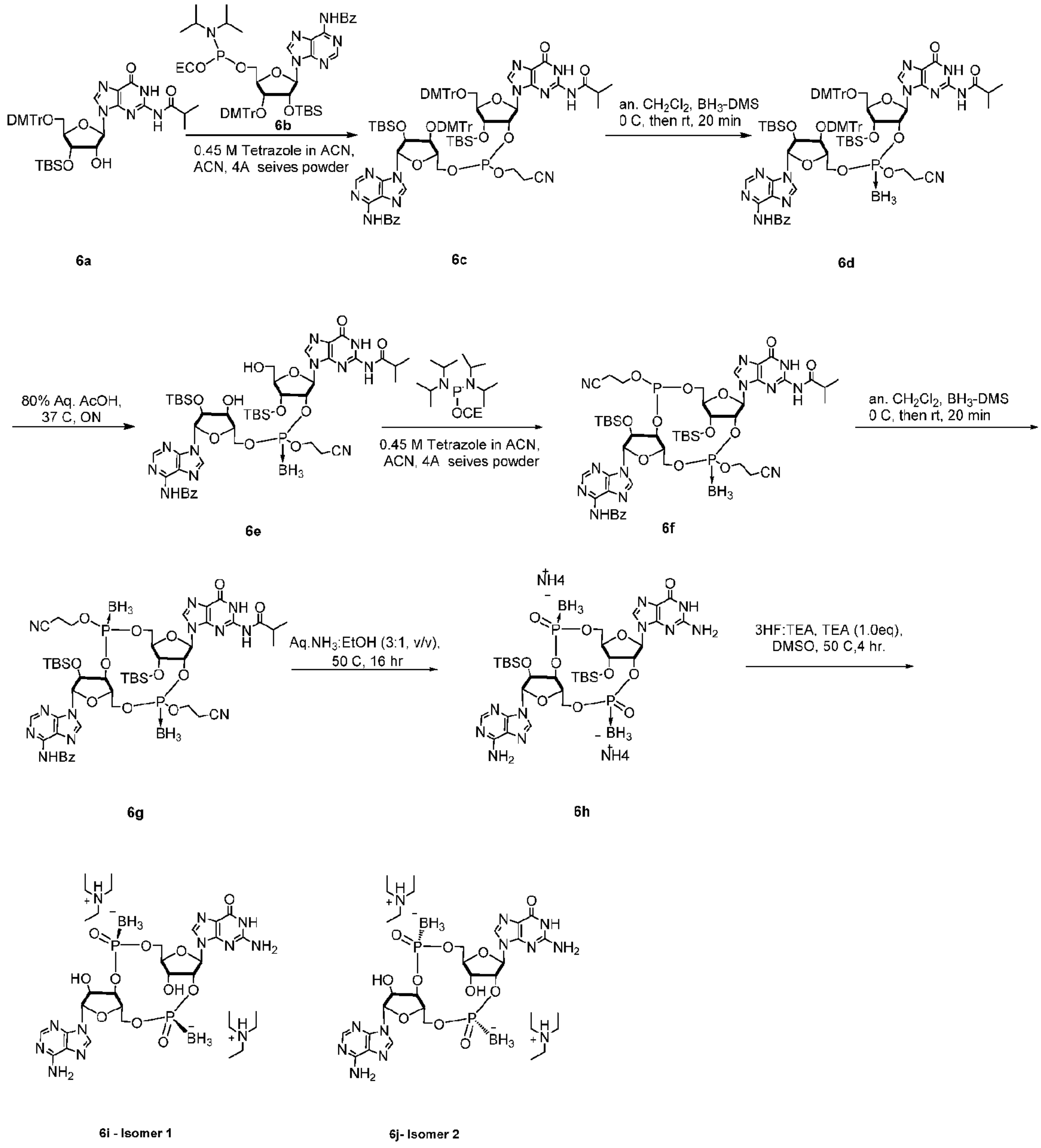
Example 6

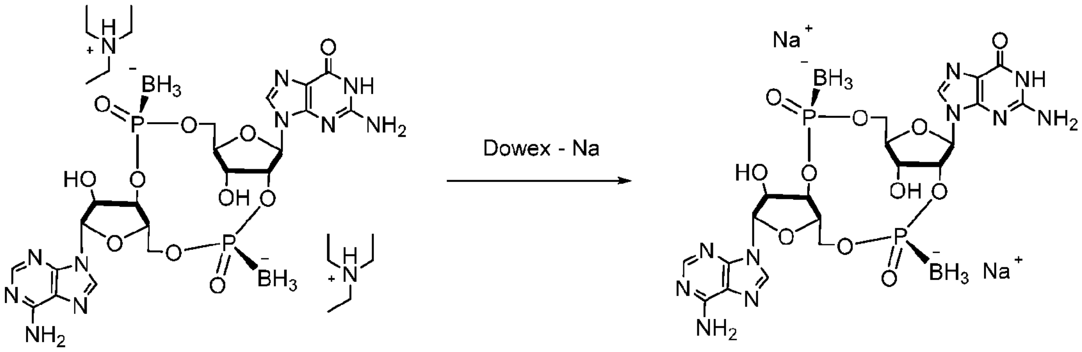


Compound **7**

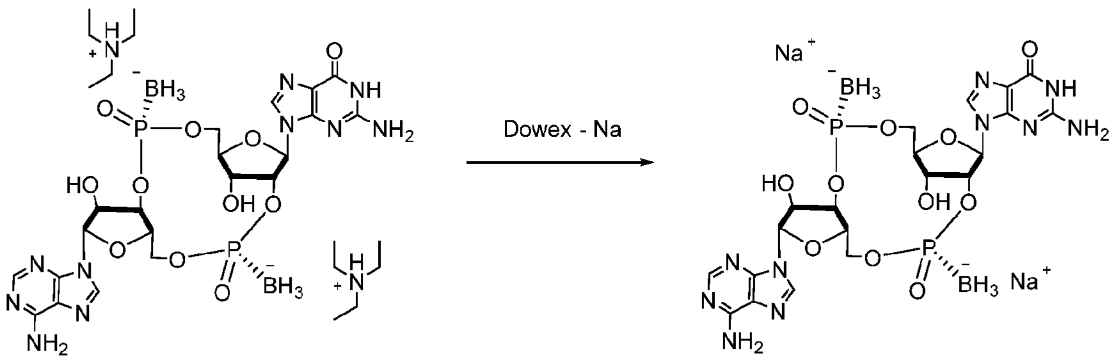


Compound **8**


6b
0.45 M Tetrazole in ACN,
ACN, 4A seives powder
an. CH_2Cl_2, BH_3-DMS
0 C, then rt, 20 min
6a
6c
6d
80% Aq. AcOH,
37 C, ON
0.45 M Tetrazole in ACN,
ACN, 4A seives powder
an. CH_2Cl_2, BH_3-DMS
0 C, then rt, 20 min
6e
6f
Aq.NH_3:EtOH (3:1, v/v),
50 C, 16 hr
3HF:TEA, TEA (1.0eq),
DMSO, 50 C,4 hr.
6g
6h
6i - Isomer 1
6j- Isomer 2



6i - Isomer 1 **Compound 7**



6j- Isomer 2 **Compound 8**

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

The nucleoside compound **6a** (1.63 g, 2.12 mmol) was co-evaporated with a mixture of anhydrous toluene: anhydrous acetonitrile (1:1, *v/v*, 3 × 20 mL), then dissolved in anhydrous acetonitrile (50 mL) and phosphoramidite **6b** (2.1 gr, 2.12 mmol). 4Å molecular sieves powder (4 .0 gr) were added to this. The resulting heterogeneous mixture was bubbled with Argon gas for 4 min. After stirring this mixture at rt for 30 min, 0.45 M tetrazole in acetonitrile (30 mL, 12.72 mmol) was added at rt. After stirring the reaction for 45 min, reaction mixture was filtered then washed with sat. aq. $NaHCO_3$ (1 x 20 mL) and sat. aq. NaCl (1 x 20 mL), dried over $MgSO_4$, and the filtrate evaporated to dryness to afford phosphite compound **6c**, which was used directly without further purification for the next step.

The crude phosphite compound **6c** was dissolved in anhydrous CH_2Cl_2 (40 mL), then 4Å molecular sieves powder (4.0 gr) was added to this. The resulting heterogeneous mixture was bubbled with Argon gas for 4 min. After stirring this mixture at rt for 30 min, borane dimethyl sulfide complex solution (2.0 M in THF, BH_3-DMS, 3.49 mL, 6.99 mmol) was added very slowly over 5 min at 0 °C. After stirring the reaction for 20 min at rt, the reaction mixture quickly filtered, then diluted EtOAc (120 mL), quenched with water (20 mL). The phases were separated and the organic phase was washed with water (1 x 20 mL), sat. aq. NaCl (1 x 20 mL), and then the aqueous phase was back-extracted with EtOAc (1 x 20 mL). The combined organic phases were evaporated to dryness, and the resulting crude material was purified by flash column chromatography on silica gel (0 - 85 % EtOAc in hexane, *v/v*) to afford boranophosphate dimer **6d** (980 mg). ESI-MS: *m/z* 1670.30 $[M+H]^+$.

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

Di-DMTr-boranophosphate dimer **6d** (2.3 gr, 1.37 mmol) was dissolved in 80% aq. AcOH:CH_3CN (3:1, *v/v*, 13 mL). After stirring the reaction mixture for 16 h at 37 °C, the mixture was diluted with EtOAc (70 mL), then washed sequentially with sat. aq. $NaHCO_3$ (3 x 20 mL) and sat. aq. NaCl (1 x 15 mL). The organic phase was evaporated to dryness, resulting in a crude residue which was purified by flash column chromatography over silica gel (20-100% Acetone in Hexane, *v/v*) to afford diol-boranophosphate dimer **6e** (0.9 g). ESI-MS: *m/z* 1066.45 $[M+H]^+$.

Step 3: preparation of compound **6f**

The diol nucleoside compound **6e** (0.55 g, 0.516 mmol) was co-evaporated with a mixture of anhydrous toluene: anhydrous acetonitrile (1:1, *v/v*, 3 x 20 mL) then dissolved in anhydrous acetonitrile (20 mL) and 4 Å molecular sieves powder (1.0 g) was added to this. The resulting heterogeneous mixture was bubbled with Argon gas for 4 min. After stirring this mixture at rt for 30 min, 0.45 M tetrazole in acetonitrile (7 mL, 3.09 mmol) was added at rt, then after stirring the reaction for 75 min, the mixture was filtered, the filtrate washed sequentially with sat. aq. $NaHCO_3$ (1 x 20 mL) and sat. aq. NaCl (1 x 20 mL), dried over $MgSO_4$ (stirred for 5 min then filtered) and evaporated to dryness to afford compound **6f**. The resulting mixture was used directly without further purification for the next step. The crude phosphite **6f** was dissolved in anhydrous CH_2Cl_2 (20 mL), then 4 Å molecular sieves powder (1.0 g) was added to this. The resulting heterogeneous mixture was bubbled with Argon gas for 4 min. After stirring this mixture at rt for 30 min, borane dimethyl sulfide complex solution (2.0 M in THF, BH_3-DMS, 0.93 mL, 1.84 mmol) was added very slowly over 5 min at 0 □C. After stirring the reaction at rt, the reaction mixture quickly filtered, then diluted with EtOAc (80 mL), and quenched with water (20 mL). The phases were partitioned and the organic phase was washed with sat. aq. NaCl (1 x 20 mL), then the aqueous phase was back-extracted with EtOAc (1 x 20 mL). The combined organic phases were evaporated to dryness, the resulting crude material was purified by flash column chromatography on silica gel (0-10% MeOH in dichloromethane, *v/v*) to afford fully-protected cyclic boranophosphate **6f** (480 mg, 75% pure). ESI-MS: *m/z* 1179.73 $[M+H]^+$.

Step 4: preparation of compound **6g**

3'-Silyl-G(iBu)-2'-silyl-A(Bz)-2',3'-cyclicdinucleotide-boranophosphate **6f** (437 mg, approximately 75% pure) was dissolved in mixture of aq. ammonia: EtOH (7 mL, 3:1, *v/v*). After stirring the reaction mixture for 16 h at 50 □C, the reaction mixture was concentrated to dryness and co-evaporated with EtOH (2 x 10 mL) and toluene (2 x 20 mL). The resulting crude solid was washed with dichloromethane (40 mL) and the precipitate was collected by filtration to afford the di-TBS protected cyclic dimer **6g** (ESI-MS: *m/z* 896.20 $[M-H]^-$), which was used for the next reaction without any further purification.

To remove the TBS groups, cyclic dimer compound **6g** (350 mg crude) was dissolved in anhydrous DMSO (5.5 mL) and to this it was added triethylamine trihydrofluoride (HF.3TEA, 2.8 mL and trimethylamine 0.6 mL). After stirring the reaction mixture for 3.5 h at 50 °C, it was neutralized with triethylamine and purified by preparative HPLC (Buffer A: 50 mM triethylammonium acetate in H_2O; Buffer B: 50 mM triethylammoniumacetate in CH_3CN, gradient: 0-30% of B over 30 min, flow rate 24 mL/min) to afford two isomers of boranophosphate **6i** (9 mg) and **6j** (4 mg) as a triethylammonium salt as a white solid. ESI-MS: *m/z* 668.6 $[M-H]^-$.

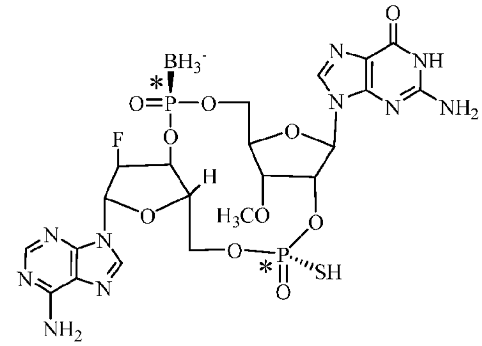
Step 5: preparation of compound 7 and compound **8 as sodium salt**.

Dowex 50W × 8, 200-400 (3 mL, H form) was added to a beaker and washed with deionized water (20 mL). Then to the resin was added 15% H_2SO_4 in deionized water, the mixture was gently stirred for 5 min, and decanted (10 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 CV), and then with deionized water until it was neutral. The resin was transferred back into the beaker, 15% NaOH in deionized water solution was added, and 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 CV), and then with deionized water until it was neutral. The triethylammonium form of both isomers of cyclic boranophosphates **6i** (9 mg) and **6j** (4 mg) was dissolved in minimum amount of deionized water, added to the top of the column, and eluted with deionized water. Appropriate fractions of CDN based on UV were pooled together and lyophilized to afford the sodium salt form of compound **7** (8.2 mg) and compound **8** (3.3 mg), respectively.

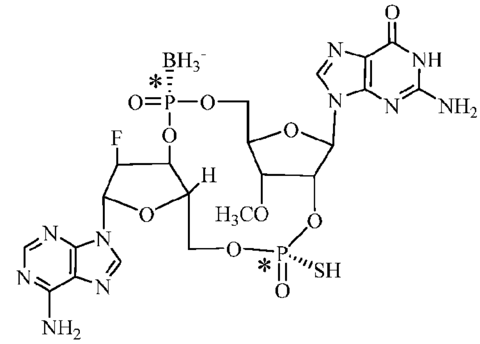
Compound 7: ^{1}H NMR (400 MHz, D_2O): □ 8.15 (s, 1H), 8.09 (s, 1H), 7.99 (s, 1H), 6.01 (s, 1H), 5.93 (d, *J* = 8.7 Hz, 1H), 4.98-4.93 (m, 1H), 4.87-4.80 (m, 1H), 4.74-4.60 (m, 1H), 4.42-4.37 (m, 2H), 4.31 (s, 1H), 4.24 -4.15 (m, 2H), 3.92-3.82 (m, 2H), 0.5 to -0.5 (very broad peak, 6H); ^{31}P NMR (162 MHz, D_2O) 94.70 (very broad peak); ESI-MS: *m/z* 668.6 $[M-1]^-$.

Compound 8: ^{1}H NMR (400 MHz, D_2O): δ 8.12 (s, 1H), 8.10 (s, 1H), 7.95 (s, 1H), 5.99 (d, *J* = 2.4 Hz, 1H), 5.92 (d, *J* = 8.4 Hz, 1H), 5.22-5.15 (m, 1H), 4.83-4.76 (m, 1H), 4.75-4.71 (m, 1H), 4.50 (d, *J* = 4 Hz, 1H), 4.40-4.30 (m, 1H), 4.31 (s, 1H), 4.25 -4.18 (m, 1H), 4.15 -4.10 (m, 1H), 4.02-3.96 (m, 1H), 3.90-3.84 (m, 1H), -0.2 to 0.9 (very broad peak, 6H); ^{31}P NMR (162 MHz, D_2O) δ 93.75 (very broad peak); ESI-MS: *m/z*: 668.7 $[M-1]^-$.

