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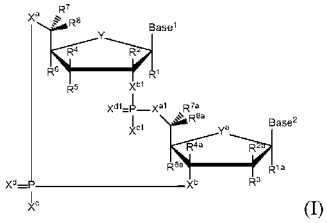
(54) Title: CYCLIC DI-NUCLEOTIDE COMPOUNDS AS STING AGONISTS



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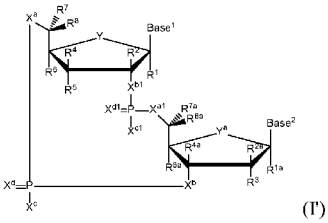

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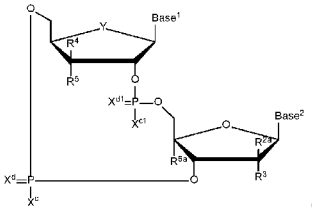

(I")

(57) Abstract: A class of polycyclic compounds of general formula (I), of general formula (I'), or of general formula (I"), wherein $Base^1$, $Base^2$, Y, Y^a, X^a, Xa^1, X^b, X^{b1}, X^c, X^{c1}, X^d, X^{d1}, R^1, R^{1a}, R^2, R^{2a}, R^3, R^4, R^{4a}, R^5, R^6, R^{6a}, R^7, R^{7a}, R^8, and R^{8a} are defined herein, that may be useful as inductors of type I interferon production, specifically as STING active agents, are provided. Also provided are processes for the synthesis and use of compounds.

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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.

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TITLE OF THE APPLICATION

CYCLIC DI-NUCLEOTIDE COMPOUNDS AS STING AGONISTS

FIELD OF THE INVENTION

The present disclosure relates to cyclic di-nucleotide compounds and derivatives thereof that may be useful as STING (Stimulator of Interferon Genes) agonists that activate the STING pathway. The present disclosure also relates to processes for the synthesis and to uses of such cyclic di-nucleotide compounds.

BACKGROUND OF THE INVENTION

The immune system has evolved to recognize and neutralize different types of threats in order to maintain the homeostasis of the host, and it is generally broken down into two arms: adaptive and innate. The adaptive immune system is specialized to recognize antigens not naturally expressed in the host as foreign and to mount an anti-antigen response through the coordinated actions of many leukocyte subsets. The hallmark of adaptive immune responses is their ability to provide "memory" or long-lasting immunity against the encountered antigen. While this specific and long-lasting effect is critical to host health and survival, the adaptive immune response requires time to generate a full-blown response.

The innate immune system compensates for this time delay and is specialized to act quickly against different insults or danger signals. It provides the first line of defense against bacteria, viruses, parasites and other infectious threats, but it also responds strongly to certain danger signals associated with cellular or tissue damage. The innate immune system has no antigen specificity but does respond to a variety of effector mechanisms. Opsonization, phagocytosis, activation of the complement system, and production of soluble bioactive molecules such as cytokines or chemokines are all mechanisms by which the innate immune system mediates its response. By responding to these damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) described above, the innate immune system is able to provide broad protection against a wide range of threats to the host.

Free cytosolic DNA and RNA are among these PAMPs and DAMPs. It has recently been demonstrated that the main sensor for cytosolic DNA is cGAS (cyclic GMP-AMP synthase). Upon recognition of cytosolic DNA, cGAS catalyzes the generation of the cyclic-dinucleotide 2'-3' cGAMP, an atypical second messenger that strongly binds to the ER-transmembrane adaptor protein STING. A conformational change is undergone by cGAMP-

bound STING, which translocates to a perinuclear compartment and induces the activation of critical transcription factors IRF-3 and NF-κB. This leads to a strong induction of type I interferons and production of pro-inflammatory cytokines such as IL-6, TNF-α and IFN-γ.

The importance of type I interferons and pro-inflammatory cytokines on various cells of the immune system has been very well established. In particular, these molecules strongly potentiate T cell activation by enhancing the ability of dendritic cells and macrophages to uptake, process, present and cross-present antigens to T cells. The T cell stimulatory capacity of these antigen-presenting cells is augmented by the up-regulation of critical co-stimulatory molecules, such as CD80 or CD86. Finally, type I interferons can rapidly engage their cognate receptors and trigger the activation of interferon-responsive genes that can significantly contribute to adaptive immune cell activation.

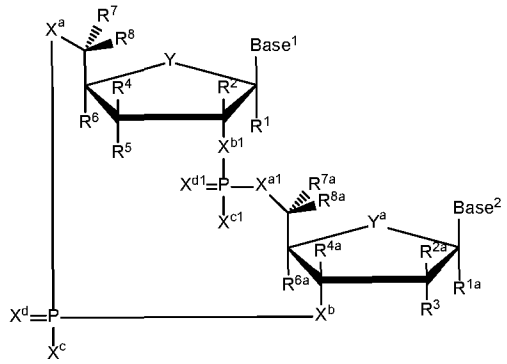
From a therapeutic perspective, type I interferons are shown to have antiviral activities by directly inhibiting human hepatitis B virus and hepatitis C virus replication, and by stimulating immune responses to virally infected cells. Compounds that can induce type I interferon production are used in vaccines, where they act as adjuvants, enhancing specific immune responses to antigens and minimizing side effects by reducing dosage and broadening the immune response.

In addition, interferons, and compounds that can induce interferon production, have potential use in the treatment of human cancers. Such molecules are potentially useful as anti-cancer agents with multiple pathways of activity. Interferons can inhibit human tumor cell proliferation directly and may be synergistic with various approved chemotherapeutic agents. Type I interferons can significantly enhance anti-tumor immune responses by inducing activation of both the adaptive and innate immune cells. Finally, tumor invasiveness may be inhibited by interferons by modulating enzyme expression related to tissue remodeling.

In view of the potential of type I interferons and type I interferon-inducing compounds as anti-viral and anti-cancer agents, there remains a need for new agents that can induce potent type I interferon production. With the growing body of data demonstrating that the cGAS-STING cytosolic DNA sensory pathway has a significant capacity to induce type I interferons, the development of STING activating agents is rapidly taking an important place in today's anti-tumor therapy landscape.

SUMMARY OF THE INVENTION

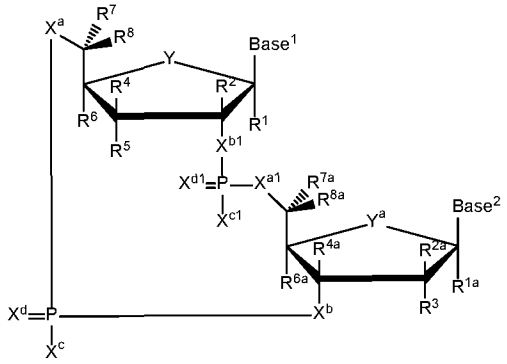
The present disclosure relates to novel cyclic di-nucleotide compounds of general formula (I), general formula (I'), and/or general formula (I''). In particular, the present disclosure relates to compounds having the general structural formula (I):



(I)

or pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof, as described herein.

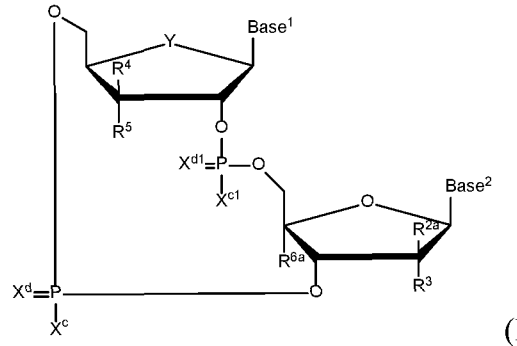
The present disclosure also relates to compounds having general structural formula (I'):



(I')

or pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof, as described herein.

The present disclosure also relates to compounds having general structural formula (I''):



(I'')

or pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof, as described herein.

Embodiments of the disclosure include compounds of general formula (I), compounds of general formula (I'), and/or compounds of general formula (I''), and pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof, as well as synthesis and isolation of compounds of

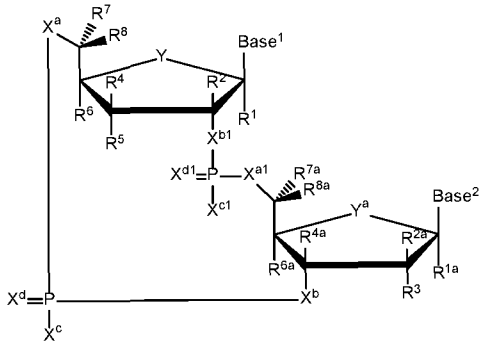
general formula (I), compounds of general formula (I'), and/or compounds of general formula (I''), and pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof. Uses of compounds of general formula (I), compounds of general formula (I'), and/or compounds of general formula (I'') are also disclosed.

Other embodiments, aspects and features of the present disclosure are either further described in or will be apparent from the ensuing description, examples and appended claims.

DETAILED DESCRIPTION OF THE INVENTION

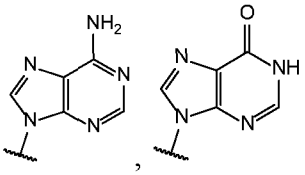
The present disclosure includes compounds of general formula (I), compounds of general formula (I'), and/or compounds of general formula (I'') above, and pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof. These compounds and their pharmaceutically acceptable salts, hydrates, solvates, and/or prodrugs are useful as agents to induce interferon production.

A first embodiment of the disclosure relates to cyclic di-nucleotide compounds of general formula (I):



(I)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of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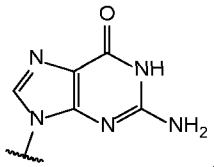
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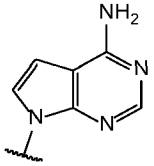
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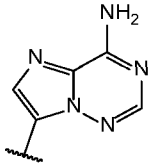
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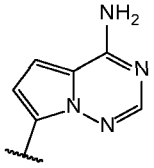
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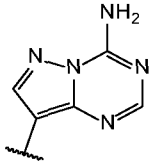
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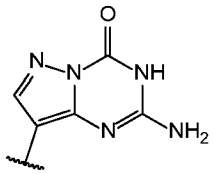
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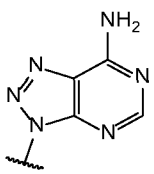
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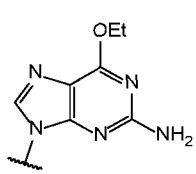
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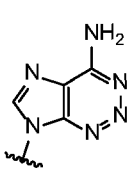
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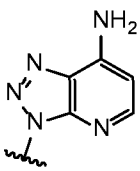
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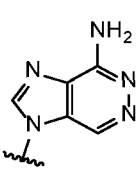
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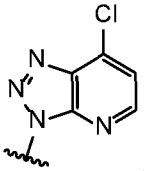
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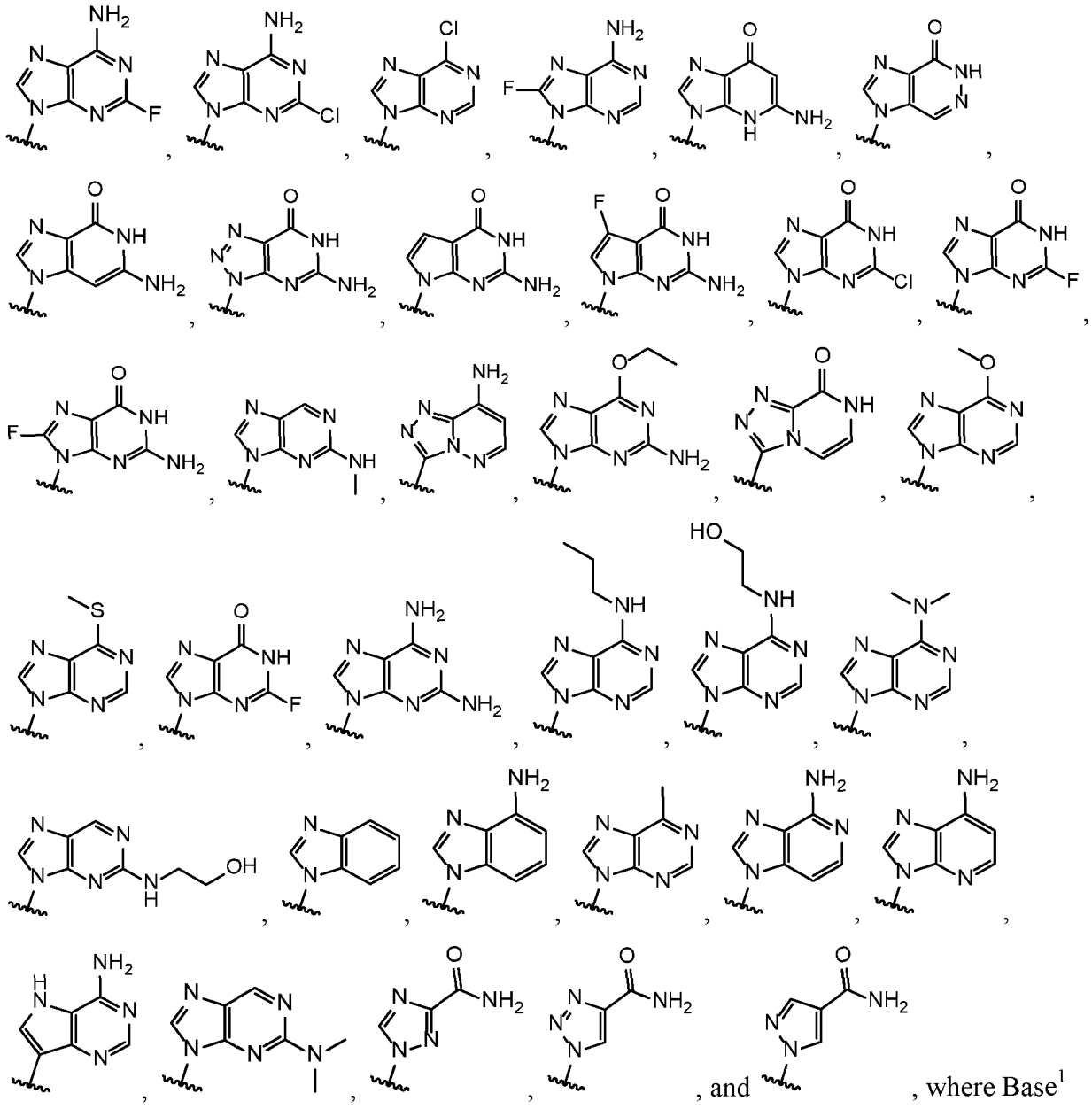
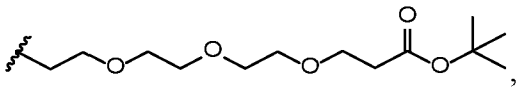
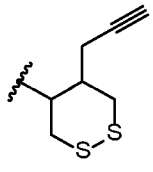
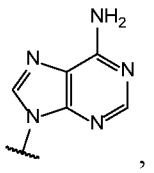
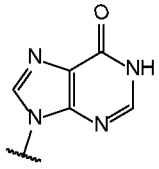
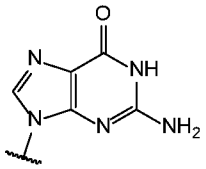
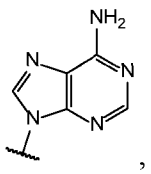
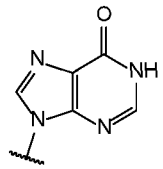
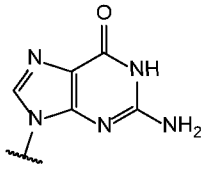
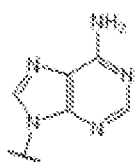
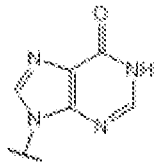
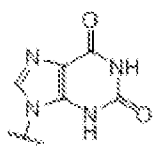
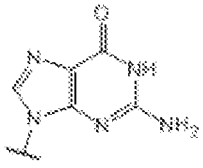


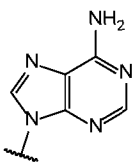
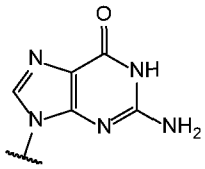
and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$; Y and Y^a are each independently selected from the group consisting of -O-, -S-, $-SO_2-$, $-CH_2-$, and $-CF_2-$; X^a and X^{a1} are each independently selected from the group consisting of O, C, and S; X^b and X^{b1} are each independently selected from the group consisting of O, C, and S; X^c and X^{c1} are each independently selected from the group consisting of O^-, S^-, OR^9, and NR^9R^9; X^d and X^{d1} are each independently selected from the group consisting of O and S; R^1 and R^{1a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl,

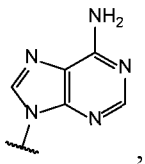
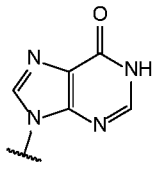
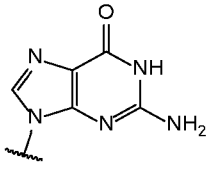
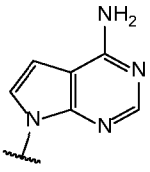
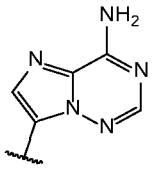
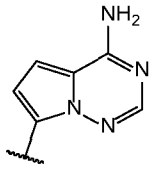
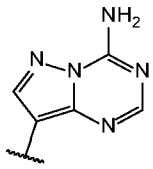
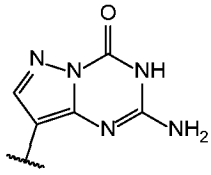
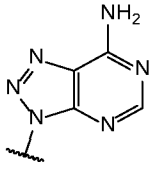
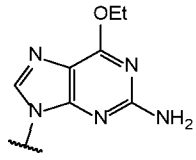
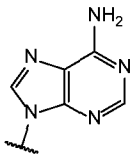
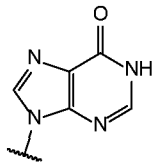
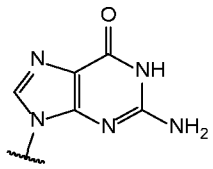
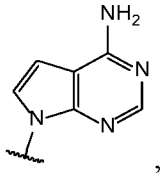
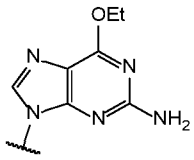
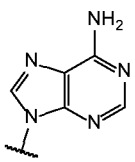
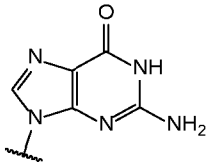
C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^1 and R^{1a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^2 and R^{2a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^3 C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^4 and R^{4a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^4 and R^{4a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^5 C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^6 and R^{6a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^6 and R^{6a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^7 and R^{7a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6

alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^7 and R^{7a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^8 and R^{8a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^8 and R^{8a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; each R^9 is independently selected from the group consisting of H, C_1-C_{20} alkyl,  , and  , where each R^9 C_1-C_{20} alkyl is optionally substituted by 0 to 3 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; optionally R^{1a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{1a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position; optionally R^{2a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{2a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position; optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position; optionally R^4 and R^5 are connected to form are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position; optionally R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position; optionally R^7 and R^8 are connected to

form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene; and optionally R^{7a} and R^{8a} are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene.

In specific aspects of this embodiment, when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH or SH, X^d and X^{d1} are each O, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group consisting of  ,  , and  , R^5 and R^3 are not both selected from the group consisting of H, F and OH. That is, when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH or SH, X^d and X^{d1} are each O, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group consisting of  ,  , and  , either only one of R^5 and R^3 is selected from the group consisting of H, F, and OH, or neither R^5 and R^3 is selected from the group consisting of H, F, and OH. In further specific instances of this aspect, when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH, X^d and X^{d1} are each O or S, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group consisting of  ,  ,  , and  , R^5 and R^3 are not both selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, where said C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I and OH.