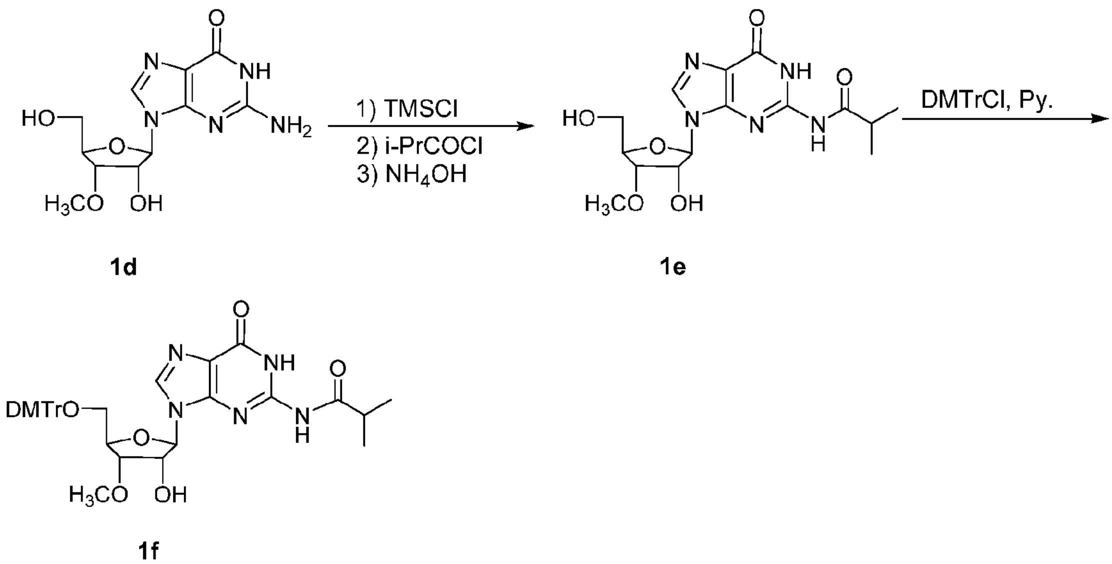
Example 7

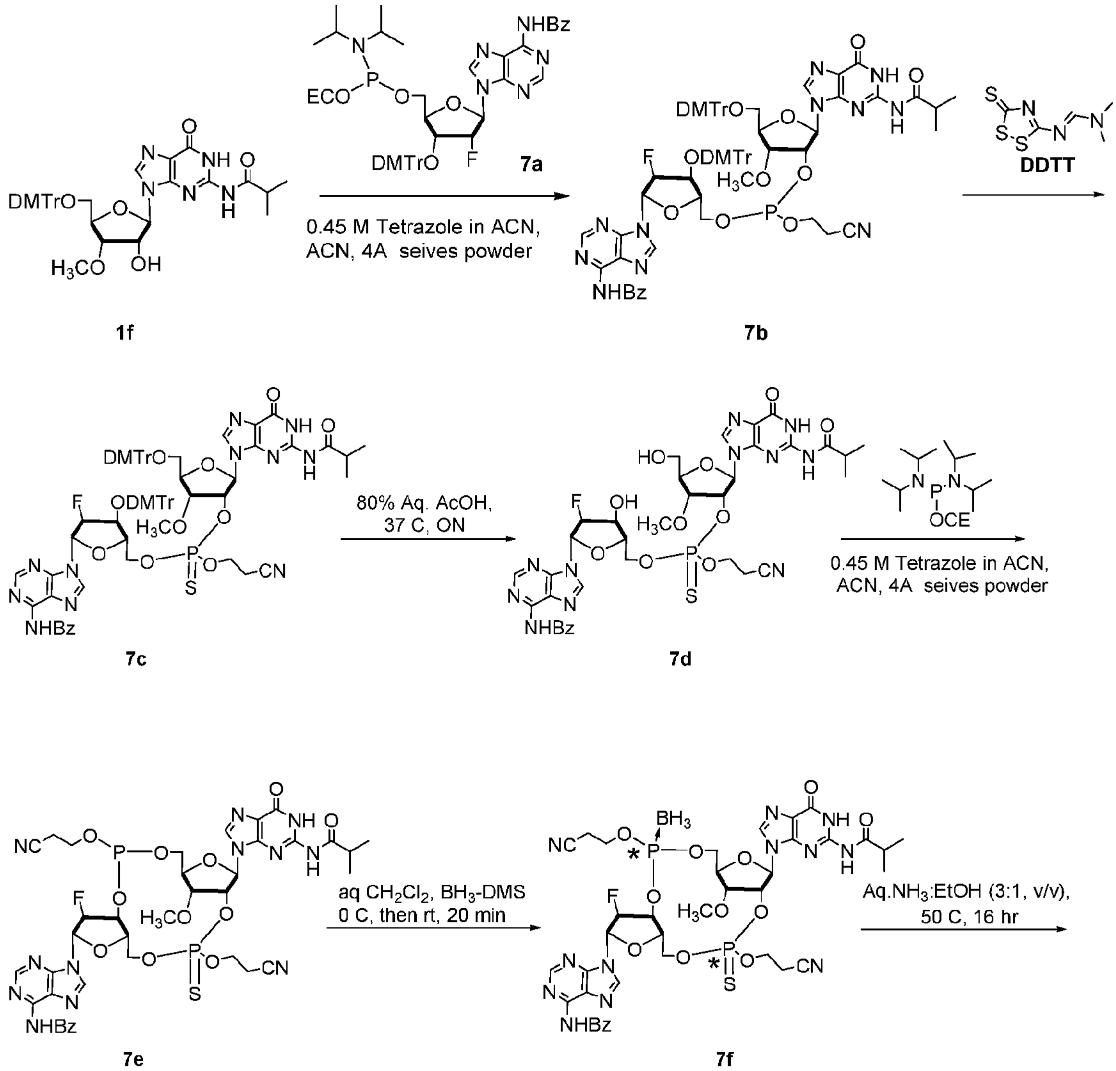


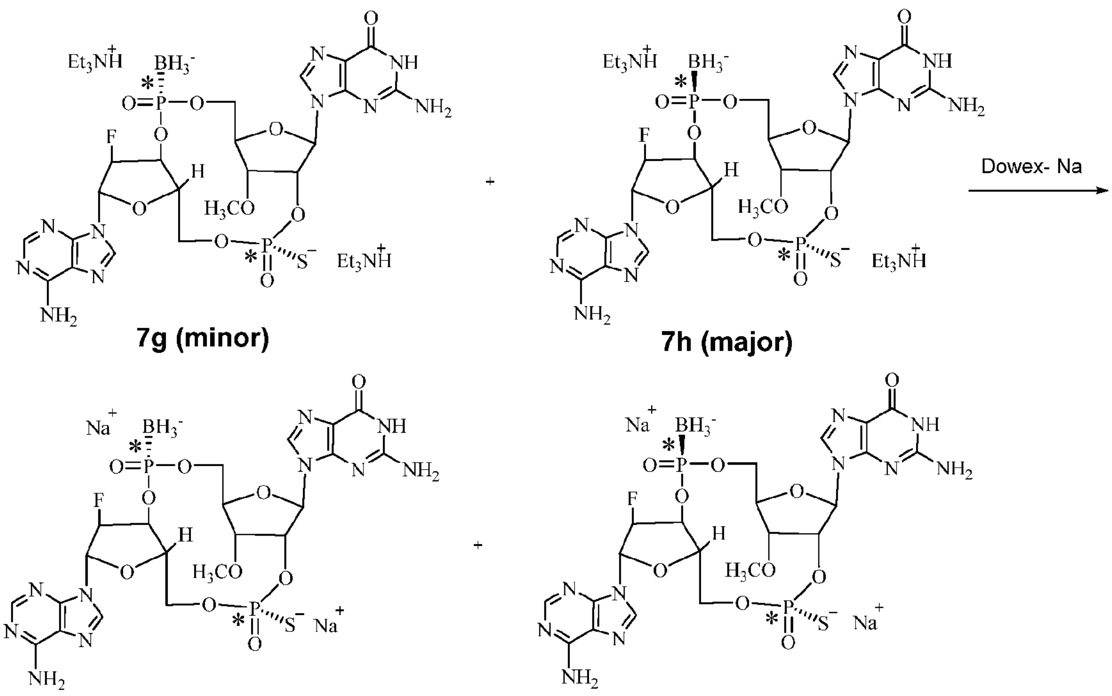
Compound **9**



Compound **10**







7g (minor) **7h (major)**

Compound 10 (minor) **Compound 9 (major)**

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

The nucleoside compound **1f** (2.3 g, 3.43 mmol) was co-evaporated with a mixture of anhydrous toluene/anhydrous acetonitrile (1:1, v/v, 3 x 50 mL), then dissolved in anhydrous acetonitrile (80 mL). 4 Å Molecular sieves powder (4.0 g) and tetrazole in acetonitrile (61 mL, 0.45 M, 27.44 mmol) were added to the reaction mixture. The resulting heterogeneous mixture was purged with bubbling $Ar_{(g)}$ for 4 min. After stirring at rt for 10 min, a solution of amidite **7a** (3.0 g, 3.43 mmol, ChemGenes Corp.) in anhydrous acetonitrile (15 mL) was added at rt. After stirring for 1 h 45 min at rt, the reaction mixture was diluted with ethyl acetate (250 mL), filtered, and the filtrate washed with saturated aqueous $NaHCO_3$ (1 x 40 mL) and brine (1 x 40 mL). The filtrate was then dried ($MgSO_4$), stirred for 5 min, filtered, and the filtrate concentrated to dryness to afford phosphite **7b** (ESI-MS: *m/z* 1444.45 $[M+1]^+$).

Crude phosphite compound **7b** was used in the next step without further purification. Crude compound **7b** was dissolved in anhydrous pyridine (100 mL) and (*E*)-*N*,*N*-dimethyl-*N'*-(3-thioxo-3H-1,2,4-dithiazol-5-yl)formimidamide (DDTT, 2.12 g, 10.29 mmol) was added at rt. After stirring for 1 h at rt, the reaction mixture was diluted with ethyl acetate (250 mL), washed sequentially with saturated aqueous $NaHCO_3$ (1 x 40 mL) and brine (1 x 40 mL), and concentrated to dryness. The aqueous phase was extracted with ethyl acetate (1 x 20 mL). The combined organic phases were concentrated to dryness under reduced pressure to afford a crude material which was purified by flash chromatography on silica gel (0-8 % MeOH in CH_2Cl_2, v/v) to afford di-DMTr-phosphorothioate dimer **7c** (4.6 g, ~92 %, approximately 90 % purity). ESI-MS: *m/z* 1476.90 $[M+H]^+$.

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

Dimer **7c** (4.5 g, 3.05 mmol) was dissolved in 80 % aqueous AcOH:acetonitrile (90 mL, 8:2, v/v). After stirring the reaction mixture for 20 h at 45 °C, the mixture was diluted with ethyl acetate (400 mL) and washed sequentially with saturated aqueous $NaHCO_3$ (3 x 80 mL) and brine (1 x 50 mL). The aqueous phase was separated and extracted with ethyl acetate (1 x 20 mL). The combined organic phases were concentrated to dryness under reduced pressure and the resulting crude residue was purified by flash chromatography on silica gel (0-15% MeOH in CH_2Cl_2, v/v) to afford dimer **7d** (1.65 g, 63 %). ESI-MS: *m/z* 872.10 $[M+H]^+$.

Step 3: preparation of compound **7f**

Dimer compound **7d** (275 mg, 0.315 mmol) was co-evaporated with a mixture of anhydrous toluene/anhydrous acetonitrile (1:1, v/v, 3 x 10 mL) then dissolved in anhydrous acetonitrile (20 mL, sonicated for 5 min for complete solubility of cpd **7d**). 4 Å Molecular sieves powder (0.6 g) and tetrazole in acetonitrile (5.6 mL, 0.45 M, 2.52 mmol) were then added. The resulting heterogeneous mixture was purged with bubbling $Ar_{(g)}$ for 4 min. After stirring the mixture at rt for 10 min, 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (142 mg, 0.473 mmol, 1.5 eq) was added at rt in five portions over 20 min. After stirring for 90 min at rt, the reaction mixture was diluted with ethyl acetate (60 mL), filtered, and the filtrate washed sequentially with saturated aqueous

$NaHCO_3$ (1 x 20 mL) and brine (1 x 20 mL). The filtrate was then dried ($MgSO_4$) while stirring for 5 min, filtered, and the filtrate concentrated to dryness under reduced pressure to afford compound **7e** (ESI-MS: *m/z* 971.10 $[M+1]^+$). The resulting residue was used in the next step without further purification.

Crude compound **7e** was dissolved in anhydrous dichloromethane (25 mL), to which was added 4 Å molecular sieves powder (0.5 g). The resulting heterogeneous mixture was purged with bubbling $Ar_{(g)}$ for 4 min. Upon stirring the mixture at rt for 10 min, the mixture was cooled to 0 °C. A borane dimethyl sulfide complex solution (2.0 M in THF, BH_3-DMS, 550 µL, 3.5 eq) was added very slowly over 5 min at 0 °C, and the reaction mixture was stirred at rt for 12 min. The mixture was then quickly filtered, diluted with ethyl acetate (80 mL), and quenched with water (20 mL). The organic phase was washed with brine (1 x 20 mL), and the aqueous layer was extracted with ethyl acetate (1 x 20 mL). The combined organic layers were concentrated to dryness under reduced pressure to afford a crude residue, which was purified by flash chromatography on silica gel (0-10% MeOH in dichloromethane, v/v) to afford a mixture of diastereoisomers **7f** (130 mg, ~42% for 2 steps). ESI-MS: *m/z* 984.95 $[M+H]^+$.

Step 4: preparation of compound **7g** and compound **7h**

The mixture of diastereoisomers **7f** (130 mg) was dissolved in a mixture of aqueous ammonia/ethanol (7 mL, 3:1, v/v). After stirring the reaction mixture for 18 h at 50 °C, the reaction mixture was concentrated to dryness and co-evaporated with ethanol (2 x 10 mL) and toluene (2 x 20 mL). The resulting crude solid was washed with dichloromethane (15 mL), collected by filtration, and purified by reverse phase preparative HPLC (column: Synergi 4µ, Hydro RP, 250 mm × 30 mm, Mobile Phase: Buffer A: 50 mM triethylammonium acetate in H_2O; Buffer B: 50 mM triethylammoniumacetate in CH_3CN, gradient: 0-30% of B over 30 min, flow rate 24 mL/min) to afford a first minor boranophosphothioate isomer **7g** (8.7 mg) and a second major boranophosphothioate isomer **7h** (13.1 mg) as triethylammonium acetate (TEAA) salts. ESI-MS: *m/z*: 703.1 $[M-1]^-$.

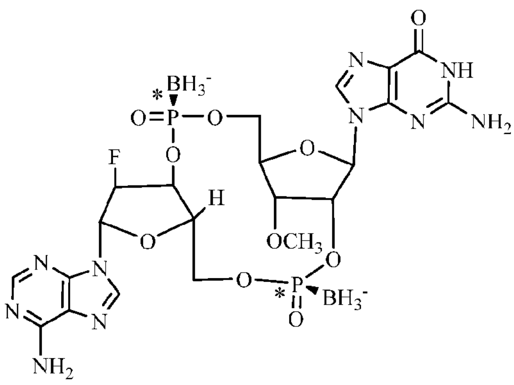
Step 5: preparation of compound **9** and compound **10**

Dowex 50W × 8, 200-400 (5 mL, H form) was added to a beaker and washed with deionized water (30 mL). Then to the resin was added 15% H_2SO_4 in deionized water, 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 CV), and then with deionized water until neutral pH 7 was attained. The resin was transferred back into the beaker, 15 % NaOH in deionized water solution was added, the mixture was gently stirred for 5 min, then decanted (1 x). The resin was transferred to the column and washed with 15 % NaOH in water (at least 4 CV), then with deionized water until neutral pH 7 was attained. Each boranophosphothioate isomer **7g** (8.7 mg) and **7h** (13.1 mg) TEAA salts was dissolved in a minimum amount of deionized water, added to the top of the column, and eluted with deionized water. Appropriate fractions of compounds **9** and **10** were pooled together and lyophilized to afford respectively compound **10** (7.8 mg) and compound **9** (12.4 mg) as a sodium salt.