In further specific aspects of this embodiment, when $Base^1$ and $Base^2$ are each selected from the group consisting of  and  , and R^{2a} is F and R^5 is F, at least one of X^c and X^{c1} is SR^9.

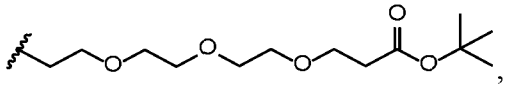
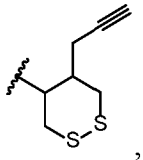
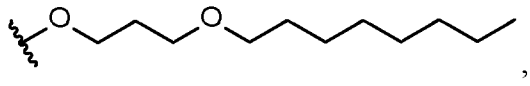
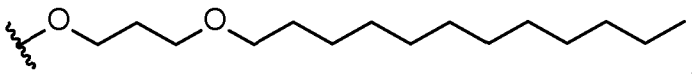
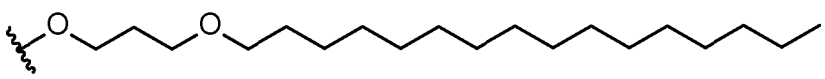
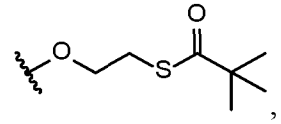
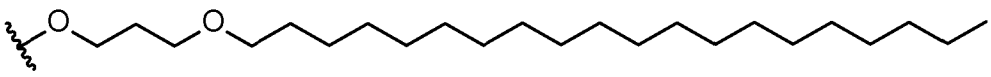
In a first aspect of the first embodiment, $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  ,  ,  ,  ,  ,  ,  ,  ,  , and  , where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$. In particular instances, $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  ,  ,  , and  , where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$. In even more particular instances, $Base^1$ and $Base^2$ are each independently selected from the group consisting of  and  , where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10}

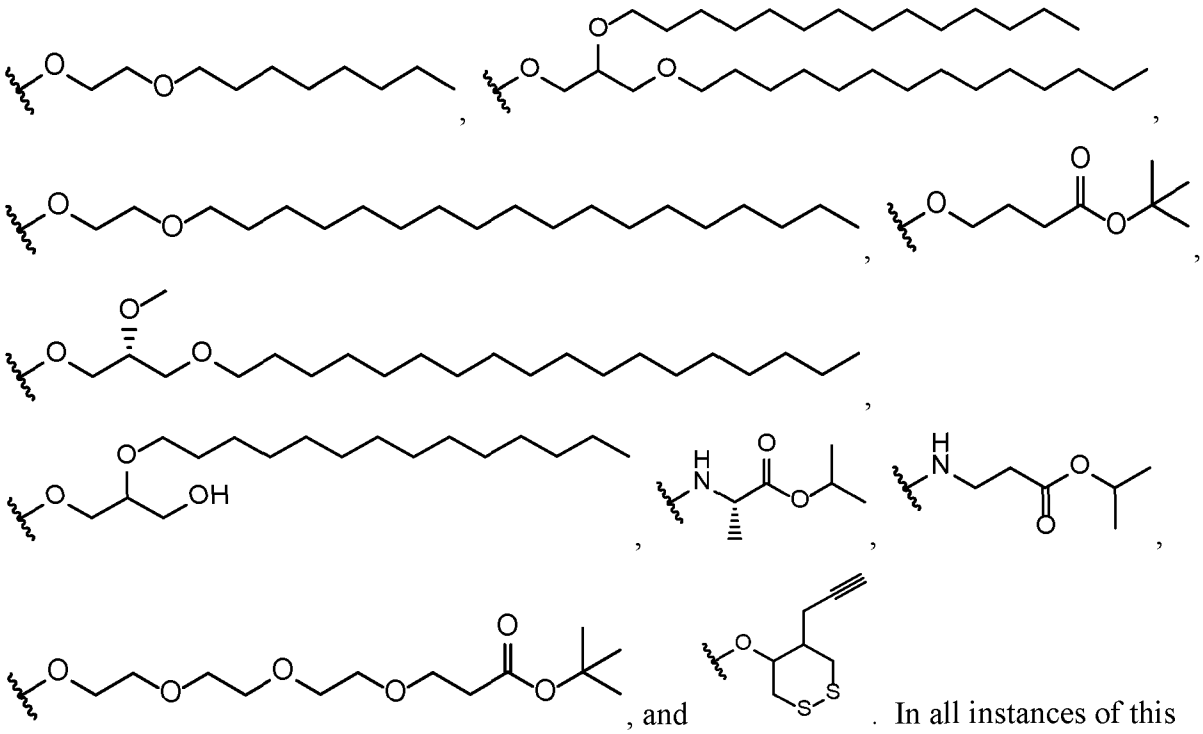
is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above.

In a second aspect of the first embodiment, Y and Y^a are each independently selected from the group consisting of -O- and -S-. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first aspect described above.

In a third aspect of the first embodiment, X^a and X^{a1} are each independently selected from the group consisting of O and S. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through second aspects described above.

In a fourth aspect of the first embodiment, X^b and X^{b1} are each independently selected from the group consisting of O and S. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through third aspects described above.

In a fifth aspect of the first embodiment, X^c and X^{c1} are each independently selected from the group consisting of O^-, S^-, OR^9, and NR^9R^9, where each R^9 is independently selected from the group consisting of H, C_1-C_{20} alkyl,  , and  , where each R^9 C_1-C_{20} alkyl is optionally substituted by 0 to 3 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl. In particular instances, X^c and X^{c1} are each independently selected from the group consisting of O^-, S^-,  ,  ,  ,  ,  .



, and . In all instances of this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through fourth aspects described above.

In a sixth aspect of the first embodiment, X^d and X^{d1} are each independently selected from the group consisting of O and S. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through fifth aspects described above.

In a seventh aspect of the first embodiment, R^1 and R^{1a} are each H. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through sixth aspects described above.

In an eighth aspect of the first embodiment, R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^2 and R^{2a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3. In particular instances, R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, CF_3, CH_3, CH_2OH, and CH_2CH_3. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through seventh aspects described above.

In a ninth aspect of the first embodiment, R^3 is selected from the group consisting H, F, Cl, I, Br, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3. In particular instances, R^3 are each independently selected from the group consisting of

H, F, Cl, I, Br, OH, CN, N_3, CF_3, CH_3, CH_2OH, and CH_2CH_3. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through eighth aspects described above.

In a tenth aspect of the first embodiment, R^4 and R^{4a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^4 and R^{4a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3. In particular instances, R^4 and R^{4a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, CF_3, CH_3, CH_2OH, and CH_2CH_3. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through ninth aspects described above.

In an eleventh aspect of the first embodiment, R^5 is selected from the group consisting of H, F, Cl, I, Br, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3. In particular instances, R^5 are each independently selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, CF_3, CH_3, CH_2OH, and CH_2CH_3. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through tenth aspects described above.

In a twelfth aspect of the first embodiment, R^6 and R^{6a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through eleventh aspects described above.

In a thirteenth aspect of the first embodiment, R^7 and R^{7a} are each H. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through twelfth aspects described above.

In a fourteenth aspect of the first embodiment, R^8 and R^{8a} are each H. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through thirteenth aspects described above.

In a fifteenth aspect of the first embodiment, R^{1a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{1a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through fourteenth aspects described above.

In a sixteenth aspect of the first embodiment, R^{2a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{2a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through fourteenth aspects described above.

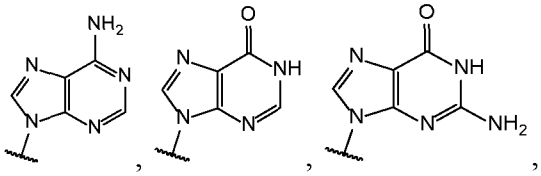
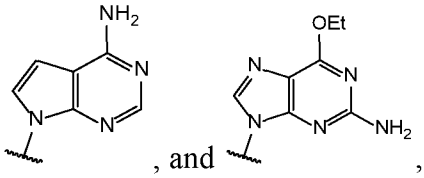
In a seventeenth aspect of the first embodiment, R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, and -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through fourteenth aspects described above.

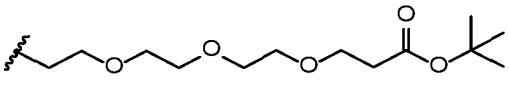
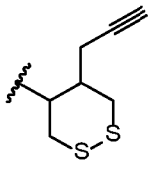
In an eighteenth aspect of the first embodiment, R^4 and R^5 are connected by C_1-C_6 alkylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through fourteenth aspects described above.

In a nineteenth aspect of the first embodiment, R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through fourteenth aspects described above.

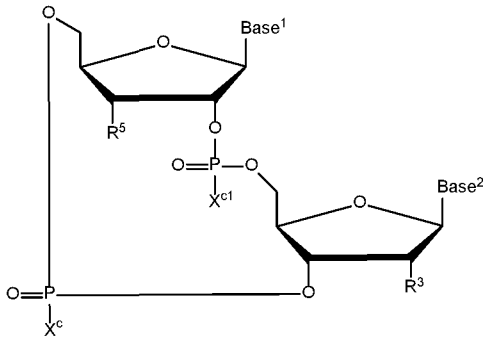
In a twentieth aspect of the first embodiment, R^7 and R^8 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through fourteenth aspects described above.

In a twenty-first aspect of the first embodiment, R^{7a} and R^{8a} are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above or in the first through fourteenth aspects described above.

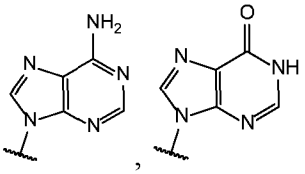
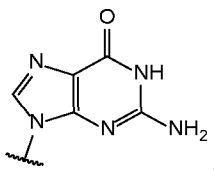
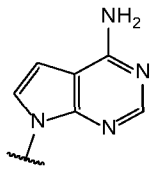
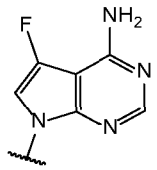
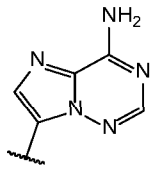
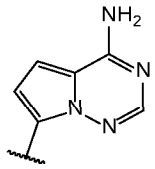
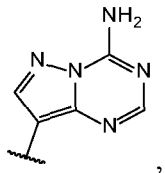
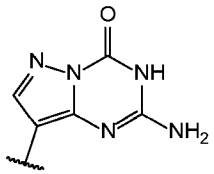
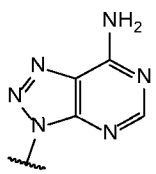
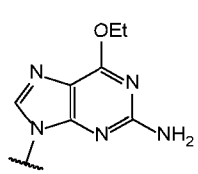
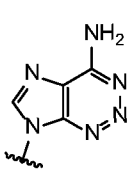
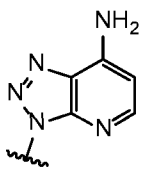
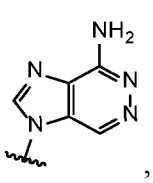
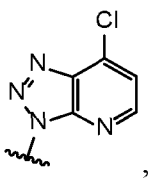
In a twenty-second aspect of the first embodiment, $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  , and , where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$; Y and Y^a are each independently selected from the group consisting of -O-, -S-, $-SO_2-$, $-CH_2-$, and $-CF_2-$; X^a and X^{a1} are each independently selected from the group consisting of O and S; X^b and X^{b1} are each independently selected from the group consisting of O and S; X^c and X^{c1} are each independently selected from the group consisting of O^-, S^-, OR^9, and NR^9R^9; X^d and X^{d1} are each independently selected from the group consisting of O and S; R^1 and R^{1a} are each H; R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^2 and R^{2a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^3 is selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^4 and R^{4a} are each independently selected from the group consisting of H, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^4 and R^{4a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^5 is selected from the group consisting of H, F, Cl, I, Br, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^6 and R^{6a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, and C_1-C_6 haloalkyl, where said R^6 and R^{6a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^7 and R^{7a} are each H; R^8 and R^{8a} are each H; each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,

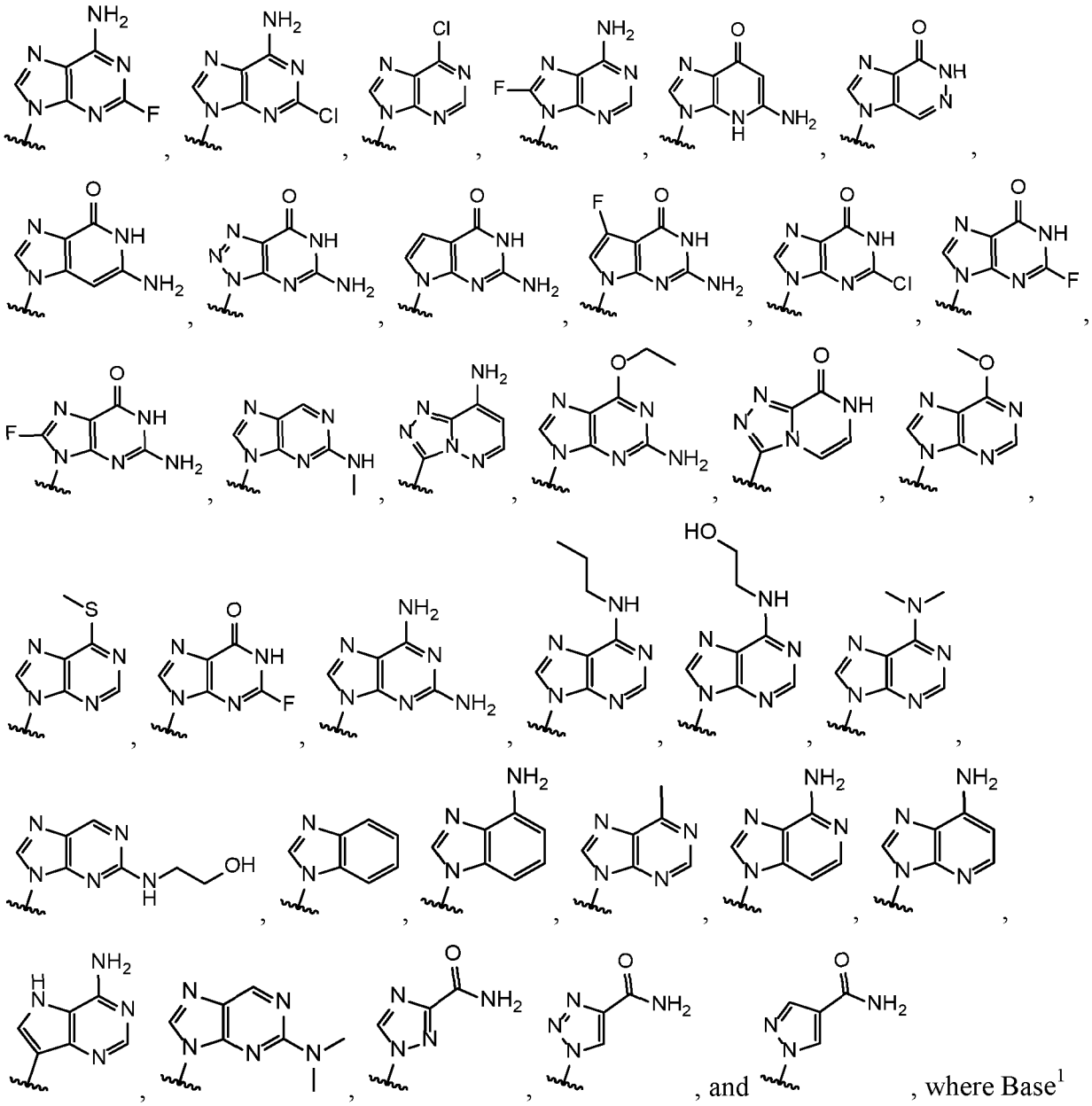
 , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, and -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position or optionally R^4 and R^5 are connected by C_1-C_6 alkylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above.

In a twenty-third aspect of the first embodiment, the compound of formula (I) is a compound of formula (Ia):

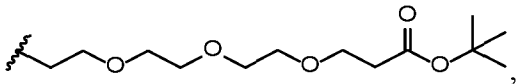
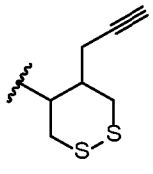


(Ia)

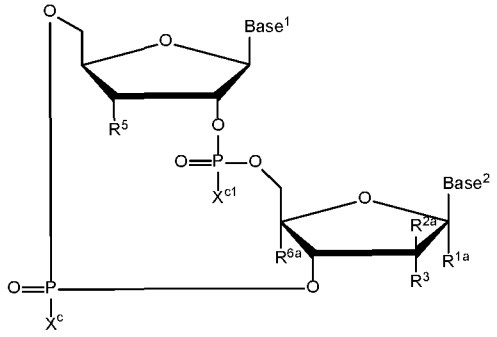
or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of  , ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  .



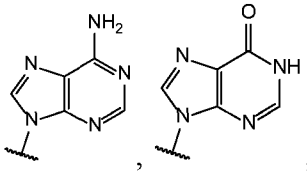
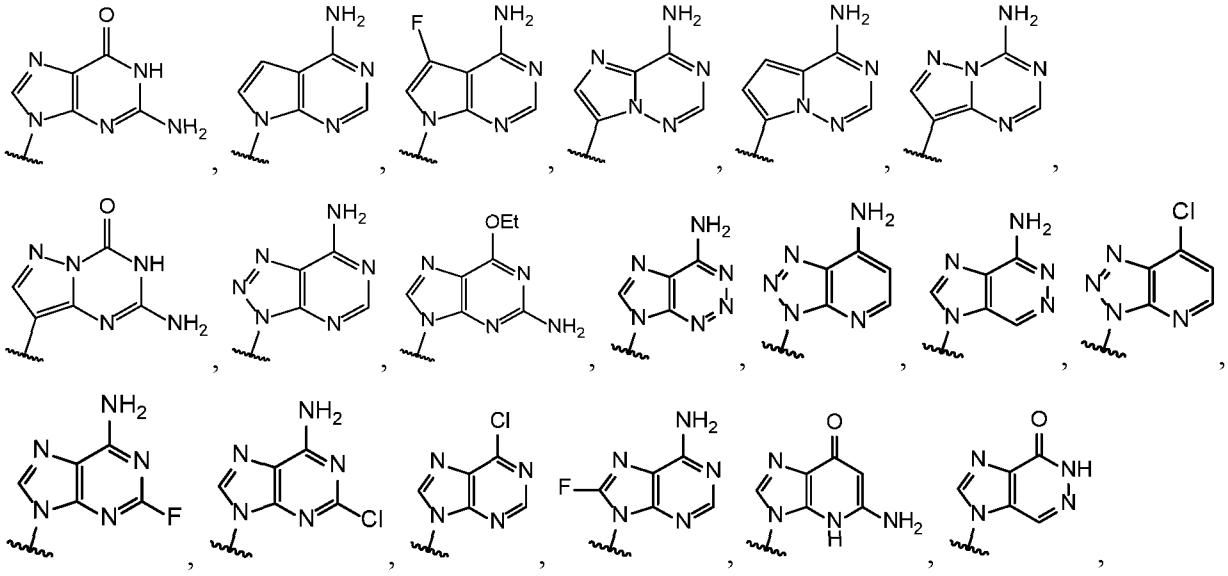
and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$; X^c and X^{c1} are each independently selected from the group consisting of O^-, S^-, OR^9, and NR^9R^9; R^3 is selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, I, Br, and OH; R^5 is selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, I, Br, and OH; R^3 and R^5 are not