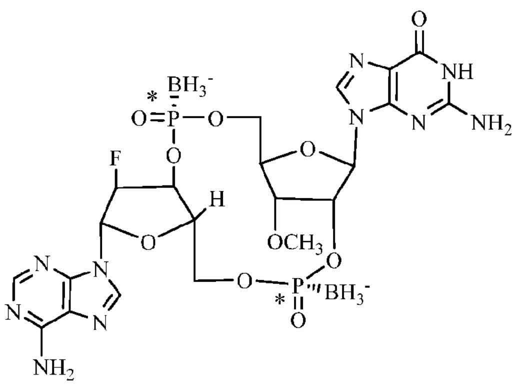
Compound 9 (major isomer): ^{1}H NMR (400 MHz, D_2O): δ 8.07 (s, 1H), 7.97 (s, 1H), 7.86 (s, 1H), 6.25 (d, J = 16.4 Hz, 1H), 5.86 (d, J = 8.4 Hz, 1H), 5.52 (d, J = 3.6 Hz, 0.5H), 5.40 (d, J = 3.6 Hz, 0.5H) 5.16-5.19 (m,1H), 4.83-4.89 (m, 1H), 4.41-4.45 (m, 2H), 4.25-4.32 (m, 2H), 4.06-4.17 (m, 2H), 3.88-3.94 (m, 1H), 3.46 (s, 3H), -0.1 to 0.65 (very broad peak, 3H); ^{31}P NMR (162 MHz, D_2O): δ 94-95 (very broad peak, boranophosphate), 52.59 (phosphorothiate); ^{19}F NMR (379 MHz, D_2O): δ -201.7 (multiplet); ESI-MS: *m/z*: 703.1 [M-1]$^-$.

Compound 10 (minor isomer): ^{1}H NMR (400 MHz, D_2O): δ 8.18 (s, 1H), 8.13 (s, 1H), 8.07 (s, 1H), 6.31 (d, J = 15.6 Hz, 1H), 5.91 (d, J = 8.4 Hz, 1H), 5.65 (d, J = 2.8 Hz, 0.5H), 5.50 (d, J = 2.8 Hz, 0.5H) 5.07-5.30 (m, 2H), 4.40-4.48 (m, 2H), 4.20-4.35 (m, 2H), 4.10-4.14 (m, 1H), 3.96-4.00 (m, 1H), 3.83-3.88 (m, 1H), 3.48 (s, 3H), 0.2 to 0.8 (very broad peak, 3H); ^{31}P NMR (162 MHz, D_2O): δ 94-95 (very broad peak, boranophosphate), 57.79 (phosphorothiate); ^{19}F NMR (379 MHz, D_2O): δ -202.4 (multiplet); ESI-MS: *m/z*: 703.1 [M-1]$^-$.

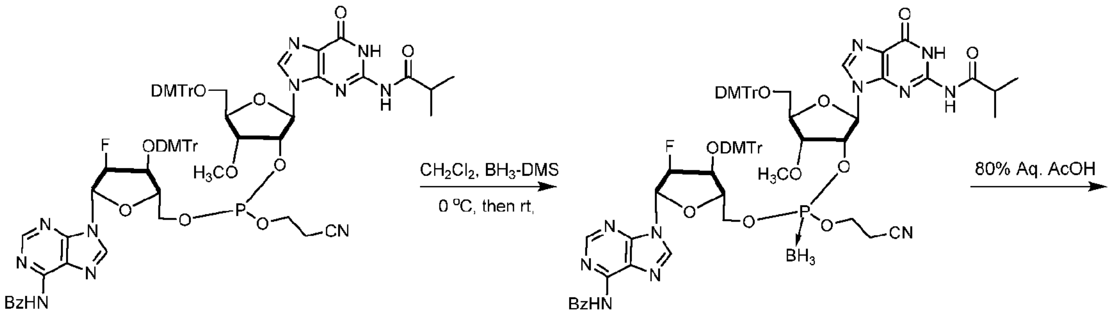
Example 8



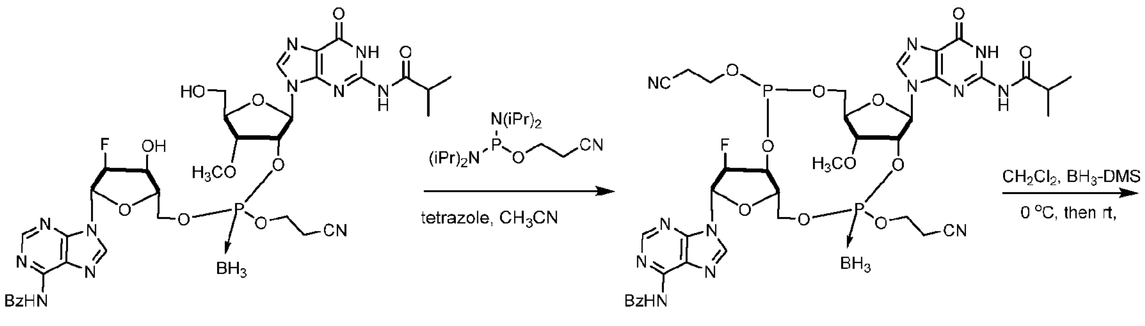
Compound 11



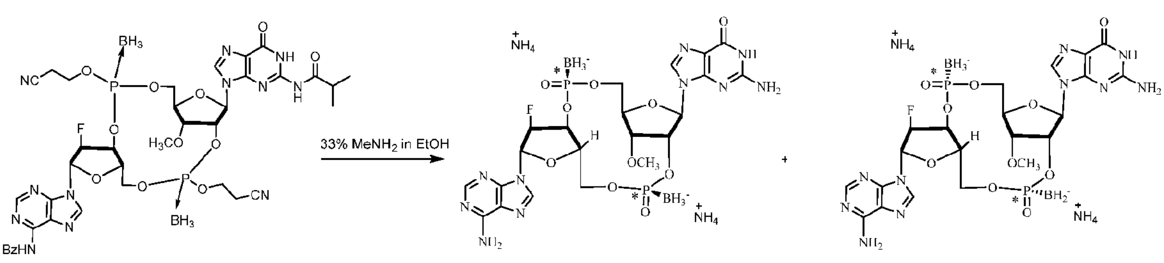
Compound 12



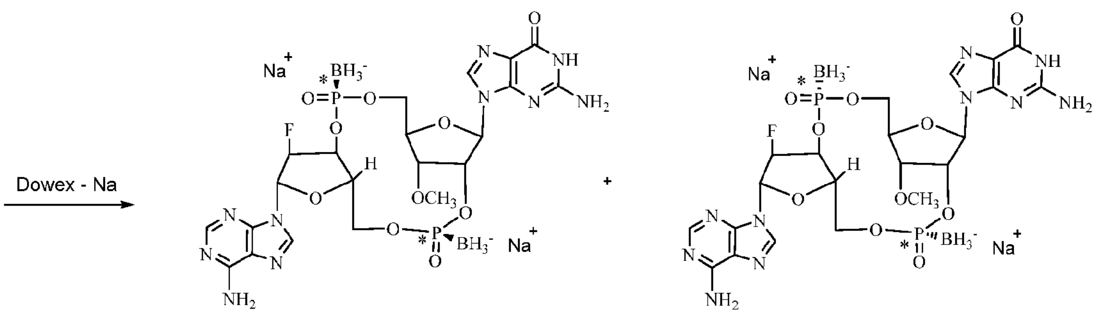
7b 8a



8b **8c**



8d **11** **12**



Cpd 11 sodium salt **Cpd 12, sodium salt**

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

To a solution of compound **7b** (8 g, 5.538 mmol) in CH_2Cl_2 (80 mL) was added 4Å molecular sieves powder (8 g) and the resulting heterogeneous mixture was bubbled with argon for 4 min. After stirring at rt for 30 min, borane dimethyl sulfide complex solution (2.0 M in

THF, BH_3-DMS, 9.138 mL, 18.277 mmol) was added very slowly over 5 min at 0 °C. After stirring the reaction for 2 h at rt, the reaction mixture was quickly filtered, diluted with CH_2Cl_2 (40 mL) and quenched with water (50 mL). The phases were separated and the organic layer was successively washed with water (1 x 50 mL), brine (1 × 50 mL) and the aqueous layers back-extracted with CH_2Cl_2 (1 × 50 mL). The combined organic layers were concentrated under reduced pressure to dryness and the resulting crude material was purified by flash column chromatography on silica gel (petroleum ether / EtOAc = 1 / 0 ~ 0 / 1) to give compound **8a** (7.5 g, 5.143 mmol) as a pale yellow oil.

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

Compound **8a** (7.5 g, 5.143 mmol) was added to a solution of CH_3CN (20 mL) in 80% acetic acid aqueous solution (60 mL) (CH_3CN / acetic acid aqueous solution = 1 / 3, acetic acid 48 mL, H_2O 12 mL, CH_3CN 20 mL). After stirring the mixture reaction overnight at 25 °C, triethylsilane was added and the reaction stirred at 25 °C for an additional 1 h. The reaction mixture was quickly filtered, diluted with EtOAc (50 mL) and quenched with water (20 mL). The pH was adjusted to 7-8 with aqueous saturated $NaHCO_3$ solution. The phases were separated and the organic layer was successively washed with water (1 x 100 mL), brine (1 x 100 mL) and concentrated under reduced pressure to dryness. The residue was purified by flash column chromatography on silica gel (petroleum / EtOAc = 0 / 1 then CH_2Cl_2 / MeOH = 1 / 0 to 20 / 1) to give compound **8b** (6.5 g, 7.126 mmol) as a white solid. ESI-MS: *m/z*= 854.1 $[M+1]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (br d, *J* = 13.5 Hz, 1H), 11.56 (d, *J* = 9.7 Hz, 1H), 11.22 (br d, *J* = 7.1 Hz, 1H), 8.72 (d, J = 3.1 Hz, 1H), 8.56 - 8.41 (m, 1H), 8.27 - 8.18 (m, 1H), 8.02 (br d, *J* = 6.2 Hz, 2H), 7.65 - 7.60 (m, 1H), 7.56 - 7.50 (m, 2H), 6.35 (br t, *J* = 19.0 Hz, 1H), 6.00 (d, J = 6.4 Hz, 1H), 5.95 (t, *J* = 6.5 Hz, 1H), 5.65 - 5.43 (m, 1H), 5.34 - 5.20 (m, 2H), 4.75 - 4.55 (m, 1H), 4.16 - 3.97 (m, 6H), 3.92 - 3.82 (m, 1H), 3.67 - 3.49 (m, 2H), 3.14 (d, *J* = 5.1 Hz, 3H), 2.81 - 2.66 (m, 3H), 1.08 (br d, *J* = 6.8 Hz, 6H), 0.59 - -0.18 (m, 3H); ^{31}P NMR (162 MHz, DMSO-d_6) 115.80 (br s, 1P).

Step 3: preparation of compound **8c**

Compound **8b** (1g, 1.096 mmol) was co-evaporated with CH_3CN (3 x 20 mL) and dissolved in anhydrous CH_3CN (44 mL). It was then added to 4 Å molecular sieves powder (1000 mg) and after stirring the mixture for 30 min, a solution of tetrazole in CH_3CN (0.45 M, 14.6 mL, 6 eq) was added at rt. The resulting mixture was stirred for 15 min at rt. It was then added dropwise a solution of 3-((bis(diisopropylamino)phosphanyl)oxy)propanenitrile (495.647 mg, 1.644 mmol) in anhydrous CH_3CN (5.0 mL) over 20 min. After stirring the reaction mixture for 1 h, an additional solution of tetrazole in CH_3CN (0.45 M, 9.7 mL, 4 eq) was added to this mixture at rt. The reaction mixture was filtered and the filtrate extracted with EtOAc (3 x 400 mL). The combined organic layers were successively washed with aqueous $NaHCO_3$ (3 x 200 mL), brine (3 x 200 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 (CH_2Cl_2 : MeOH 100 : 0 to CH_2Cl_2: MeOH 90 : 10) to afford compound **8c** (600 mg, 0.63 mmol) as a white solid.

Step 4: preparation of compound **8d**

To a solution of compound **8c** (600 mg 0.63 mmol) in CH_2Cl_2 (15 mL) was added a solution of 2M borane-dimethyl sulfide complex in THF (1039.276 µL, 2.079 mmol) very slowly over 5 min at 0 °C. After stirring the reaction mixture at 0 °C for 15 min, the reaction was quenched with MeOH (20 mL) at 0 °C, then concentrated under reduced pressure. The reaction was repeated a second time using the same scale. The two crude batches were combined and purified by flash column chromatography on silica gel (gradient eluent: CH_2Cl_2 : MeOH 100 : 0 to CH_2Cl_2 : MeOH 90 : 10) to give compound **8d** (1 g, 1.035 mmol, combined batches) as a white solid. ESI-MS: m/z= 966.4 $[M+1]^+$.

Step 5: preparation of compounds **11** and **12**

Compound **8d** (1.0 g, 1.035 mmol) was treated with a solution of $MeNH_2$ in EtOH (33%, 10 mL). After stirring at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure and the residue was purified by preparative high-performance liquid chromatography (Prep HPLC condition: Column: Phenomenex Kinetex XB-C18 150mm x 30mm, 5 µm; Conditions: H_2O (A)-CH_3CN (B); Begin B: 0; End B: 20; Flow Rate: 25 mL /

min). The pure fractions were collected and lyophilized to dryness to give crude compound **11** (0.11 g, 0.160 mmol) and crude compound **12** (0.14 g, 0.204 mmol) as white solids.

The crude compounds **11** and **12** were further purified by preparative high-performance liquid chromatography (Prep HPLC conditions: Column: DuraShell 150 x 25mm x 5um; Condition: water (10 mM NH_4HCO_3) (A)-CH_3CN (B); Begin B : 0; End B: 15; Flow Rate: 35 mL / min). The pure fractions were collected and lyophilized to dryness to afford compound **11** (0.08 g, 0.117 mmol) and compound **12** (0.04 g, 0.058 mmol), each as a white solid.

Compound **11**: ^{1}H NMR (400 MHz, D_2O) δ 8.21 (d, *J* = 13.2 Hz, 2H), 8.05 (s, 1H), 6.36 (d, *J* = 16.3 Hz, 1H), 5.93 (d, *J* = 8.2 Hz, 1H), 5.73 - 5.55 (m, 1H), 5.19 - 5.02 (m, 2H), 4.55 - 4.48 (m, 2H), 4.34 - 4.24 (m, 2H), 4.16 (d, *J* = 4.4 Hz, 1H), 4.05 - 3.94 (m, 2H), 3.57 (s, 3H), 0.76 - 0.13 (m, 3H), -0.32 (br s, 3H); ESI-MS: *m/z* = 686.9 $[M+1]^+$; ^{19}F NMR (376 MHz, D_2O) -202.02 (td, *J* = 20.0, 50.3 Hz, 1F); ^{31}P NMR (162 MHz, D_2O) δ ppm - 94.42 (br s, 1P).

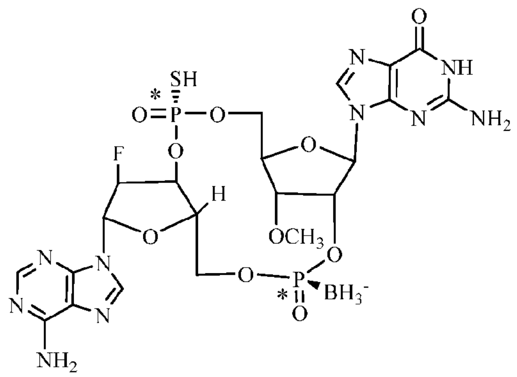
Compound **12**: ^{1}H NMR (400 MHz, D_2O) δ 8.31 (s, 1H), 8.23 (s, 1H), 7.86 (br s, 1H), 6.40 (br d, J = 15.4 Hz, 1H), 5.89 (br d, *J* = 8.6 Hz, 1H), 5.64 - 5.47 (m, 1H), 5.35 (br s, 1H), 5.00 - 4.86 (m, 1H), 4.54 - 4.45 (m, 2H), 4.38 (br d, *J* = 11.7 Hz, 1H), 4.20 (br d, J = 17.0 Hz, 2H), 4.07 (br d, *J* = 8.2 Hz, 1H), 3.96 (br d, *J* = 10.1 Hz, 1H), 3.53 (s, 3H), 0.24 (br s, 6H); ESI-MS: *m/z* = 686.9 $[M+1]^+$; ^{19}F NMR (376 MHz, D_2O) -200.77 - -202.57 (m, 1F); ^{31}P NMR (162 MHz, D_2O) 99.68 - 84.67 (m, 1P).