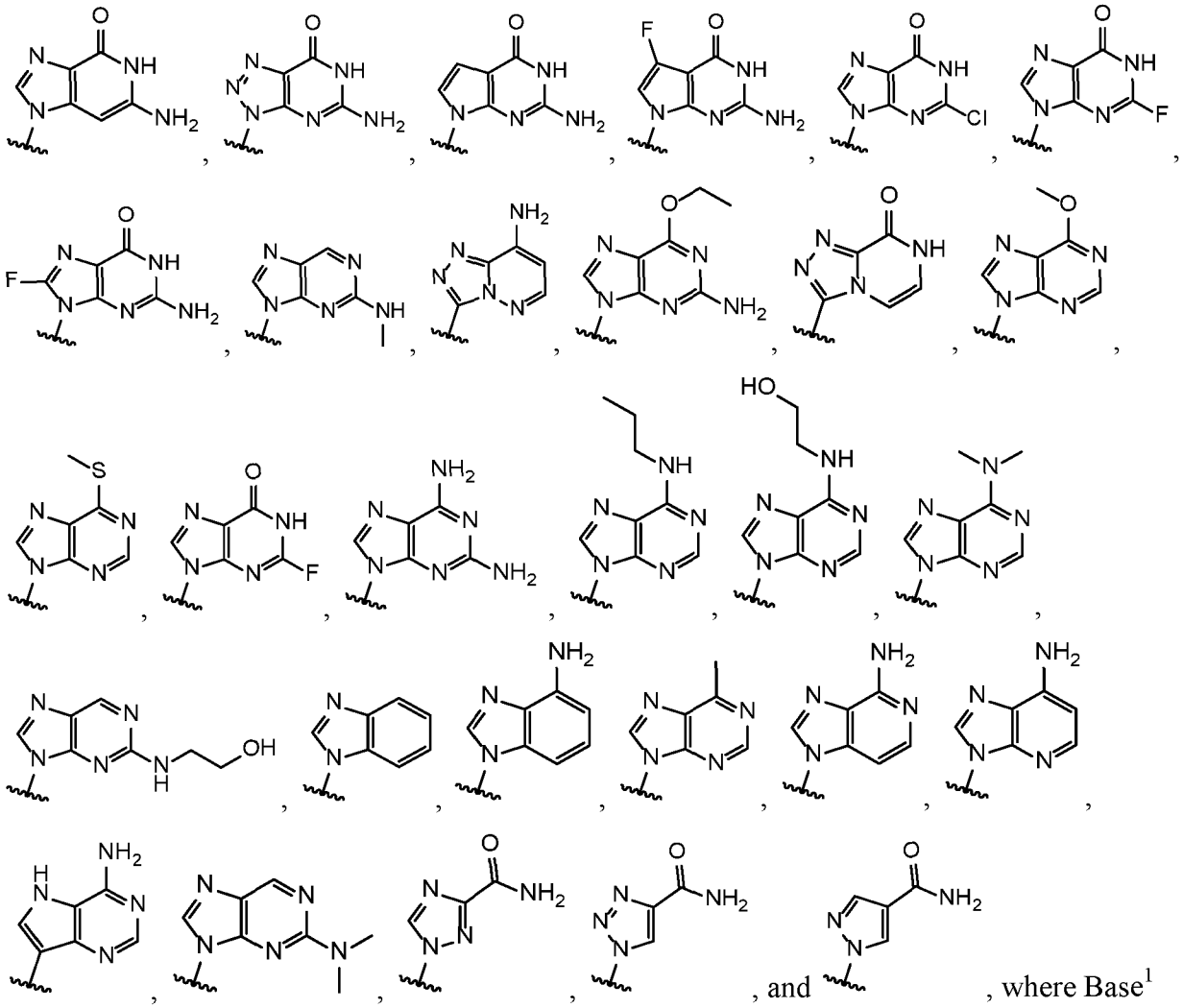
both selected from the group consisting of OH, C_1-C_6 alkyl substituted with OH, and C_1-C_6 haloalkyl substituted with OH; and each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,  , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above.

In a twenty-fourth aspect of the first embodiment, the compound of formula (I) is a compound of formula (Ib):

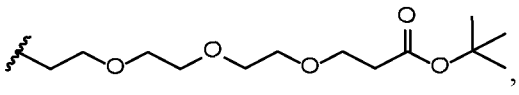
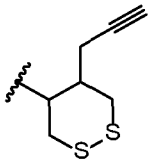


(Ib)

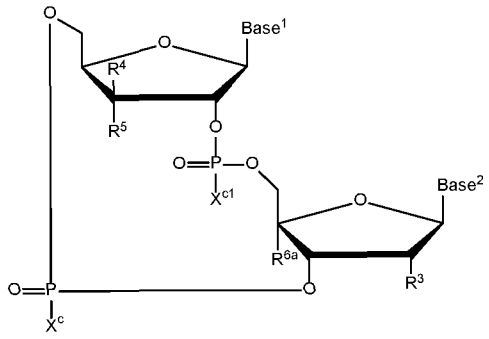
or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of  , 



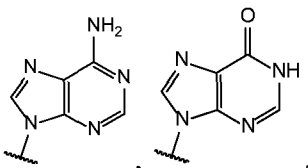
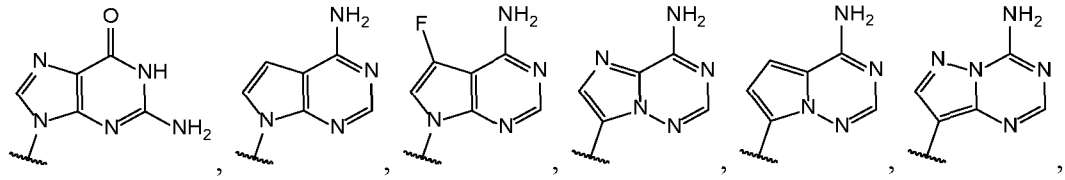
and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$; X^c and X^{c1} are each independently selected from the group consisting of O^-, S^-, OR^9, and NR^9R^9; R^{1a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^{1a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^{2a} is selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^{2a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, I, Br, and OH; R^3 is selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3

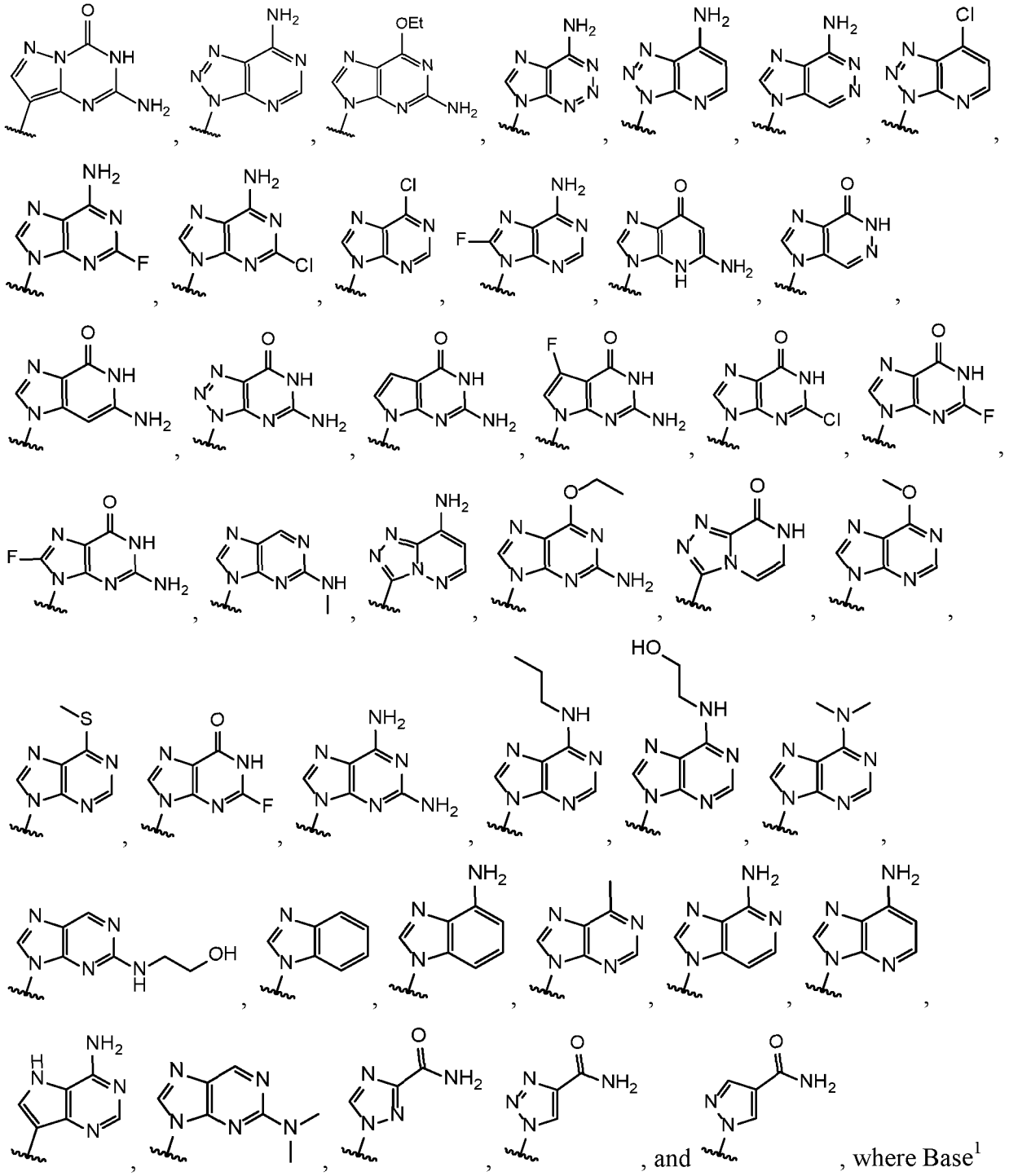
C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, I, Br, and OH; R^5 is selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, I, Br, and OH; R^3 and R^5 are not both selected from the group consisting of OH, C_1-C_6 alkyl substituted with OH, and C_1-C_6 haloalkyl substituted with OH; R^{6a} is selected from the group consisting of H, F, Cl, I, Br, OH, C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl; each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,  , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; and optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, and -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above.