Step 6: preparation of compound **11 sodium salt** and compound **12 sodium salt**

Compound **11 sodium salt**. Dowex 50W x 8, 200-400 (H form, 50 g) was added to a beaker (for 45 mg of cpd **11**) and washed with deionized water (2x), then added to the resin (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 volumes), and then with deionized water until the resin was neutral. The resin was transferred back into the beaker, and a NaOH solution (15% NaOH in water 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 water (at least 4 column volumes) and then with water until it was neutral (at least 4 column volumes). Compound **11** was dissolved in deionized water (50 mg in 40 mL), added to the top of the column and eluted with deionized water. Compound **11** was eluted from the column in early fractions as detected by TLC (UV). The product was lyophilized to give compound **11 sodium salt** (24.3 mg, 0.033 mmol) as a white solid. ESI-MS: *m/z* = 686.9 $[M+1]^+$; ^1H NMR (400 MHz, D_2O) δ 7.81 (2 H, d, *J* = 3.6 Hz), 7.71 (1 H, s), 5.99 (1 H, d, *J* = 15.6 Hz), 5.61 (1 H, d, *J* = 8.2 Hz), 5.16 - 5.32 (1 H, m), 4.65 - 4.84 (2 H, m), 4.19 - 4.28 (2 H, m), 3.96 - 4.11 (2 H, m), 3.88 (1 H, d, *J* = 4.2 Hz), 3.69 - 3.80 (2 H, m), 3.31 (3 H, s), -1.07 - 0.45 (6 H, m); ^{19}F NMR (377 MHz, D_2O) -202.06 (1 F, s); ^{31}P NMR (162 MHz, D_2O) 94.39 (1 P, br s).

Compound **12 sodium salt**. Dowex 50W x 8, 200-400 (H form, 50 g) was added to a beaker (for 50 mg of cpd **12**) and washed with deionized water (2x) then added to the resin (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 volumes), and then with deionized water until the resin was neutral. The resin was transferred back into the beaker, and a NaOH solution (15% NaOH in water 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 water (at least 4 column volumes) and then with water until it was neutral (at least 4 column volumes). Compound **12** was dissolved in deionized water (50 mg in 40 mL), added to the top of the column and eluted with deionized water. Compound **12** eluted from the column in early fractions as detected by TLC (UV). The product was lyophilized to give compound **12 sodium salt** (43.3 mg, 0.059 mmol) as a white solid. ESI-MS: *m/z* = 686.9 $[M+1]^+$; ^1H NMR (400 MHz, D_2O) δ 8.11 (s, 1 H), 8.06 (s, 1H), 7.94 (s, 1H), 6.28 (d, *J* = 16.06 Hz, 1H), 5.89 (d, *J* = 8.53 Hz, 1H), 5.44 - 5.60 (m, 1H), 5.27 (td, *J* = 8.97, 4.39 Hz, 1H), 4.86 - 5.00 (m, 1H), 4.46 - 4.55 (m, 2H), 4.37 (br d, *J* = 11.80 Hz, 1H), 4.19 - 4.28 (m, 1H), 4.19 - 4.28 (m, 1H), 4.15 (br dd, *J* = 11.67, 2.13 Hz, 1H), 4.02 (br d, *J* = 12.30 Hz, 1H), 3.54 (s, 3H), -0.01 - 0.75 (m, 6H); ^{19}F NMR (377 MHz, D_2O) -201.59 (s, 1 F); ^{31}P NMR (162 MHz, D_2O) 94.01 (br s, 1 P).

Example 9



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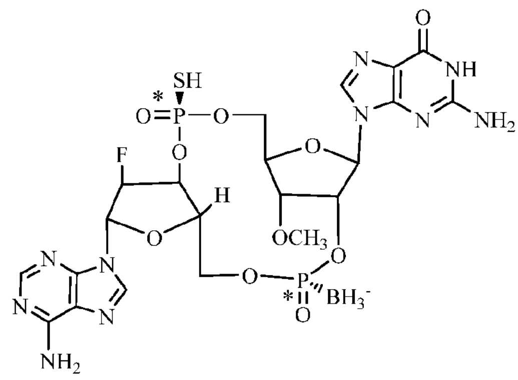

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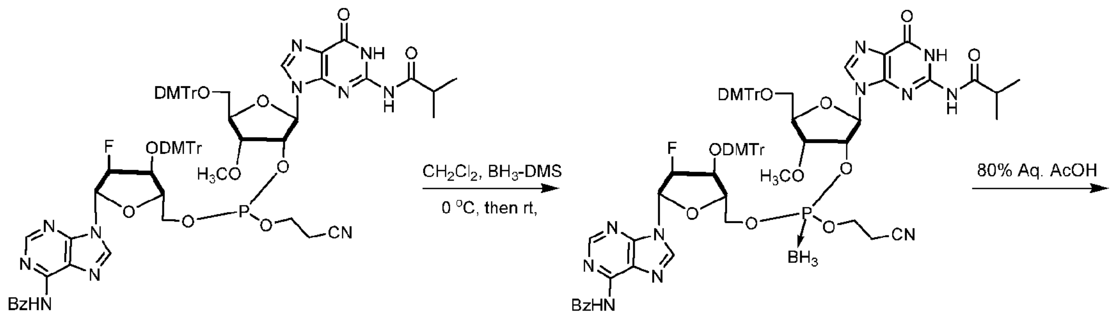
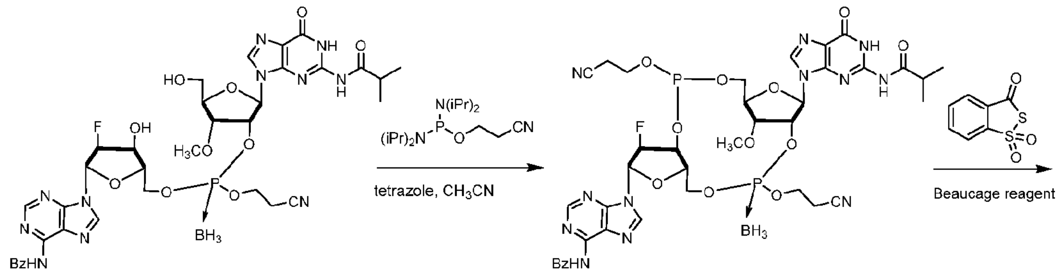
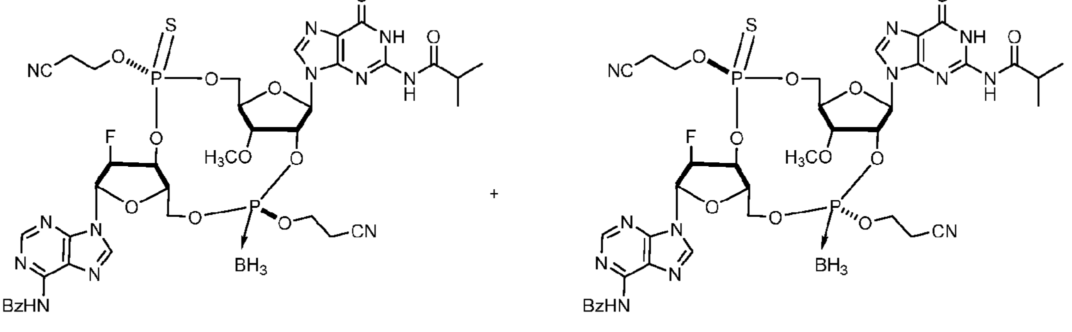


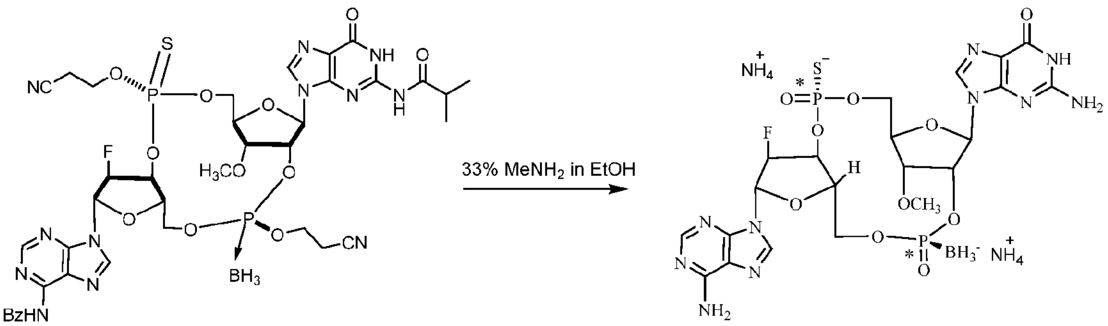
7b 8a



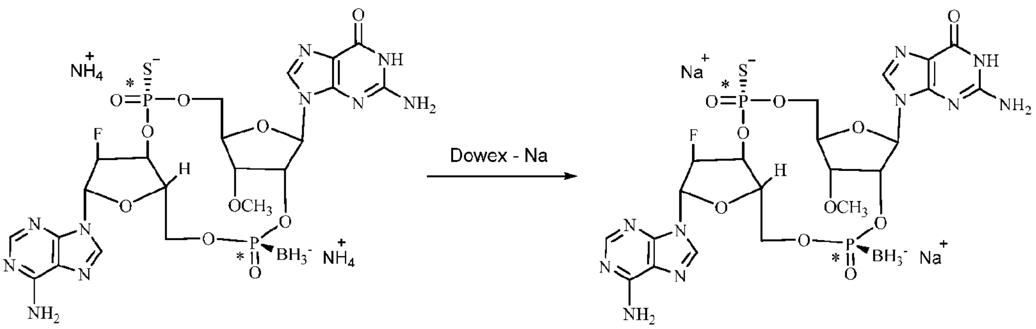
8b 8c



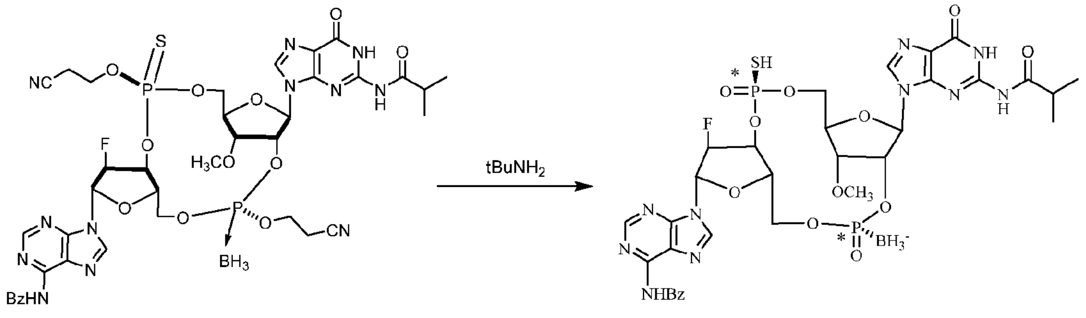
9a 9b



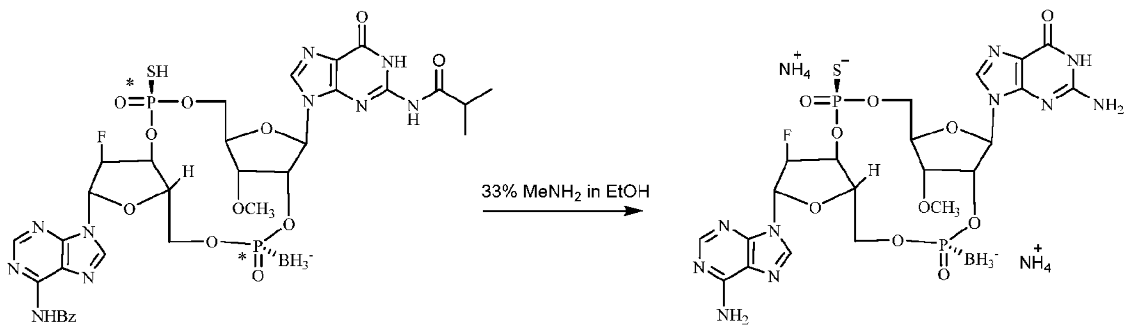
9a Cpd 13



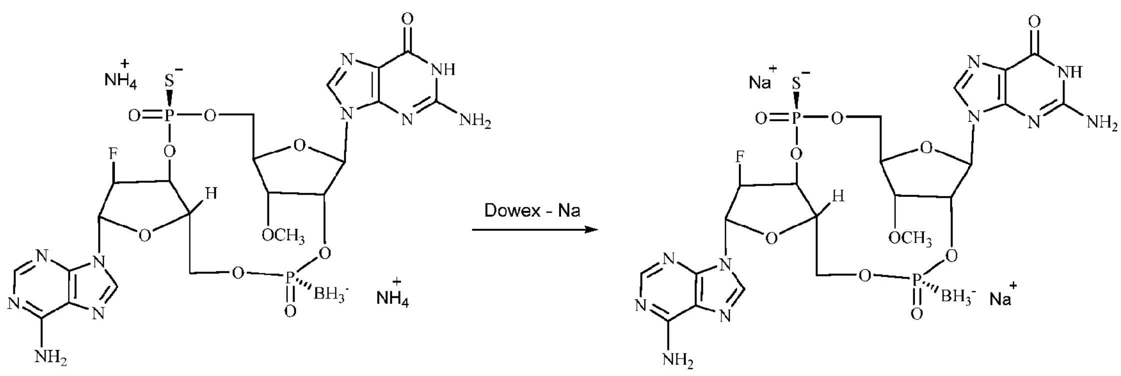
Compound 13, ammonium salt **Compound 13, sodium salt**



9b 9c



9c Cpd 14



Compound 14, ammonium salt **Compound 14, sodium salt**

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

Compound **7b** (10.46 g, 7.241 mmol) was dissolved in anhydrous CH_2Cl_2 (100 mL) and 4Å molecular sieves powder (4.0 g) was added to this solution. The resulting heterogeneous mixture was degassed under reduced pressure and purged with Argon several times. After stirring this mixture at 25 °C for 30 min, borane dimethyl sulfide complex solution (2.0 M in THF, BH_3-DMS, 11.948 mL, 23.897 mmol) was added very slowly for 5 min at 0 °C. After stirring the reaction for 20 min at 25 °C, the reaction mixture was quickly filtered, diluted with CH_2Cl_2 (120 mL) and quenched with water (20 mL). The organic phase was successively washed with water (50 mL) and brine (50 mL). The aqueous layers were back-extracted with EtOAc (50 mL). The combined organic layers were concentrated under reduced pressure and the resulting crude material was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH=100/1 to 10/1) to give compound **8a** (11.1 g, 7.612 mmol) as a yellow solid.

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

Compound **8a** (11.1 g, 7.612 mmol) was dissolved in 80% aqueous AcOH/CH_3CN/Et_3SiH (3/1/1, v/v, 200 mL). After stirring the mixture for 16 h at 37 °C, the reaction mixture was diluted with EtOAc (500 mL) and then neutralized with saturated aqueous $NaHCO_3$. The organic layer was successively washed with water (500 mL) and brine (2 x 250 mL), then dried over Na_2SO_4, filtered, and the filtrate evaporated to dryness to give a residue. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH = 100/1 to 10/1) to give compound **8b** (3.6 g, 4.218 mmol) as a white foam. ESI-MS: *m/z* 854.2 $[M + H]^+$; ^{19}F NMR (376 MHz, CD_3CN) δ -203.09 (s, 1F), -203.36 (s, 1F); ^{31}P NMR (162 MHz, CD_3CN) δ 116.95-116.34 (m, 1P).

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

Compound **8b** (1.5 g, 1.757 mmol) was co-evaporated with a mixture of anhydrous toluene / CH_3CN (1/1, v/v, 3 x 20 mL) to give a white solid. The solid was then dissolved in anhydrous CH_3CN (80 mL) and 4Å molecular sieves powder (2.0 g) was added to this solution. After stirring this mixture at 25°C for 30 min, a solution of tetrazole in CH_3CN (0.45 M, 31.242 mL, 14.059 mmol) was added to the solution at 25 °C. The reaction mixture was stirred for 20 min at 25 °C. 2-Cyanoethoxybis-(N,N-diisopropylamino) phosphine (794.519 mg, 2.636 mmol) was added to the solution over 20 min (in five portions). After stirring for 60 min, another portion of solution of tetrazole in CH_3CN (0.45 M, 10 mL) was added to this solution. After stirring for another 1 h, the reaction mixture was diluted with EtOAc (100 mL) and filtered. The organic layer was washed with saturated aqueous $NaHCO_3$ (50 mL), brine (50 mL), dried over $MgSO_4$ (after stirring for 5 min followed by filtration), and the filtrate evaporated to dryness to give compound **8c** (1.76 g, crude) as a white solid. The resulting solid was used directly without further purification for the next step. Step 3 was repeated a second time using the same scale.

Step 4: preparation of compounds **9a** and **9b**

To a solution of compound **8c** (1.76 g, 1.848 mmol) in MeCN (30 mL) was added 3H-benzo[c][1,2]dithiol-3-one 1,1-dioxide (1.85 g, 9.238 mmol) at 25 °C. After stirring at 25 °C for 1 h, the reaction mixture was filtered, and the resulting cake was washed with CH_2Cl_2/MeOH (10/1, 20 mL x 3). The combined filtrates were concentrated under pressure to give a residue. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH = 100/1 to 10/1) to give compound **9a** (652 mg) as a yellow foam and compound **9b** (660 mg) as a yellow foam.

Compound **9a** was re-purified by reverse phase preparative HPLC (column: Phenomenex Gemini C18 250x50 10μm; mobile phase: water (10 mM NH_4HCO_3)-ACN, Begin B:30; End B: 60; Flow Rate: 25 mL/min Gradient Time: 15 min) to give compound **9a** (225 mg, 0.229 mmol) as a white solid. Compound **9b** was re-purified by reverse phase preparative HPLC (column: Waters Xbridge 150x25 5μm; mobile phase: water (10 mM NH_4HCO_3)-ACN, Begin B:37; End B: 67; Flow Rate: 25 mL/min Gradient Time: 8 min) to give **9b** (351 mg, 0.356 mmol, 19.294% yield) as a white solid. ESI-MS: *m/z* 985.5 $[M + H]^+$.

Step 5: Preparation of compound **13, ammonium salt**

Compound **9a** (100 mg, 0.102 mmol) was treated with $MeNH_2$ (33% in EtOH, 5 mL) and stirred at 25 °C for 12 h. The reaction 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 (10 mM NH_4HCO_3)-ACN, Begin B: 0, End B:

15 %, flow rate: 35 mL/min, Gradient Time: 10 min) to give compound **13 ammonium salt** (56 mg, 0.08 mmol) as a white solid. ESI-MS: *m/z* 704.8 $[M + H]^+$. ^1H NMR (400 MHz, D_2O) δ 8.30 (br, s, 1H), 7.93 (br s, 2H), 6.41(br, d, *J* =15.8 Hz, 1H), 5.98 - 5.68 (m, 2H), 5.57 (br, s, 1H), 5.24 - 4.95 (m, 1H), 4.62- 4.35 (m, 3H), 4.29 - 4.12 (m, 3H), 4.02 (br d, J=9.3 Hz, 1H), 3.60 (s, 3H), -0.48 (br, s, 3H); ^{19}F NMR (376 MHz, D_2O) -199.64--201.37 (m, 1F); ^{31}P NMR (162 MHz, D_2O) 91.37 (s, 1P), 91.31 (s, 1P), 90.52 (s, 1P), 89.94 (s, 1P), 52.36 (s, 1P), 52.26 (s, 1P).

Step 6: preparation of compound **13 sodium salt**

Dowex 50W x 8, 200-400 (H form, 50 g) was added to a beaker (for 56 mg of cpd **13**) and washed with deionized water (2x). then added to the resin (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 volumes), and then with deionized water until the resin was neutral. The resin was transferred back into the beaker, and a NaOH solution (15% NaOH in water 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 water (at least 4 column volumes) and then with water until it was neutral (at least 4 column volumes). Compound **12** was dissolved in deionized water (50 mg in 40 mL), added to the top of the column and eluted with deionized water. Compound **12** eluted from the column in early fractions as detected by TLC (UV). Compound **13** was dissolved in deionized water (56 mg in 30 mL), added to the top of the column, and eluted with deionized water. A product eluted from the column in early fractions as detected by TLC (UV). The product was lyophilized to afford compound **13 sodium salt** (45.4 mg, 0.064 mmol) as a white solid. ^1H NMR (400 MHz, D_2O) δ 8.25 (s, 1H), 8.21 (s, 1H), 7.95 (s, 1H), 6.42 (d, *J* =14.8 Hz, 1H), 5.91 - 5.84 (m, 1.5H), 5.76 (d, *J* =3.8 Hz, 0.5H), 5.60 - 5.51 (m, 1H), 5.19 - 5.04 (m, 1H), 4.61 - 4.53 (m, 2H), 4.52 - 4.44 (m, 1H), 4.27 - 4.18 (m, 3H), 4.07 (dd, *J* =4.0, 12.0 Hz, 1H), 3.60 (s, 3H), 0.47 - -0.89 (m, 3H); ^{19}F NMR (377 MHz, D_2O) -201.93 (s, 1F); ^{31}P NMR (162 MHz, D_2O) δ 92.47 (br dd, *J* =26.4, 73.4 Hz, 1P), 91.98 (s, 1P), 91.71 (s, 1P), 91.24 (s, 1P), 91.19 (s, 1P), 91.10 (s, 1P), 90.97 (s, 1P), 52.78 (s, 1P), 52.64 (s, 1P); ESI-MS: *m/z* 704.8 $[M + H]^+$.

Step 7: preparation of Compound **9c**

To a solution of compound **9b** (311 mg, 0.316 mmol) in MeCN/EtOH(1/1, 5 mL) was added tert-butylamine (5 mL). After stirring for 2 h, the reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse phase preparative HPLC (column: Waters Xbridge 150x25 5μm; mobile phase: water (10mM NH_4HCO_3)-ACN, Begin B: 8; End B: 38; Flow Rate: 25 mL/min Gradient Time: 8 min) to give compound **9c** (243 mg, 0.277 mmol) as a white solid.

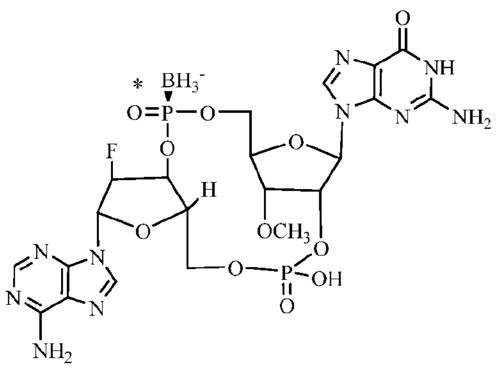
Step 8: preparation of compound **14 ammonium salt**

Compound **9c** (243 mg, 0.277 mmol) was treated with $MeNH_2$ (33% in EtOH, 10 mL) and stirred at 25 °C for 12 h. The reaction mixture was then 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)-ACN, Begin B: 0, End B: 15 %, flow rate: 35 mL/min, Gradient Time: 10 min) to afford compound **14 ammonium salt** (125 mg, 0.177 mmol) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 8.23 (d, J = 2.5 Hz, 2H), 8.01 (s, 1H), 6.43 (d, J = 15.1 Hz, 1H), 5.95 - 5.76 (m, 2H), 5.56 (dt, J = 4.3, 8.9 Hz, 1H), 5.21 - 5.05 (m, 1H), 4.64 - 4.46 (m, 3H), 4.33 - 4.15 (m, 4H), 3.56 (s, 3H), 0.96 - -0.39 (m, 3H); ^{19}F NMR (377 MHz, D_2O) δ -201.77 (s, 1F); ^{31}P NMR (162 MHz, D_2O) δ 93.50 (s, 1P), 92.44 (s, 1P), 52.51 (s, 1P); ESI-MS: m/z 704.8 $[M + H]^+$.

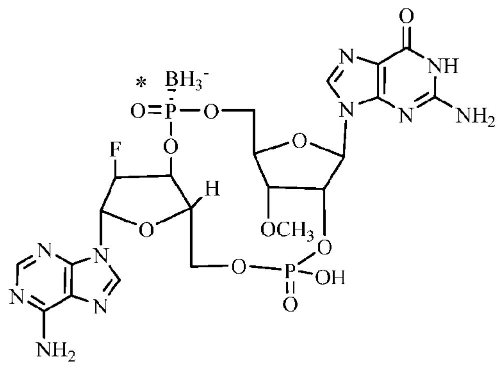
Step 9: preparation of compound **14 sodium salt**

Dowex 50W x 8, 200-400 (H form, 50 g) was added to a beaker (for 125 mg of cpd **14**) and washed with deionized water (2x) then added to the resin (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 volumes), and then with deionized water until the resin was neutral. The resin was transferred back into the beaker, and a NaOH solution (15% NaOH in water 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 water (at least 4 column volumes) and then with water until it was neutral (at least 4 column volumes). Compound **14** was dissolved in deionized water (125 mg in 40 mL), added to the top of the column, and eluted with deionized water. Product was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to afford **compound 14 sodium salt** (105.4 mg, 0.141 mmol) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 8.21 (br, d, J = 9.0 Hz, 2H), 7.89 (s, 1H), 6.38 (d, J = 15.3 Hz, 1H), 5.89 - 5.72 (m, 2H), 5.67 (br, s, 1H), 5.19 - 5.02 (m, 1H), 4.62 - 4.42 (m, 3H), 4.27 - 4.17 (m, 3H), 4.07 (br d, J = 9.8 Hz, 1H), 3.57 (s, 3H), 0.36 (br, s, 3H); ^{19}F NMR (377 MHz, D_2O) δ -201.25 (s, 1F); ^{31}P NMR (162 MHz, D_2O) δ 92.42 - 90.93 (m, 1P), 52.35 (s, 1P); ESI-MS: m/z 704.8 $[M + H]^+$.

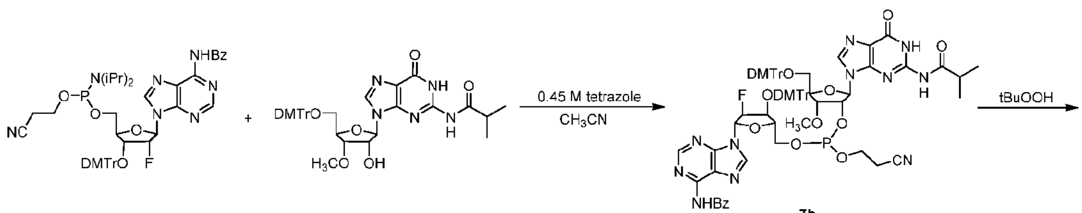
Example 10



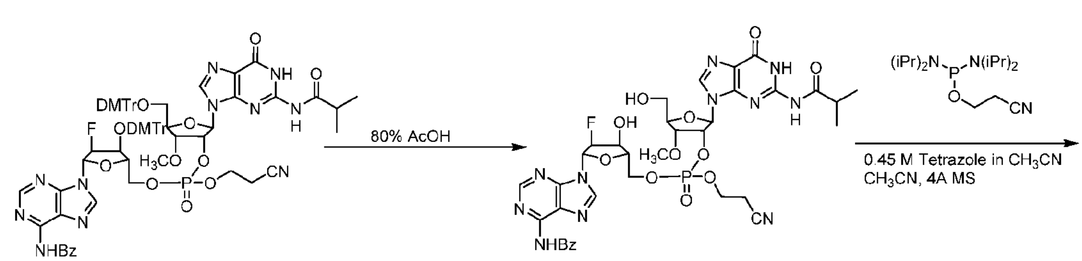
Compound 15



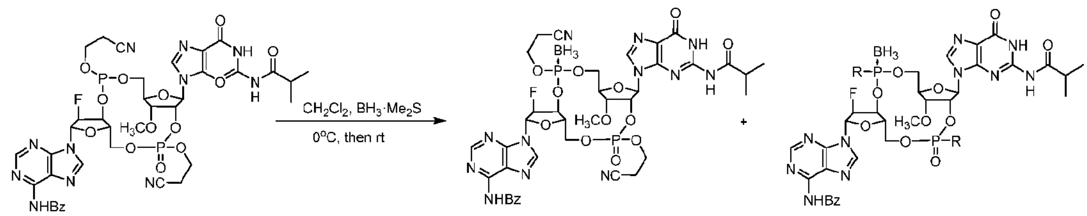
Compound 16



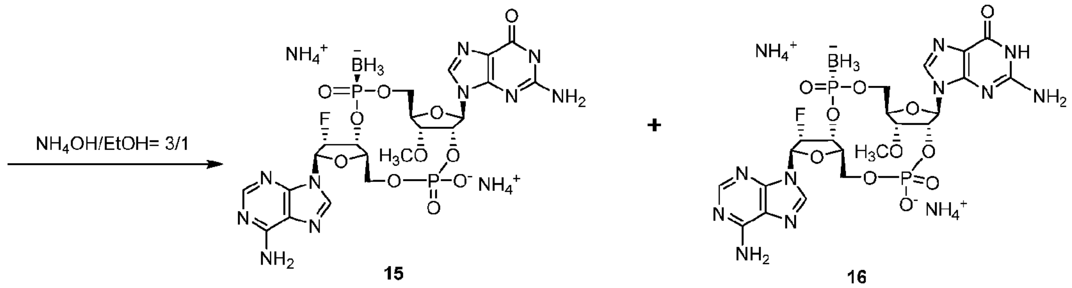
7a 1f 7b



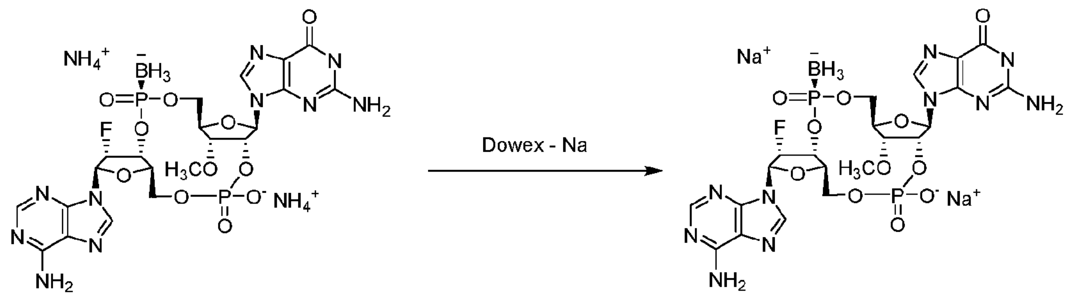
10a **10b**



10c **10d** **10e, R=OH and/or OCH$_2$CH$_2$CN**



15 **16**



15 **Compound 15, sodium salt**

Step 1: Preparation of Compound **7b**

To a solution of compound **1f** (5.0 g, 7.47 mmol) in acetonitrile (180 mL) was added 1H-tetrazole (0.45 M, 132.7 mL) at 25 °C. After stirring the solution for 10 min at 25 °C, a solution of compound **7a** (6.87g, 7.84 mmol) in acetonitrile (20 mL) was added dropwise. After stirring for 2 hours at 25 °C, the solution was used into the next step without any further purification.

Step 2: Preparation of Compound **10a**

To the previous solution of compound **7b** (332.7 mL, 7.44 mmol) in acetonitrile was added tert-butylhydroperoxide (3.35 g, 37.22 mmol) at 25 °C. After stirring at 25 °C for 1.5 h, the solution was diluted with EA (100 mL) and washed with aqueous saturated $NaHCO_3$ (2 x 100 mL) and brine (2 x 100 mL). The organic layer was successively dried over anhydrous Na_2SO_4, filtered and the solvent evaporated under reduced pressure to give compound **10a** (10 g) as a white solid.

Step 3: Preparation of Compound **10b**

To a solution of compound **10a** (10 g, crude) in acetonitrile (50 mL) was added triethylsilane (40 mL) and 80% acetic acid in acetonitrile (200 mL) at 25 °C. After stirring the solution at 50 °C for 12 hours, the mixture was neutralized with aqueous saturated $NaHCO_3$ to pH 8. The mixture was diluted with EtOAc (500 mL) and the organic layer was successively washed with aqueous saturated $NaHCO_3$ (100 mL), brine (100 mL) and evaporated under reduced pressure to dryness. The aqueous layer was extracted with EtOAc (2 x 200 mL) and evaporated under reduced pressure to dryness. The combined crude material was purified by flash column chromatography on silica gel (0-9 % MeOH in CH_2Cl_2, v/v) to give compound 10b (5 g) as a white solid. ESI-MS: *m/z* 856.2 $[M + H]^+$.

Step 4: Preparation of Compound **10c**

To a solution of compound **10b** (1.5 g, 1.4 mmol) in tetrahydrofuran (2 mL) and acetonitrile (75 mL) was added 1H-tetrazole (0.45 M, 15.58 mL, 7.01 mmol) at 25 °C. It was then added a solution of 3-((bis(diisopropylamino)phosphino)oxy)propanenitrile (845.34 mg, 2.8 mmol) in acetonitrile (5 mL) at 25 °C. After stirring for 1.5 h at 25 °C, the solution was washed with aqueous saturated $NaHCO_3$ (50 mL), brine (50 mL) and evaporated under reduced pressure to dryness to give compound **10c** (1.8 g) as a yellow solid which was used into the next step without any further purification. ESI-MS: *m/z* 955.5 $[M + H]^+$.