In a twenty-fifth aspect of the first embodiment, the compound of formula (I) is a compound of formula (Ic):

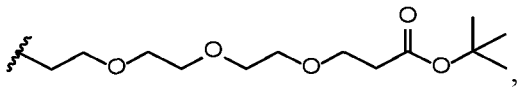
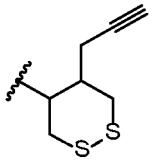


(Ic)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  .



and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$; X^c and X^{c1} are each independently selected from the group consisting of O^-, S^-, OR^9, and NR^9R^9; R^3 is selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting

of F, Cl, I, Br, and OH; R^4 is selected from the group consisting of H, F, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^4 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, I, Br, and OH; R^5 is selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, I, Br, and OH; R^3 and R^5 are not both selected from the group consisting of OH, C_1-C_6 alkyl substituted with OH, and C_1-C_6 haloalkyl substituted with OH; R^{6a} is selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^{6a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, I, Br, and OH; each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,  , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; and optionally R^4 and R^5 are connected by C_1-C_6 alkylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position. In this aspect, all other groups are as provided in the general formula (I) of the first embodiment above.

A twenty-sixth aspect of the first embodiment relates to a pharmaceutical composition, said pharmaceutical composition comprising (a) a compound according to any one of general formula (I) of the first embodiment above or in the first through twenty-fifth aspects described above or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof; and (b) a pharmaceutically acceptable carrier.

A twenty-seventh aspect of the first embodiment relates to methods of inducing an immune response in a subject, comprising administering a therapeutically effective amount of a compound according to any one of general formula (I) of the first embodiment above or in the first through twenty-fifth aspects described above or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof to the subject.

A twenty-eighth aspect of the first embodiment relates to methods of inducing an immune response in a subject, comprising administering a therapeutically effective amount of a composition according to the twenty-sixth aspect described above to the subject.

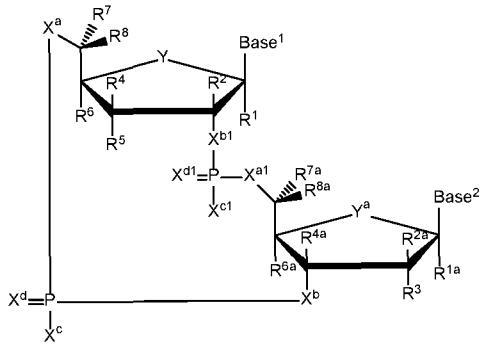
A twenty-ninth aspect of the first embodiment relates to methods of inducing a STING-dependent type I interferon production in a subject, comprising administering a therapeutically effective amount of a compound according to any one of general formula (I) of the first embodiment above or in the first through twenty-fifth aspects described above or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof to the subject.

A thirtieth aspect of the first embodiment relates to methods of inducing a STING-dependent type I interferon production in a subject, comprising administering a therapeutically effective amount of a composition according to the twenty-sixth aspect described above to the subject.

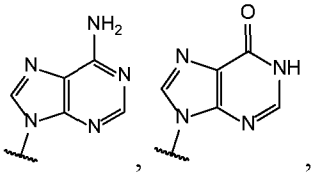
A thirty-first aspect of the first embodiment relates to methods of inducing a STING-dependent cytokine production in a subject, comprising administering a therapeutically effective amount of a compound according to any one of general formula (I) of the first embodiment above or in the first through twenty-fifth aspects described above or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof to the subject.

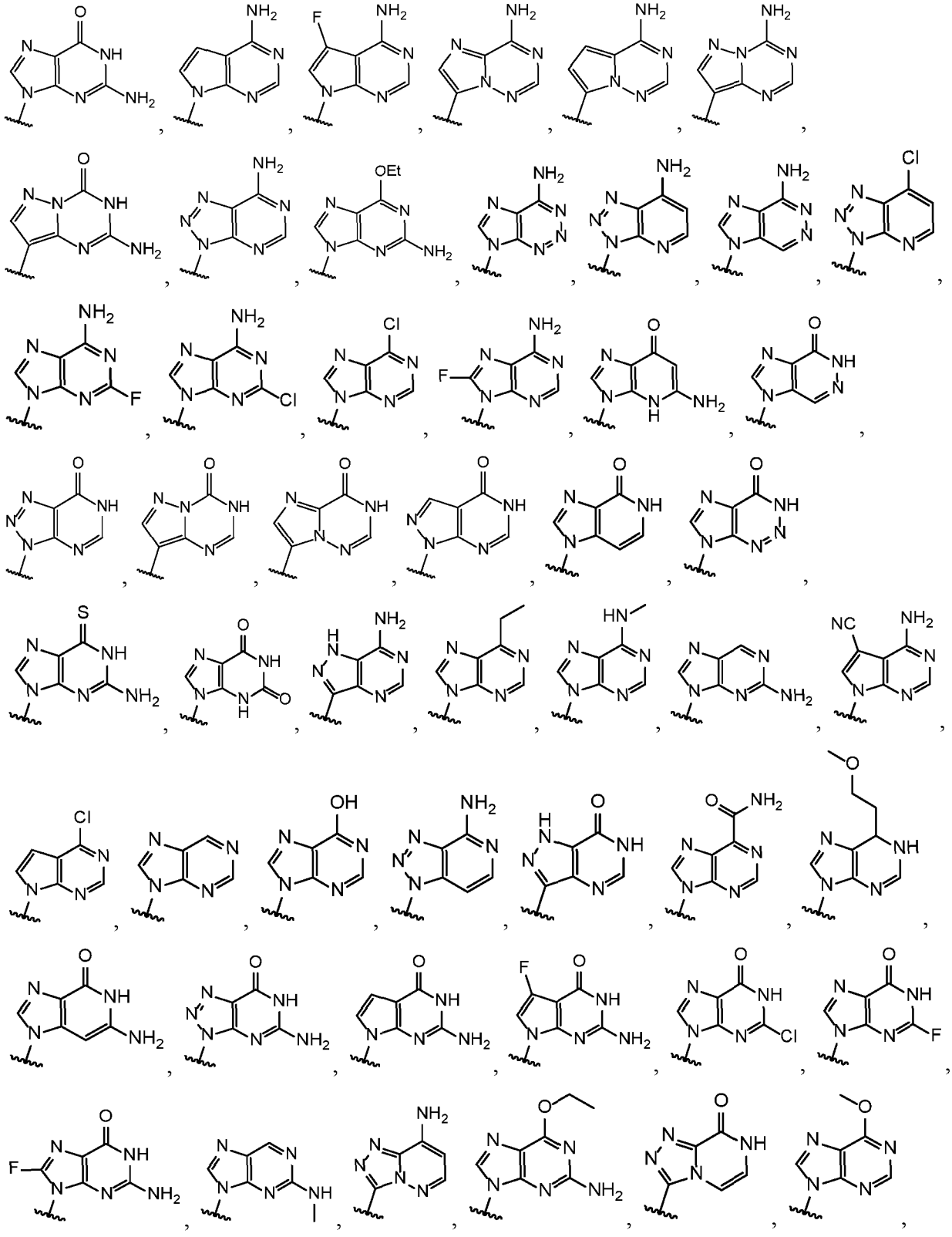
A thirty-second aspect of the first embodiment relates to methods of inducing a STING-dependent cytokine production in a subject, comprising administering a therapeutically effective amount of a composition according to the twenty-sixth aspect described above to the subject.

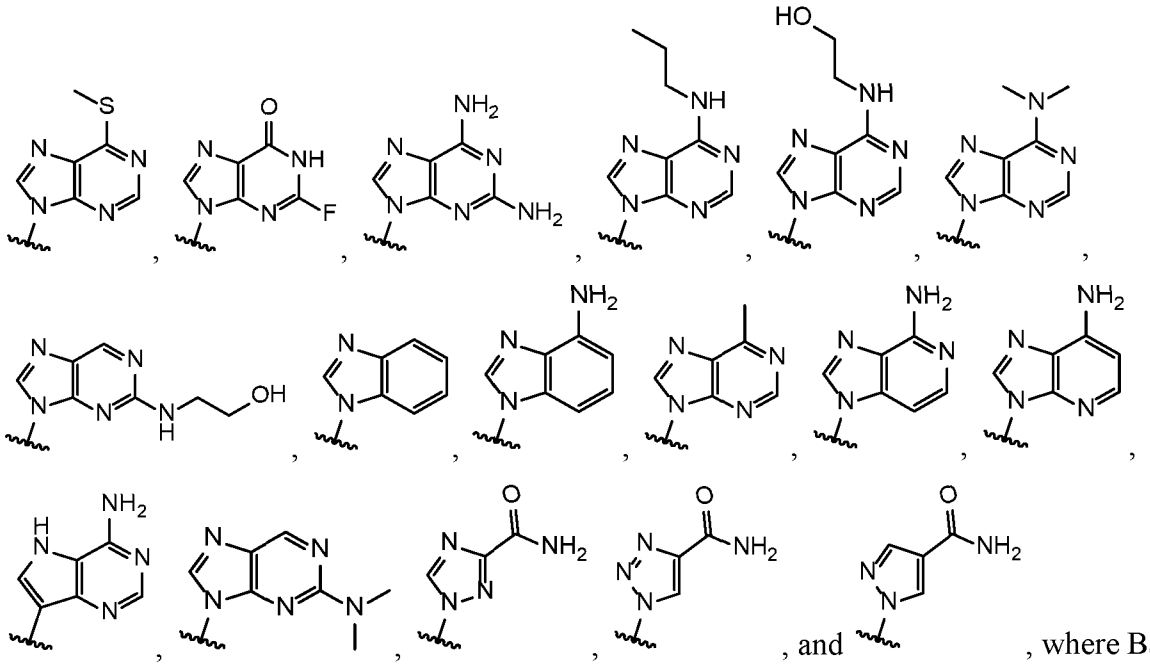
A second embodiment of the disclosure relates to cyclic di-nucleotide compounds of general formula (I'):