Preparation of compound 15

Step 5: Preparation of Compounds **10d** + **10e**

To a solution of compound **10c** (1.5 g, 1.57 mmol) in DCM (100 mL) was added Borane dimethyl sulfide (2.36 mL, 4.71 mmol) at 0 °C for 2 mins. After stirring the mixture at 25 °C for 15 min, water (30 mL) was added. The resulting solution was filtered and the filtrate concentrated under reduced pressure to give a yellow solid. The solid was diluted with DCM (100 mL) and the organic layer was successively washed with water (2 x100 mL), brine (3 x 100 mL) and concentrated under pressure to give a residue. The crude solid was purified by flash column chromatography on silica gel (0-9 % MeOH in CH_2Cl_2, v/v) to give a mixture of compounds **10d** and **10e** (500 mg, crude) as a yellow solid. ESI-MS: *m/z* 969.3 $[M + H]^+$

Step 6: Preparation of **compound 15**

A solution of compounds **10d** and **10e** (500 mg, crude) in a mixture of ethanol (14 mL) and NH_4OH (42 mL) was stirred at 50 °C for 12 hours. The solution was concentrated under pressure to give a yellow solid. The yellow solid was purified by reverse phase preparative HPLC (Column: Synergi Polar-RP 100 x 30 5μM; Condition: water (10mM NH_4HCO_3)-ACN; Begin B: 0, End B: 20; Gradient Time (min): 12; Flow Rate (ml/min): 25) to afford compound **15** (110 mg) as a white solid. Compound **15** was purified a second time by reverse phase preparative HPLC (Column: Phenomenex Kinetex XB-C18 150mm x 30mm, 5μM; Condition: water (10mM NH4HCO3)-ACN; Begin B: 0, End B: 5; Gradient Time(min): 7; Flow Rate (ml/min): 30) to give compound **15** ammonium salt (45 mg) as a white solid. ^1H NMR (400 MHz, D_2O) δ 8.31 (br, s, 1H), 8.15 (s, 1H), 7.94 (br, s, 1H), 6.43 (br, d, J=16.3 Hz, 1H), 5.98 (br, d, J=7.7 Hz, 1H), 5.67 - 5.47 (m, 1H), 5.28 (br, s, 1H), 4.93 (br, s, 1H), 4.61 - 4.49 (m, 2H), 4.43 (br, d, J=9.7 Hz, 1H), 4.26 (br, s, 2H), 4.18 (br, s, 1H), 4.05 (br s, 1H), 3.60 (s, 3H), 0.33 (br s, 3H). ^{19}F NMR (376 MHz, D_2O) δ -201.82 (s, 1F). ^{31}P NMR (162 MHz, D_2O) δ 96.09 (br, s, 1P), -2.40 (s, 1P). ESI-MS: *m/z* 688.9 $[M + H]^+$.

Step 7: Preparation of **compound 15 sodium salt**

Dowex 50W x 8, 200-400 (H form, 5 mL) was added to a beaker (for 45 mg of cpd **15 ammonium salt**) and washed with deionized water (2x) then added to the resin (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 volumes), and then with deionized water until the resin was neutral. The resin was transferred back into the beaker, and a NaOH solution (15% NaOH in water 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 water (at least 4 column volumes) and then with water until it was neutral (at least 4 column volumes). Compound **15 ammonium salt** was dissolved in deionized water (45 mg in 5 mL), added to the top of the column, and eluted with deionized water. Product was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to afford **compound 15 sodium salt** P1 (42.6 mg) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 8.21 (s, 1H), 8.09 (s, 1H), 7.97 (s, 1H), 6.39 (br, d, J=16.1 Hz, 1H), 6.00 (br, d, J=8.3 Hz, 1H), 5.70 - 5.52 (m, 1H), 5.23 (dt, J=4.5, 8.3 Hz, 1H), 5.04-4.88 (m, 1H), 4.63-4.51 (m, 3H), 4.44 (br, d, J=11.8 Hz, 1H), 4.31-4.19 (m, 3H), 4.06 (br, d, J=10.8 Hz, 1H), 3.59 (s, 3H), 0.67-0.12 (m, 3H). ^{19}F NMR (376 MHz, D_2O) δ -201.80 (s, 1F). ^{31}P NMR (162 MHz, D_2O) δ 95.45 (s, 1P), -2.26 (s, 1P). ESI-MS: *m/z* 688.8 $[M + H]^+$

Preparation of compound 15 and compound 16

Step 5: Preparation of Compounds **10d** + **10e**

To a solution of compound **10c** (2.3 g, 2.41 mmol) in DCM (100 mL) was added Borane dimethyl sulfide (3.61 mL, 7.23 mmol) at 0 °C for 2 mins. After stirring the mixture at 25 °C for 15 min, water (20 mL) was added. The resulting solution was filtered and the filtrate concentrated under reduced pressure to give a yellow solid. The solid was diluted with DCM (100 mL) and the organic layer was successively washed with water (2 x100 mL), brine (3 x 100 mL) and concentrated under pressure to give a yellow residue. The crude solid was purified by reverse phase preparative HPLC (Column: Agela Durashell C18 150 x 25 5µM; Condition: water (10 mM NH_4HCO_3)-ACN; Begin B: 25, End B: 55; Gradient Time (min): 12; Flow

Rate(ml/min): 25) to give a mixture of compounds **10d** and **10e** (65 mg,) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.81 - 8.71 (m, 1H), 8.50 (d, J=6.2 Hz, 1H), 8.08 (br, d, J=8.4 Hz, 2H), 7.69 - 7.54 (m, 3H), 6.59 - 6.50 (m, 1H), 6.37 - 6.26 (m, 2H), 6.02 - 5.86 (m, 1H), 5.26 - 5.04 (m, 2H), 4.77 - 4.68 (m, 1H), 4.63 - 4.54 (m, 3H), 4.43 (br, d, J=6.2 Hz, 2H), 4.34 - 4.27 (m, 2H), 3.86 - 3.69 (m, 2H), 2.98 - 2.90 (m, 3H), 2.71 (br, dd, J=5.8, 13.1 Hz, 1H), 2.61 - 2.49 (m, 1H), 1.22 (td, J=3.3, 6.7 Hz, 7H). ESI-MS: *m/z* 969.3 $[M + H]^+$.

Step 6: Preparation of **compound 15 and compound 16**

A solution of compounds **10d** and **10e** (65 mg, crude) in a mixture of ethanol (4 mL) and NH_4OH (12 mL) was stirred at 50 °C for 12 hours. The solution was concentrated under pressure to give a residue. The residue was purified by reverse phase preparative HPLC (Column: Synergi Polar-RP 100 x 30 5μM; Condition: water (10mM NH_4HCO_3)-ACN; Begin B: 0, End B: 20; Gradient Time (min): 12; Flow Rate (ml/min): 25) to afford compound **15** (37 mg) and compound **16** (17 mg) as white solids.

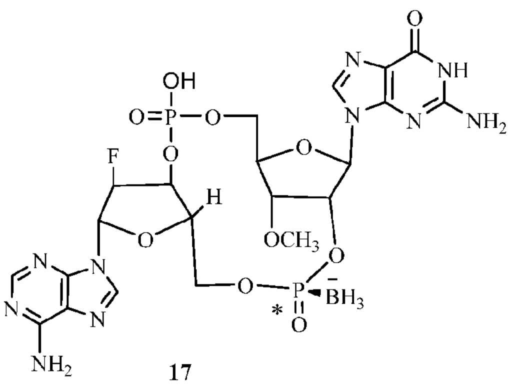
Compound **15 ammonium salt**: ^{1}H NMR (400 MHz, D_2O) δ 8.41 (br, s, 1H), 8.23 (br, s, 1H), 7.98 (br, s, 1H), 6.46 (br, d, J=16.3 Hz, 1H), 5.99 (br, s, 1H), 5.74 - 5.46 (m, 1H), 5.27 (br, s, 1H), 5.07-4.91 (m, 1H), 4.61 - 4.50 (m, 2H), 4.42 (br, s, 1H), 4.26 (br, s, 2H), 4.16 (br, s, 1H), 4.05 (br, s, 1H), 3.61 (s, 3H), 0.71 - 0.07 (m, 2H). ^{19}F NMR (376 MHz, D_2O) δ -75.63 (s, 1F). ^{31}P NMR (162MHz, D_2O) δ 94.56 (s, 1P), -3.69 (s, 1P). ESI-MS: *m/z* 688.9 $[M + H]^+$

Compound **16 ammonium salt**: ^{1}H NMR (400 MHz, D_2O) δ 8.37 (br s, 1H), 8.24 (br s, 1H), 7.85 (s, 1H), 6.42 (d, J=14.1 Hz, 1H), 5.91 - 5.88 (m, 1H), 5.8 (br s, 1H), 5.51 (br s, 1H), 5.38 (br s, 1H), 5.32 - 5.20 (m, 1H), 4.56 - 4.48 (m, 2H), 4.45 - 4.36 (m, 1H), 4.28 - 4.17 (m, 3H), 4.09 (br d, J=11.0Hz, 1H), 3.59 (s, 3H), 0.61 - 0.10 (m, 3H). ^{19}F NMR (376 MHz, D_2O) δ -202.9 (s, 1F). ^{31}P NMR (162 MHz, D_2O) δ 96.67 (m, 1P), -3.02 (s, 1P). ESI-MS: *m/z* 689.2 $[M + H]^+$

Step 7: Preparation of Compound **15 sodium salt**

Dowex 50W x 8, 200-400 (H form, 5 mL) was added to a beaker (for 37 mg of cpd **15 ammonium salt**) and washed with deionized water (2x) then added to the resin (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 volumes), and then with deionized water until the resin was neutral. The resin was transferred back into the beaker, and a NaOH solution (15% NaOH in water 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 water (at least 4 column volumes) and then with water until it was neutral (at least 4 column volumes). Compound **15 ammonium salt** was dissolved in deionized water (37 mg in 5 mL), added to the top of the column, and eluted with deionized water. Product was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to afford **compound 15 sodium salt** P1 (28.4 mg) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 8.25 (s, 1H), 8.12 (s, 1H), 7.99 (s, 1H), 6.42 (br, d, *J*=15.8 Hz, 1H), 6.02 (br, d, *J*=8.3 Hz, 1H), 5.70 - 5.52 (m, 1H), 5.30 - 5.21 (m, 1H), 5.06 - 4.91 (m, 1H), 4.62 - 4.54 (m, 2H), 4.44 (br, d, *J*=12.8 Hz, 1H), 4.30 (br, d, *J*=4.3 Hz, 2H), 4.21 (br, d, *J*=12.0 Hz, 1H), 4.06 (br, d, *J*=11.8 Hz, 1H), 3.60 (s, 3H), 0.71 - 0.09 (m, 3H). ^{19}F NMR (376 MHz, D_2O) δ -75.62 (s, 1F), -201.89 (s, 1F). ^{31}P NMR (162 MHz, D_2O) δ 96.08 (s, 1P), -2.24 (s, 1P). ESI-MS: *m/z* 688.8 $[M + H]^+$

Example 11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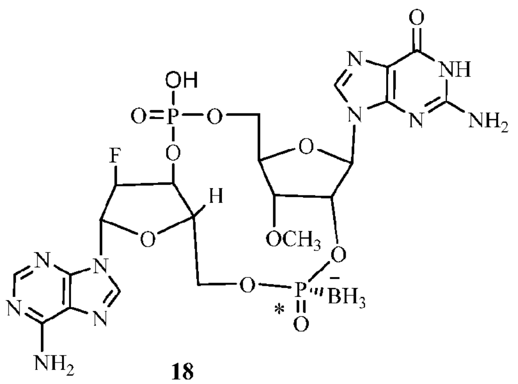
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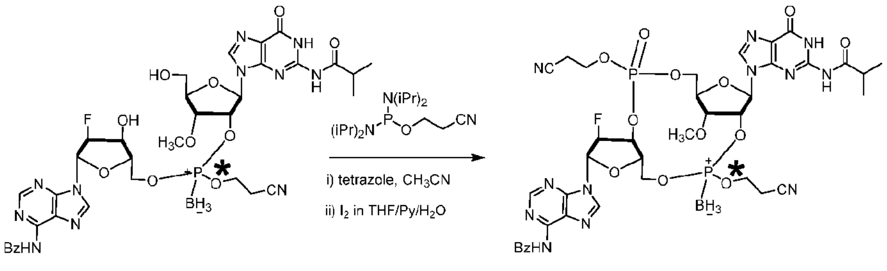
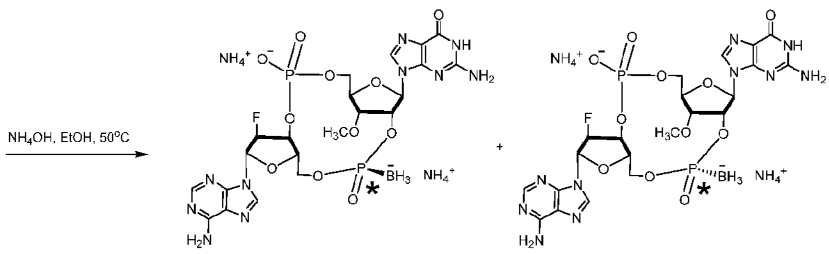
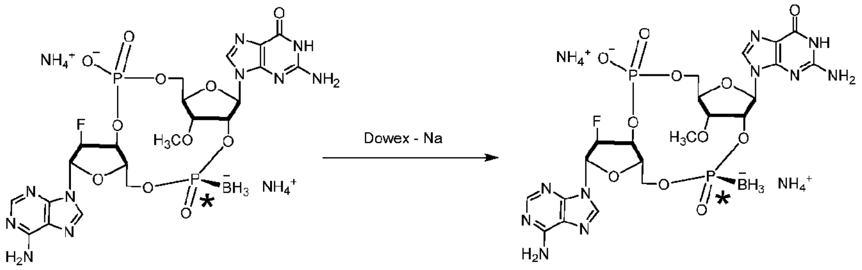


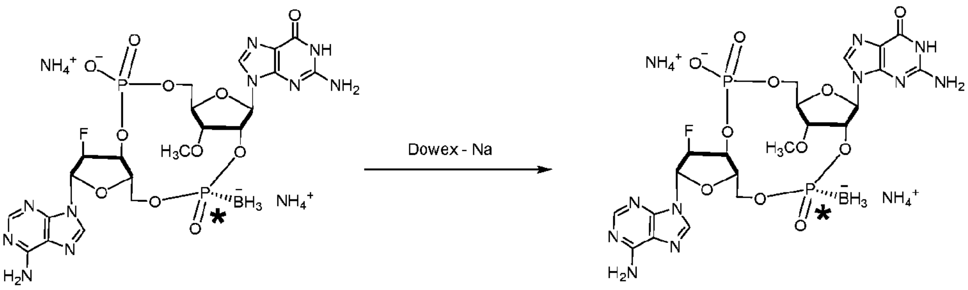
8b 11a



17 18



17 Compound 17, sodium salt



18 Compound 18, sodium salt

Step 1: preparation of (**11a**)

To a solution of **8b** (100 mg, 0.11 mmol) in CH_3CN/THF (1:1, v/v, 4.4 mL) was added 4Å molecular sieves (1 g) and 1H-tetrazole in CH_3CN (1.94 mL, 0.9 mmol). After stirring the mixture at 25 ℃ for 0.5 h, 2-cyanoethyl N,N,N',N'-tetraisopropylphospho-rodiamidite (49.56 mg,0.16 mmol) in CH_3CN was added to the mixture. The mixture was stirred at 25 °C for 2 h and 1H-tetrazole in CH_3CN (0.49 mL, 0.22 mmol, 0.45M) was added to the mixture. After stirring the mixture at 25 °C for 0.5 h, a solution of 0.5 M of I_2 in THF: Py : H_2O (8: 1: 1; V/V/V) (0.66 mL, 0.33 mmol) was added to the reaction. After stirring the mixture at 25 °C for 2 h, a saturated aqueous solution of sodium thiosulfate (2 mL) was added; the resulting mixture was filtered and the filtrate was concentrated under reduced pressure to dryness. The residue was purified by reverse phase preparative HPLC (Agela Durashell C18 150 x 25 5µM; Condition: water (10mM NH_4HCO_3)-CAN A: water (10mM NH_4HCO_3) B: MeCN; Begin B: 25% to B: 55%, Gradient Time (min) 12; 100%B Hold Time (min) 2.2; Flow Rate(ml/min) 25). The pure fractions were collected, solvent concentrated under reduced pressure and aqueous layer was lyophilized to dryness to give **11a** as a white solid (20 mg). ESI-MS: m/z 969.3 [M+1]+.

Step 2: preparation of **17** and **18**

To a solution of **11a** (20 mg 0.017 mmol) in EtOH (1.5 mL) was added $NH_3.H_2O$ (1.5 mL, 25%). After stirring the solution at 50°C for 3 d, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure to dryness. The residue was purified by reverse phase preparative HPLC (Column: Syneri Polar-RP 100 x 30 5µM Condition: water (10mM NH_4HCO_3)-MeCN A: water (10mM NH_4HCO_3) B: MeCN; Begin B: 0% to B: 20%, Gradient Time (min) 12; 100% B Hold Time (min) 2.2; Flow Rate(ml/min) 25). The pure fractions were collected and the solvent was evaporated under reduced pressure to afford **17** (25 mg) and **18** (20 mg) as white solid.