(I')

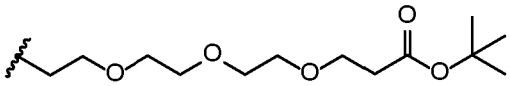
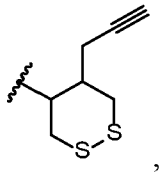
or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,

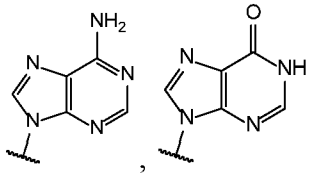


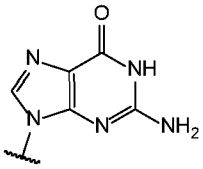
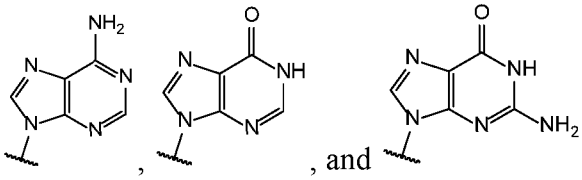
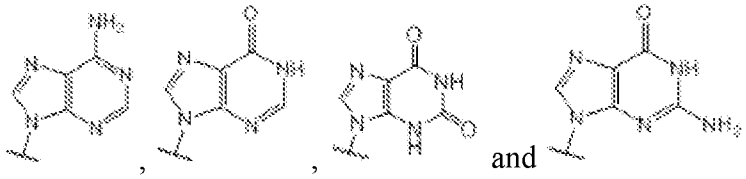


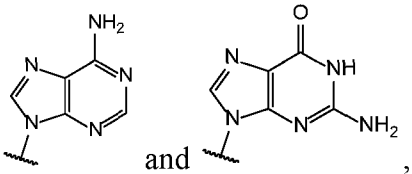
and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$; Y and Y^a are each independently selected from the group consisting of -O- and -S-; X^a and X^{a1} are each independently selected from the group consisting of O, and S; X^b and X^{b1} are each independently selected from the group consisting of O, and S; X^c and X^{c1} are each independently selected from the group consisting of OR^9, SR^9, and NR^9R^9; X^d and X^{d1} are each independently selected from the group consisting of O and S; R^1 and R^{1a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^1 and R^{1a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^2 and R^{2a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6

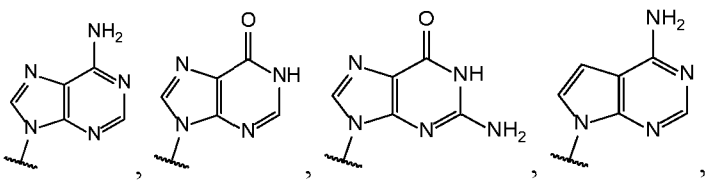
haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^3 C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^4 and R^{4a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^4 and R^{4a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^5 C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, NR^9R^9, and N_3; R^6 and R^{6a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^6 and R^{6a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^7 and R^{7a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^7 and R^{7a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^8 and R^{8a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^8 and R^{8a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; each R^9 is independently selected

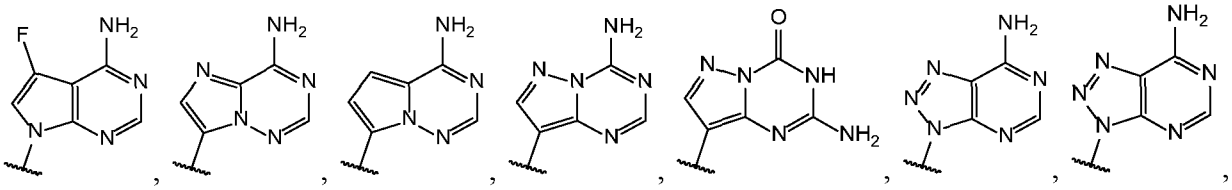
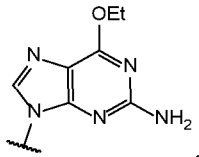
from the group consisting of H, C_1-C_{20} alkyl,  , and  , where each R^9 C_1-C_{20} alkyl is optionally substituted by 0 to 3 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; optionally R^{1a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{1a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position; optionally R^{2a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{2a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position; optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position; optionally R^4 and R^5 are connected to form are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position; optionally R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position; optionally R^7 and R^8 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene; and optionally R^{7a} and R^{8a} are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene.

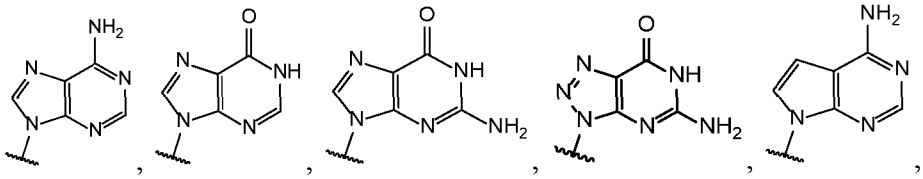
In specific aspects of this embodiment, when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH or SH, X^d and X^{d1} are each O, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group consisting of  , and

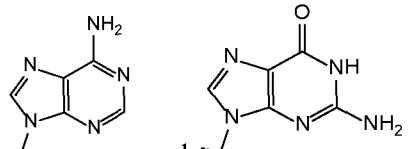
 , R^5 and R^3 are not both selected from the group consisting of H, F and OH. That is, when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH or SH, X^d and X^{d1} are each O, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group consisting of  , either only one of R^5 and R^3 is selected from the group consisting of H, F, and OH, or neither R^5 and R^3 is selected from the group consisting of H, F, and OH. In further specific instances of this aspect, when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH, X^d and X^{d1} are each O or S, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group consisting of  , R^5 and R^3 are not both selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, where said C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I and OH.

In further specific aspects of this embodiment, when $Base^1$ and $Base^2$ are each selected from the group consisting of  , and R^{2a} is F and R^5 is F, at least one of X^c and X^{c1} is SR^9.

In a first aspect of the second embodiment, $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,

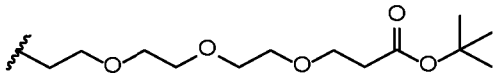
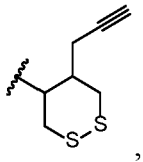
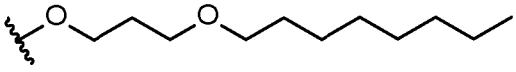
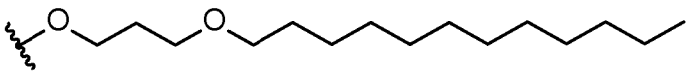
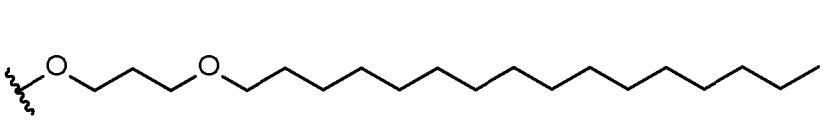
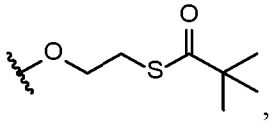
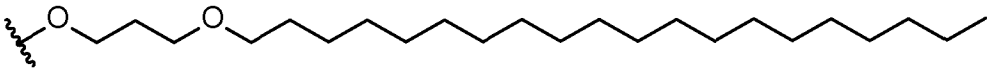
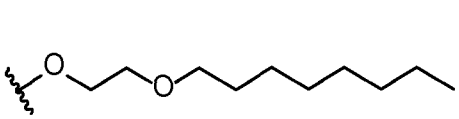
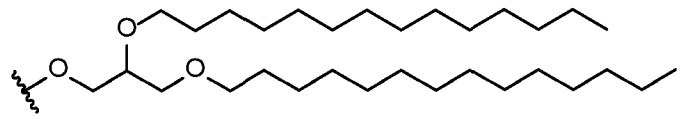
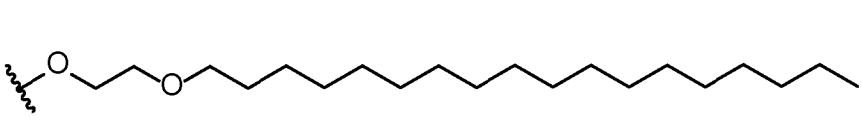
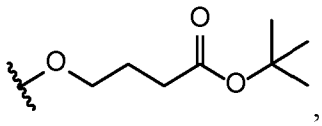
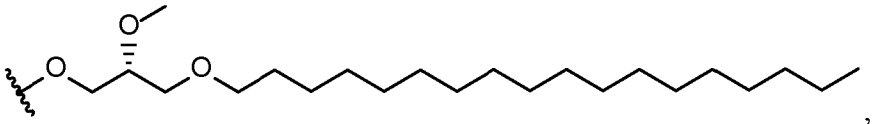
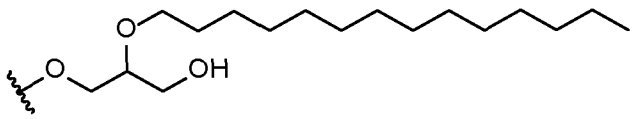
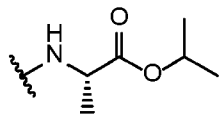
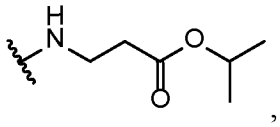
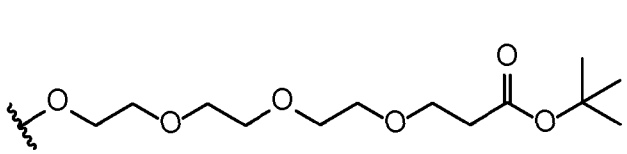
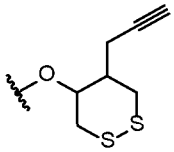



and , where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$. In particular instances, $Base^1$ and $Base^2$ are each independently selected from the group consisting of 

, and , where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$. In even more particular instances, $Base^1$ and $Base^2$ are each independently selected from the group consisting of 

and , where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above.