Analogue **17** ammonium salt: ESI-MS: m/z 688.8 [M+1]+. ^{1}H NMR (400 MHz, D_2O) δ 8.26 (br s, 1 H) 8.18 (s, 1 H) 7.77 (br s, 1 H) 6.38 (br d, J=14.31 Hz, 1 H) 5.77 (br d, J=8.03 Hz, 1 H)5.64(br s, 1 H) 5.30 - 5.52 (m, 1 H) 4.95 - 5.13 (m, 1 H) 4.50 (br d, J=9.03 Hz, 1 H) 4.34 - 4.44 (m, 2 H) 4.07 - 4.25 (m, 3 H) 3.99 (br d, J=11.04 Hz,1 H) 3.52 (s,3 H) -0.92 - 0.05 (m, 3 H).

Analogue **18** ammonium salt: ESI-MS: m/z 688.8 [M+1]+ . ^{1}HNMR (400 MHz, D_2O) δ 8.29 (s, 1 H) 8.18 (s, 1 H) 7.75 (s, 1 H) 6.35 (d, J=14.05 Hz, 1 H) 5.76 (s, 2 H) 5.30 - 5.52 (m, 1 H) 4.99 -5.20 (m, 1 H) 4.35 - 4.50 (m, 3 H) 4.09 - 4.22 (m, 3 H) 3.94 - 4.03 (m, 1 H) 3.47 (s, 3 H) 0.05 (s, 3 H).

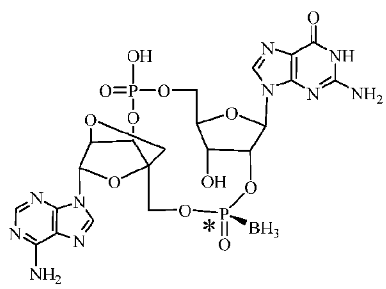
Step 3: preparation of **17** sodium salt

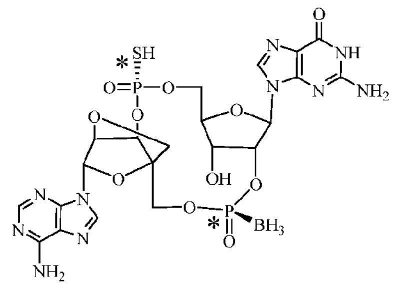
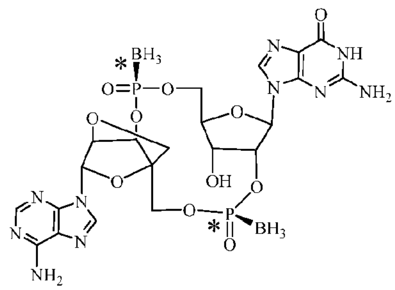
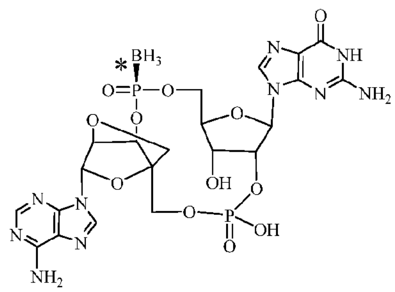
Dowex 50W x 8, 200-400 (H form, 25 g) was added to a beaker (for 33 mg of cpd **17**) and washed with deionized water (2 x 10mL) then added to the resin 15% H_2SO_4 in deionized water (80 mL). The mixture was stirred for 15 min and decanted (1 x 10mL). The resin was transferred to a column with 15% H_2SO_4 in deionized water and washed with 15% H_2SO_4 (at least 4 column volumes), and then with deionized water until the resin was neutral. The resin was transferred back into the beaker, and a NaOH solution (15% NaOH in water solution, 50 mL) was added. The mixture was stirred for 15 min and decanted (1 x 10mL). The resin was transferred to the column and washed with 15% NaOH in water (at least 4 column volumes) and then with water until it was neutral (at least 4 column volumes). Compound **17** ammonium salt was dissolved in deionized water (33 mg in 5 mL), added to the top of the column, and eluted with deionized water. Product was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to afford **compound 17 sodium salt** (12.4 mg) as a white solid. ESI-MS: *m/z*= 688.8 $[M+1]^+$. ^{1}H NMR (400 MHz, D_2O) □ 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) δ -196.87 (s, 1F); ^{31}P NMR (162 MHz, D_2O) δ 7.80 (s, 1P), -1.22 (s, 1P).

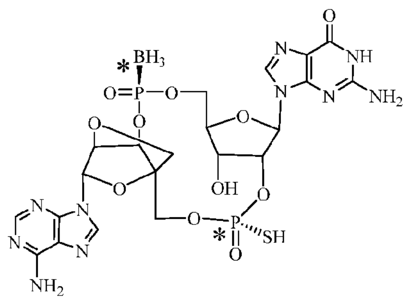
Step 4: preparation of **18** sodium salt

Dowex 50W x 8, 200-400 (H form, 15 g) was added to a beaker (for 30 mg of cpd **18**) and washed with deionized water (2 x 10mL) then added to the resin 15% H_2SO_4 in deionized water (80 mL). The mixture was stirred for 15 min and decanted (1 x 10mL). 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 volumes), and then with deionized water until the resin was neutral. The resin was transferred back into the beaker, and a NaOH solution (15% NaOH in water solution, 50 mL) was added. The mixture was stirred for 15 min and decanted (1 x 10mL). The resin was transferred to the column and washed with 15% NaOH in water (at least 4 column volumes) and then with water until it was neutral (at least 4 column volumes). Compound **18** ammonium salt was dissolved in deionized water (30 mg in 5 mL), added to the top of the column, and eluted with deionized water. Product was eluted out in early fractions as detected by TLC (UV). The product was lyophilized to afford **compound 18 sodium salt** (13.2 mg) as a white solid. ESI-MS: *m/z*= 688.8 $[M+1]^+$; ^1H NMR (400 MHz, D_2O) δ 8.22 (s, 1 H) 8.14 (s, 1 H) 7.76 (s, 1 H) 6.34 (d, J=14.05 Hz, 1 H) 5.77 (d, J=8.28 Hz, 1 H) 5.60 - 5.69 (m, 1 H) 5.30 - 5.48 (m, 1 H) 4.94 - 5.11 (m, 1 H) 4.49 (br d, J=9.29 Hz, 1 H) 4.35 - 4.45 (m, 2 H) 4.17 - 4.23 (m, 1 H) 4.12 - 4.17 (m, 1 H) 4.09 (br d, J=4.52 Hz, 1 H) 3.99 (br dd, J=12.05, 4.52 Hz, 1 H) 3.52 (s, 3 H) -0.87 - 0.01 (m, 3 H). ^{31}P NMR (162 MHz, D_2O) δ 92.38 (br s, 1 P) -1.31 (s, 1 P). ^{19}F NMR (376 MHz, D_2O) δ -75.64 (s, 1 F) -202.91 (br s, 1 F).

By the method of Example 3, substituting appropriate reagents, the following compounds may be prepared by one of skill in the art:

Cpd No.	Structure
19	

Cpd No.	Structure
20	
21	
22	

Cpd No.	Structure
23	

Biological Examples

In Vitro Assays

Biological Example 1

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 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.

<u>Full length human STING binding assay</u>

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 37°C, 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 2.

Table 2.

Cpd No.	hSTING SPA IC50 (μM)*	human cell reporter EC50 (μM)*	SPR human STING KD (μM)	ThermoFluor KD (μM)	human IFN-β (ranking value)	In vivo activity (%TGI)	In vivo activity (cures)
1	>100	>100	>100	>83.33	ND	ND	ND
2	<0.01	0.064	0.003	0.020	2205	87.1	2/8
3	>88.25	>10	ND	>66.67	ND	ND	ND
4	<0.01	0.09	0.002	0.001	2247	93.7	6/8
5	<0.01	0.16	0.008	0.084	2737	93.3	7/8
6	0.023	0.12	0.017	0.310	27	73.9	1/8
7	0.06	1.12	0.049	1.270	2260	94.3	5/8
8	0.035	0.11	0.042	0.510	2054	95.3	4/8
9	< 0.01	0.64	0.000195	ND	2240	89.8	ND
10	0.012	0.54	0.00158	ND	1321		
11	< 0.01	0.49			3840		
12	< 0.01	0.22			3860		
13	< 0.01	0.59			2900		
14	< 0.01	0.45			4964		

Cpd No.	hSTING SPA IC50 (μM)*	human cell reporter EC50 (μM)*	SPR human STING KD (μM)	ThermoFluor KD (μM)	human IFN-β (ranking value)	In vivo activity (%TGI)	In vivo activity (cures)
15		1.108					
17		2.88					

ND-not done, human IFN-β ranking value determined by Ranking value determined by total cumulative IFN-β induction over the dose range tested (0.78 to 50uM) in THP-1 cells.

* IC_{50} and EC_{50} are means of at least three values.

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).

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

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 3: 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 3.

Cpd No.	IL-6	IL-10	IFN-γ	IL-1b	IFN-α	TNFa	IL-12p40	IL-12p70	G-CSF	IFN-β
1	1.1	2.9	401.3	0.6	1.3	26.1	5.2	0.0	0.1	0.0
2	0.3	1.8	133.2	0.1	1.4	5.1	1.4	nt	0.0	nt
2	7.1	42.7	19.1	11.9	6.3	11.5	1.4	25.4	0.8	13.6
3	0.6	2.0	370.4	0.2	2.6	10.9	0.5	nt	0.0	nt
3	4.8	39.8	0.7	3.5	0.2	0.9	4.0	1.5	3.6	0.2
4	0.0	0.4	0.1	0.0	0.1	0.1	0.7	nt	0.0	nt
5	1.4	2.9	605.1	0.9	1.4	50.4	2.1	nt	0.1	nt
7	1.0	6.4	502.1	0.8	38.0	29.5	0.5	420.5	0.0	62.2
8	0.6	1.2	133.7	0.3	6.5	12.8	0.5	nt	0.0	nt
2'3'-cGAMP	2.5	5.9	17.4	1.4	6.8	5.6	1.0	5.5	0.4	16.0
PBS	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
DMSO	0.0	1.2	0.2	0.0	0.1	0.1	0.8	nt	0.0	nt

Table 4: 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 4.

Cpd No.	Top Conc (μM)	IL-6	IL-10	IFNγ	IL-1β	IFNα2	TNFα	IL12 p40	IL12 p70	G-CSF	IFNβ1
1	111.1	0.7	0.4	2.0	1.7	0.9	3.4	0.7	35.2	1.1	nt
2	40	2523.6	61.0	3225.5	544.8	27.0	643.4	9.6	252.7	227.0	18.2
3	40	491.4	21.2	4.0	102.8	1.4	7.2	3.1	3.7	7.4	0.4
4	111.1	0.1	0.0	1.0	0.1	0.3	0.6	0.0	7.0	0.0	nt
5	111.1	0.2	0.0	3.1	2.2	0.5	4.4	0.1	34.5	0.3	nt
7	111.1	321.9	4.1	2088.8	113.2	1033.1	244.6	0.6	27.7	2.6	14.1
8	111.1	0.4	0.0	2.1	0.7	0.6	1.4	0.0	39.4	0.1	nt
9	40	5084.7	121.4	4776.7	5072.5	106.4	1003.1	26.6	640.0	775.6	22.1
10	40	2791.9	37.7	3104.9	342.6	41.1	555.4	25.5	274.0	58.4	20.7
14	40	2209.3	24.1	4760.1	288.8	44.7	811.7	22.9	574.2	295.5	18.7
13	40	2536.1	50.0	6065.9	445.5	39.5	881.8	32.0	686.4	246.2	16.6
2'3'-cGAMP	40	454.0	12.1	1919.1	251.2	27.8	117.1	1.8	17.1	14.1	13.5
DMSO		0.5	0.3	0.4	0.6	0.5	0.4	0.5	0.6	1.2	nt

Table 5. 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 5.

Cpd No.	EC50 (μM)
2	8.24E-04

Cpd No.	EC50 (μM)
3	88119.3
7	8.51E-05

Table 6. 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.

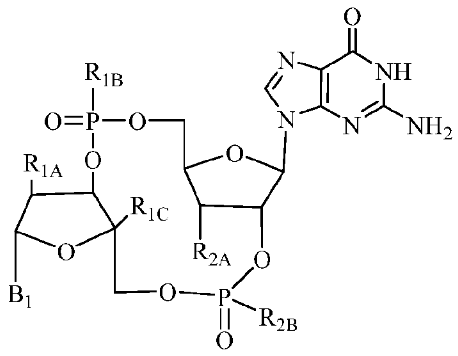
Table 6.

Cpd No.	MFI
2	5.0
3	2.0
7	5.1
2'3'-cGAMP	4.5
PBS	1.0

While the foregoing specification teaches the principles of the present invention, with examples provided for the purposes 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)



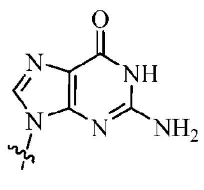
Formula (I)

wherein

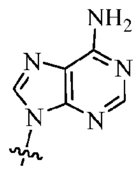
R_{1A} is hydroxy or fluoro and R_{1C} is hydrogen; or, R_{1A} is –O– and R_{1C} is CH_2 such thatR_{1A} and R_{1C} are taken together with the atoms to which they are attached to form a 5-membered ring;

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

B_1 is selected from the group consisting of rings **b1** and **b2**



b1 and



b2;

R_{2A} is selected from the group consisting of hydroxy and methoxy;

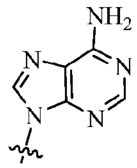
R_{2B} is selected from the group consisting of hydroxy, thiol, and BH_3^-;

provided that the compound of Formula (I) is other than (1*R*,6*R*,8*R*,9*R*,10*R*,15*R*,17*R*,18*R*)-17-(2-Amino-6-oxo-6,9-dihydro-1*H*-purin-9-yl)-8-(6-

amino-9*H*-purin-9-yl)-9-fluoro-3,12,18-trihydroxy-2,4,7,11,13,16-hexaoxa-3λ^5, 12λ^5-diphosphatricyclo[13.2.1.0^{6,10}]octadecane-3,12-dione, bis-ammonium salt;

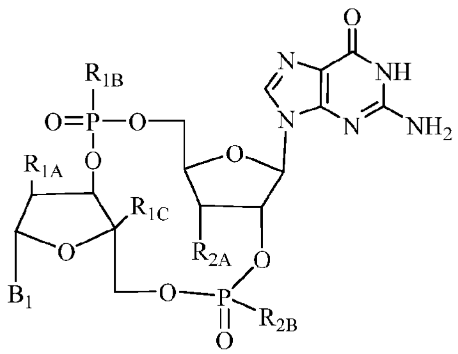
or an enantiomer, diastereomer, or pharmaceutically acceptable salt form thereof.

2. The compound of claim 1 wherein B_1 is **b2**



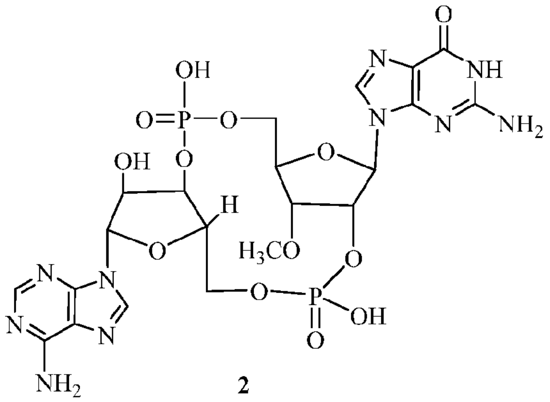
b2.

3. A compound of Formula (I)



Formula (I)

selected from the group consisting of



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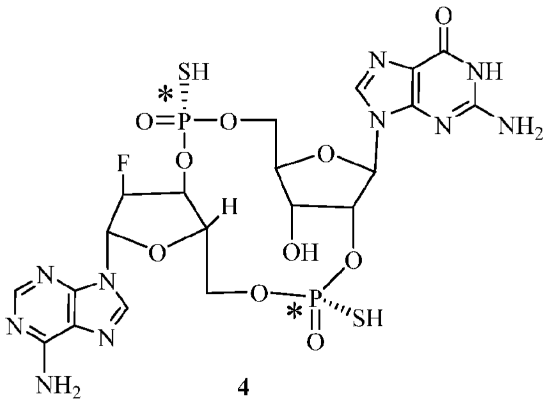

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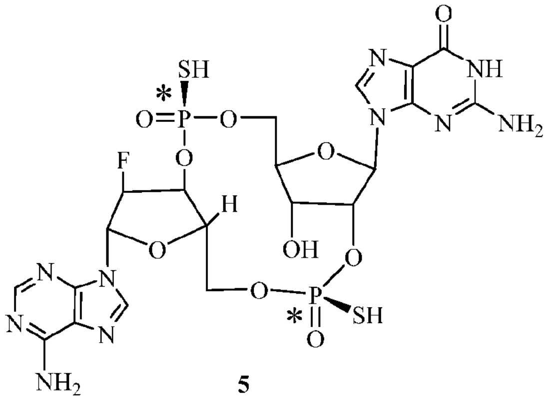

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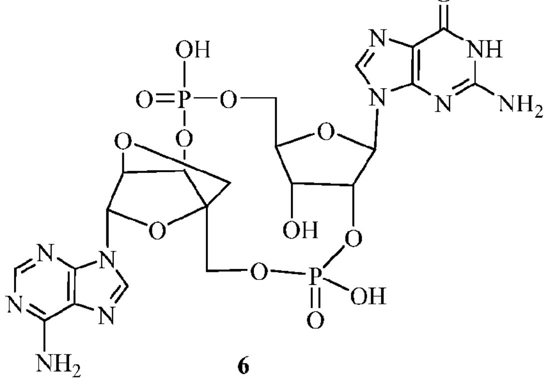

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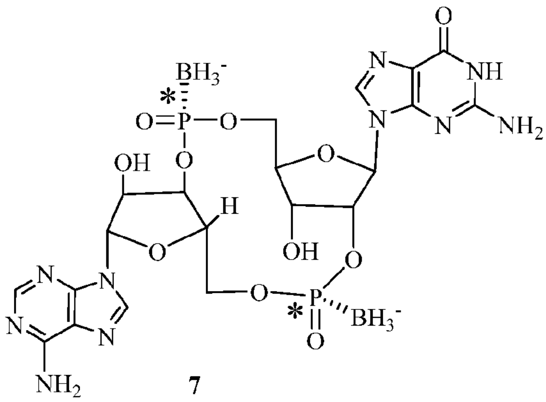

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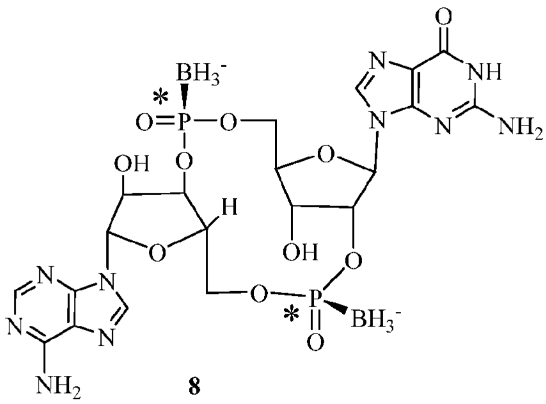

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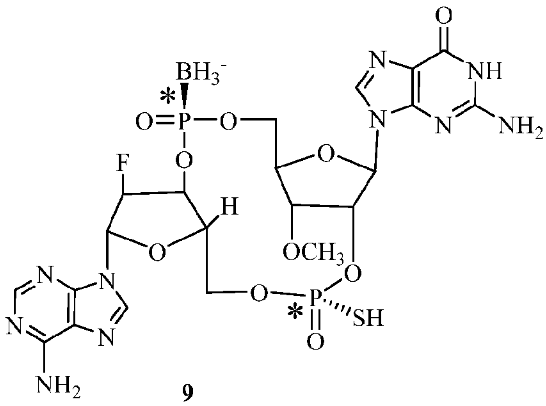

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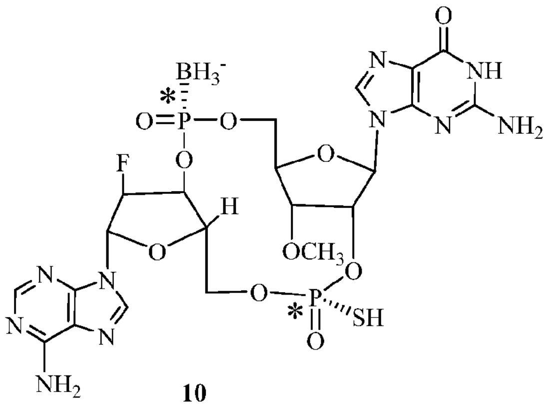

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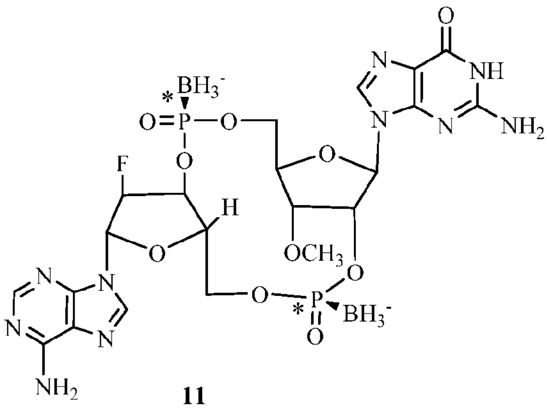

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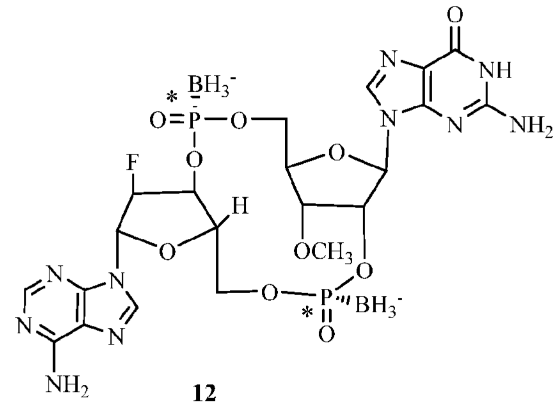

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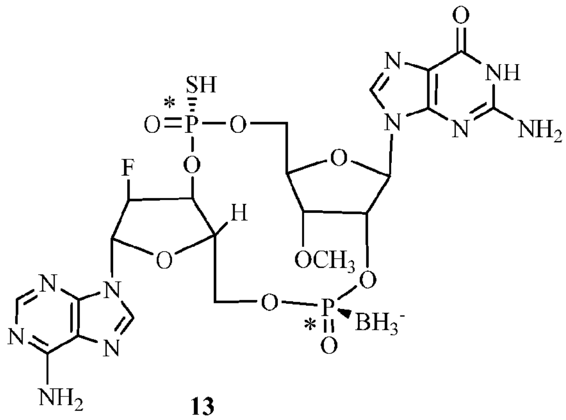

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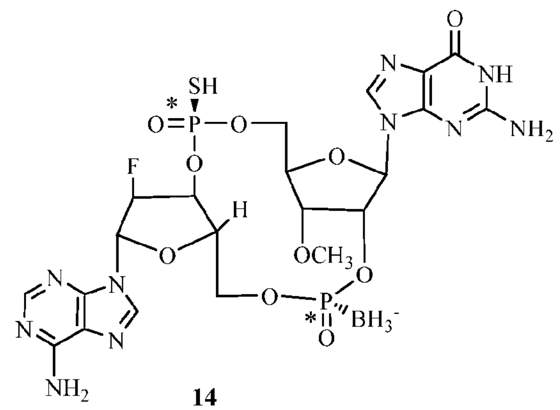

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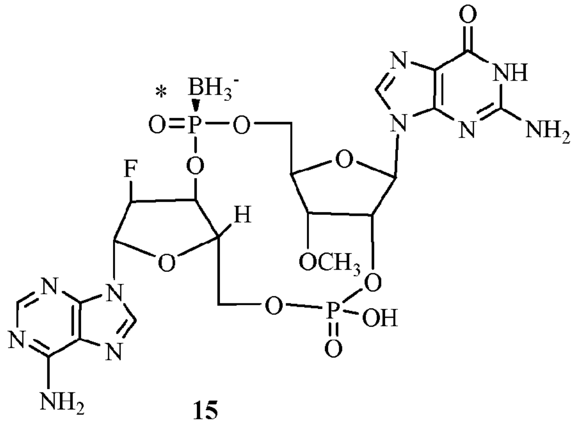

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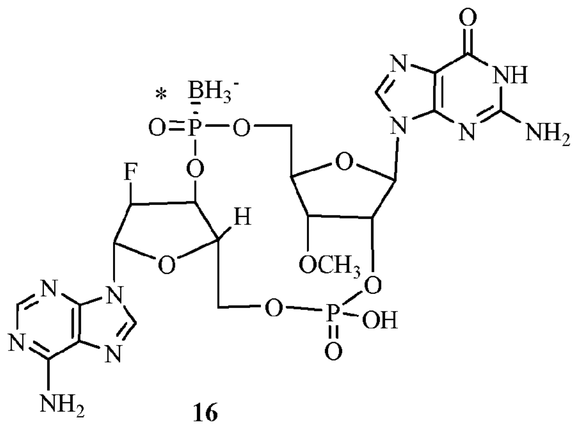

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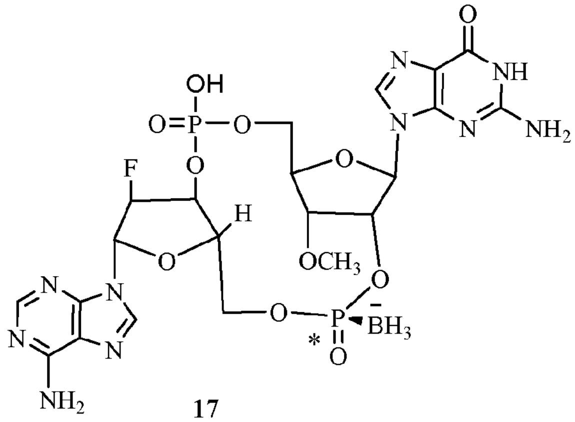

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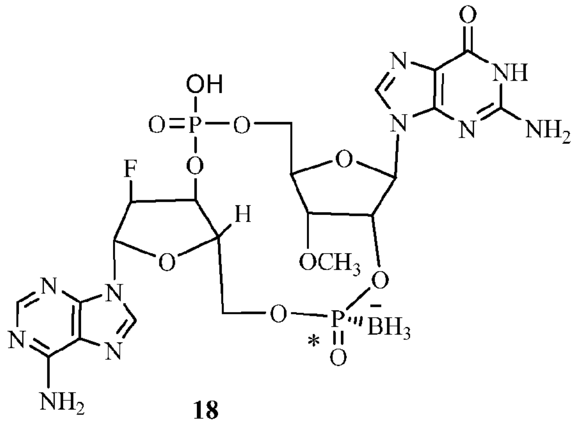

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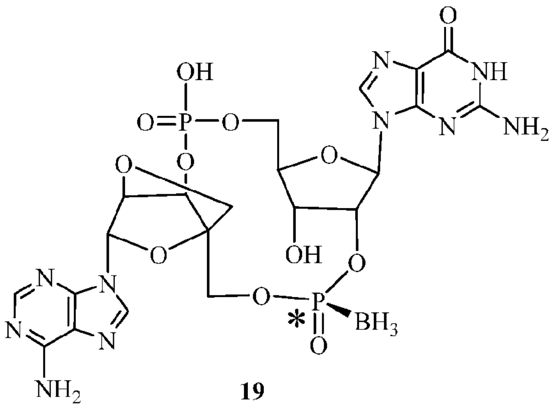

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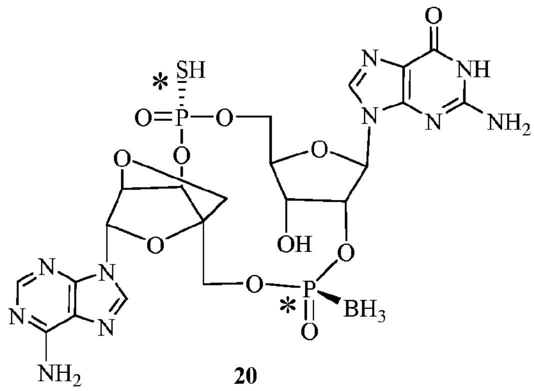

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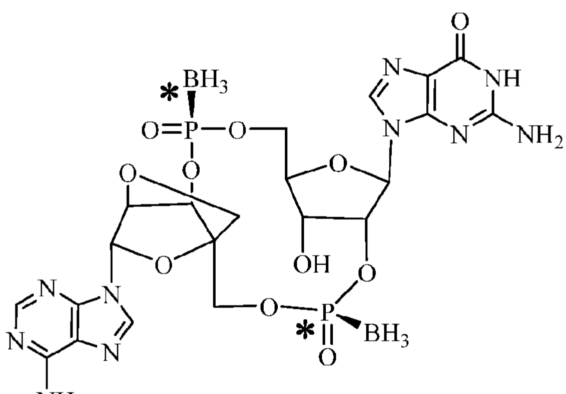

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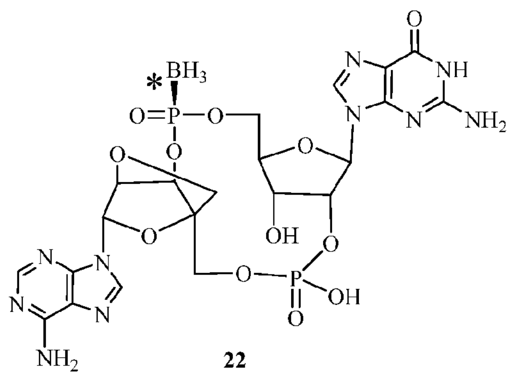

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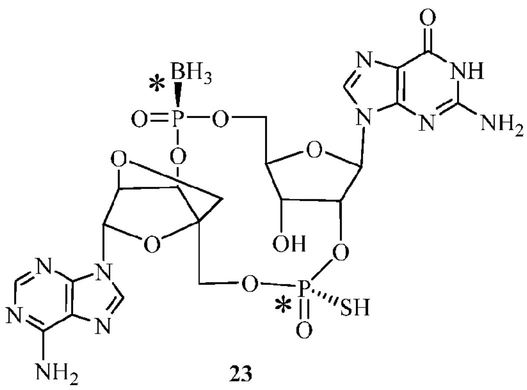

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;

or a pharmaceutically acceptable salt form thereof.

4. A pharmaceutical composition comprising a compound of any one of claims 1 to 3 and at least one of a pharmaceutically acceptable carrier, a pharmaceutically acceptable excipient, and a pharmaceutically acceptable diluent.

5. The pharmaceutical composition of claim 4, wherein the composition is a solid oral dosage form.

6. The pharmaceutical composition of claim 4, wherein the composition is a syrup, an elixir or a suspension.

7. A pharmaceutical composition comprising a compound of claim 3 and at least one of a pharmaceutically acceptable carrier, a pharmaceutically acceptable excipient, and a pharmaceutically acceptable diluent.

8. 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.

9. 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.

10. The method of claim 9 wherein said disease, syndrome, or condition is cancer.

11. The method of claim 10 wherein said cancer is selected from the group consisting of melanoma, colon cancer, breast cancer, prostate cancer, lung cancer, and fibrosarcoma.

12. The method of claim 9, wherein said disease, syndrome, or condition is viral infection.

13. The method of claim 11, wherein the viral infection is hepatitis B.

14. 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 4.

15. 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.

16. 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.

17. The method of any one of claims 13-15, wherein the viral infection is hepatitis B.

18. The method of claim 17, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of claim 3.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/062901

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07H21/02 A61K31/7084 A61P35/00 A61P31/12
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07H A61K A61P

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

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

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/120305 A1 (GLAXOSMITHKLINE IP DEV LTD [GB]) 4 August 2016 (2016-08-04)	1,2, 4-11, 14-16
Y	page 9; compound b and e	12,13, 17,18
X	US 2014/341976 A1 (DUBENSKY JR THOMAS W [US] ET AL) 20 November 2014 (2014-11-20)	1-11, 14-16
Y	the whole document in particular Figs 4 and 6	12,13, 17,18
X	WO 2014/189805 A1 (AURO BIOTECH INC [US]; UNIV CALIFORNIA [US]) 27 November 2014 (2014-11-27)	1-11, 14-16
Y	the whole document in particular Figs 2B,2C,4,6	12,13, 17,18

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 2018	09/04/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Klein, Didier

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FANG GUO ET AL: "STING Agonists Induce an Innate Antiviral Immune Response against Hepatitis B Virus", ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 59, no. 2, 1 February 2015 (2015-02-01), pages 1273-1281, XP055367110, ISSN: 0066-4804, DOI: 10.1128/AAC.04321-14 the whole document	12,13, 17,18
X,P	WO 2017/027646 A1 (MERCK SHARP & DOHME [US]; ALTMAN MICHAEL D [US]; ANDRESEN BRIAN [US];) 16 February 2017 (2017-02-16) the whole document in particular claim 22	1-18
X,P	WO 2017/075477 A1 (ADURO BIOTECH INC [US]; NOVARTIS AG [CH]) 4 May 2017 (2017-05-04) the whole document	1-18
X,P	WO 2017/093933 A1 (GLAXOSMITHKLINE IP DEV LTD [GB]) 8 June 2017 (2017-06-08) the whole document	1-18

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/062901

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