In a second aspect of the second embodiment, X^c and X^{c1} are each independently selected from the group consisting of OR^9, SR^9, and NR^9R^9, where each R^9 is independently selected

from the group consisting of H, C_1-C_{20} alkyl,  , and  , where each R^9 C_1-C_{20} alkyl is optionally substituted by 0 to 3 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl. In particular instances, X^c and X^{c1} are each independently selected from the group consisting of O^-, S^-,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , and  . In all instances of this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first aspect described above.

In a third aspect of the second embodiment, R^1 and R^{1a} are each H. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through second aspects described above.

In a fourth aspect of the second embodiment, R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^2 and R^{2a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3. In particular instances, R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, CF_3, CH_3, CH_2OH, and CH_2CH_3. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through third aspects described above.

In a fifth aspect of the second embodiment, R^3 is selected from the group consisting H, F, Cl, I, Br, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3. In particular instances, R^3 are each independently selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, CF_3, CH_3, CH_2OH, and CH_2CH_3. In even more particular instances, R^3 is selected from NH_2 and N_3. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through fourth aspects described above.

In a sixth aspect of the second embodiment, R^4 and R^{4a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^4 and R^{4a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3. In particular instances, R^4 and R^{4a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, CF_3, CH_3, CH_2OH, and CH_2CH_3. In even more particular instances, R^4 and R^{4a} are each F. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through fifth aspects described above.

In a seventh aspect of the second embodiment, R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, NH_2, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, NR^9R^9, and N_3. In particular instances, R^5 are each independently selected from the group consisting of H, F, Cl, I, Br, OH, CN, N_3, CF_3, CH_3, CH_2OH, and CH_2CH_3. In even more particular instances, R^5 is selected from NH_2 and N_3. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through sixth aspects described above.

In an eighth aspect of the second embodiment, R^6 and R^{6a} are each independently selected from the group consisting of H, F, Cl, I, Br, OH, C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6

alkynyl. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through seventh aspects described above.

In a ninth aspect of the second embodiment, R^7 and R^{7a} are each independently selected from the group consisting of H and C_1-C_6 alkyl. In particular instances, R^7 and R^{7a} are each independently selected from the group consisting of H and CH_3. In more particular instances, R^{7a} is CH_3. In additional instances, R^7 and R^{7a} are each H. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through eighth aspects described above.

In a tenth aspect of the second embodiment, R^8 and R^{8a} are each independently selected from the group consisting of H and C_1-C_6 alkyl. In particular instances, R^8 and R^{8a} are each independently selected from the group consisting of H and CH_3. In more particular instances, R^{8a} is CH_3. In additional instances, R^8 and R^{8a} are each H. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through ninth aspects described above.

In an eleventh aspect of the second embodiment, R^{1a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{1a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through tenth aspects described above.

In a twelfth aspect of the second embodiment, R^{2a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{2a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through eleventh aspects described above.

In a thriteenth aspect of the second embodiment, R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, and -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through eleventh aspects described above.

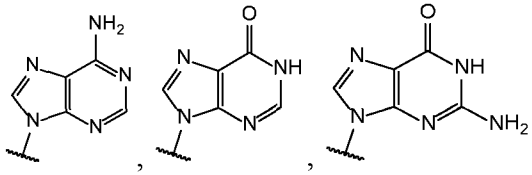
In a fourteenth aspect of the second embodiment, R^4 and R^5 are connected by C_1-C_6 alkylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4

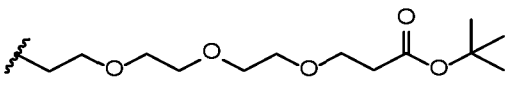
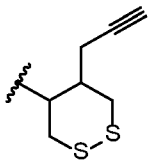
and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through eleventh aspects described above.

In a fifteenth aspect of the second embodiment, R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through eleventh aspects described above.

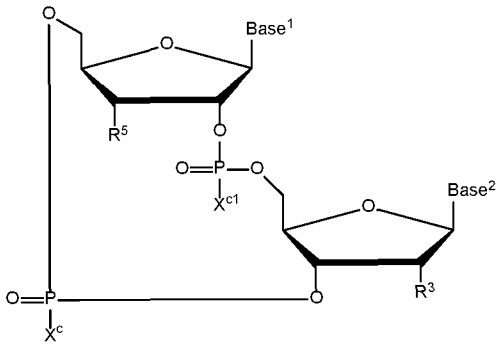
In a sixteenth aspect of the second embodiment, R^7 and R^8 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through eleventh aspects described above.

In a seventeenth aspect of the second embodiment, R^{7a} and R^{8a} are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above or in the first through eleventh aspects described above.

In an eighteenth aspect of the second embodiment, $Base^1$ and $Base^2$ are each independently selected from the group consisting of  , where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, O(C_{1-3} alkyl), O(C_{3-6} cycloalkyl), S(C_{1-3} alkyl), S(C_{3-6} cycloalkyl), NH(C_{1-3} alkyl), NH(C_{3-6} cycloalkyl), N(C_{1-3} alkyl)$_2$, and N(C_{3-6} cycloalkyl)$_2$; Y and Y^a are each independently selected from the group consisting of -O- and -S-; X^a and X^{a1} are each independently selected from the group consisting of O and S; X^b and X^{b1} are each independently selected from the group consisting of O and S; X^c and X^{c1} are each independently selected from the group consisting of OR^9, SR^9, and NR^9R^9; X^d and X^{d1} are each independently selected from the group consisting of O and S; R^1 and R^{1a} are

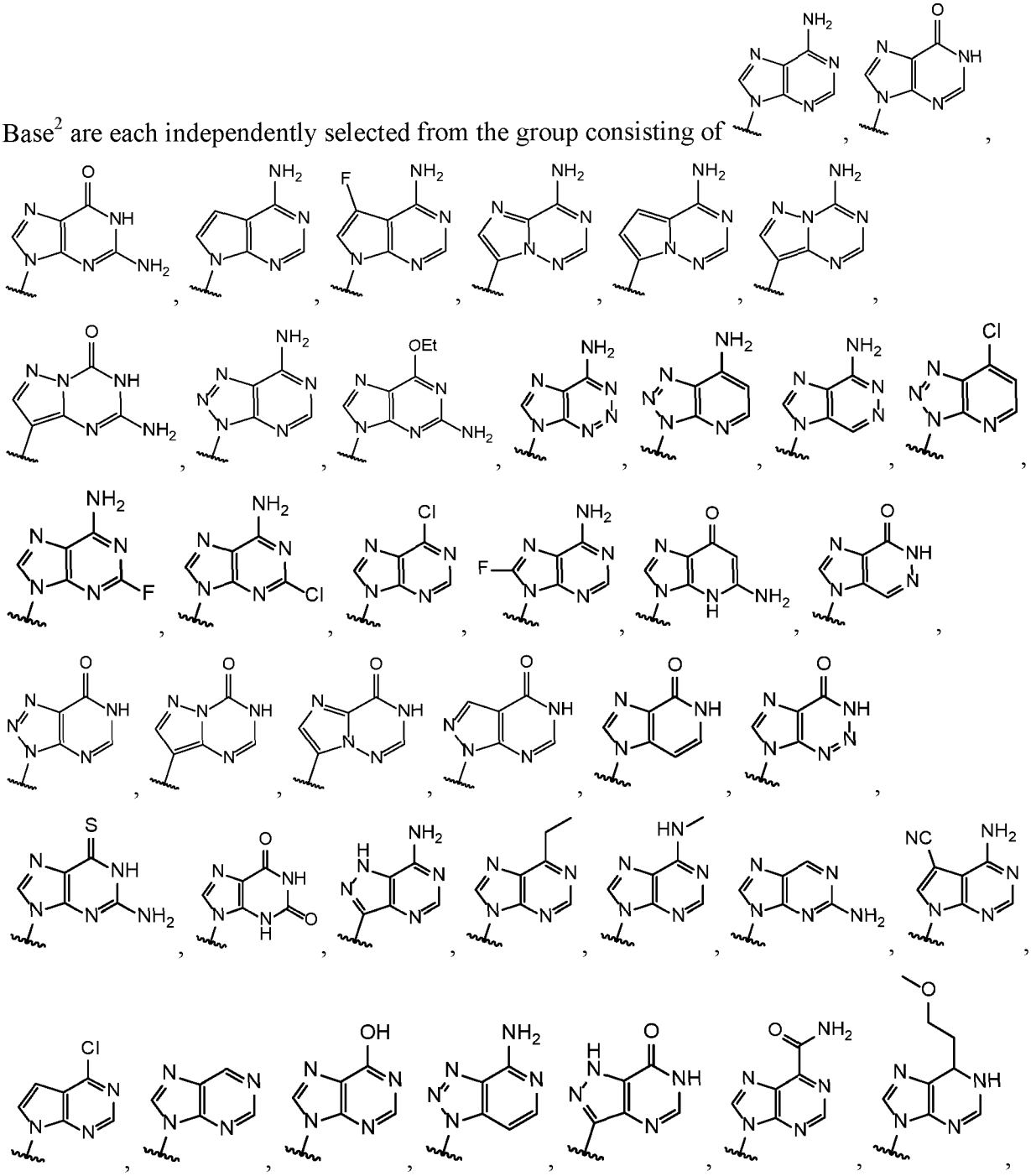
each H; R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^2 and R^{2a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^4 and R^{4a} are each independently selected from the group consisting of H, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^4 and R^{4a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, NH_2, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, NR^9R^9, and N_3; R^6 and R^{6a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, and C_1-C_6 haloalkyl, where said R^6 and R^{6a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^7 and R^{7a} are each H; R^8 and R^{8a} are each H; each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,  , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, and -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position or optionally R^4 and R^5 are connected by C_1-C_6 alkylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position. In all instances of this aspect, all other groups are as provided in the general formula (I') of the second embodiment above.

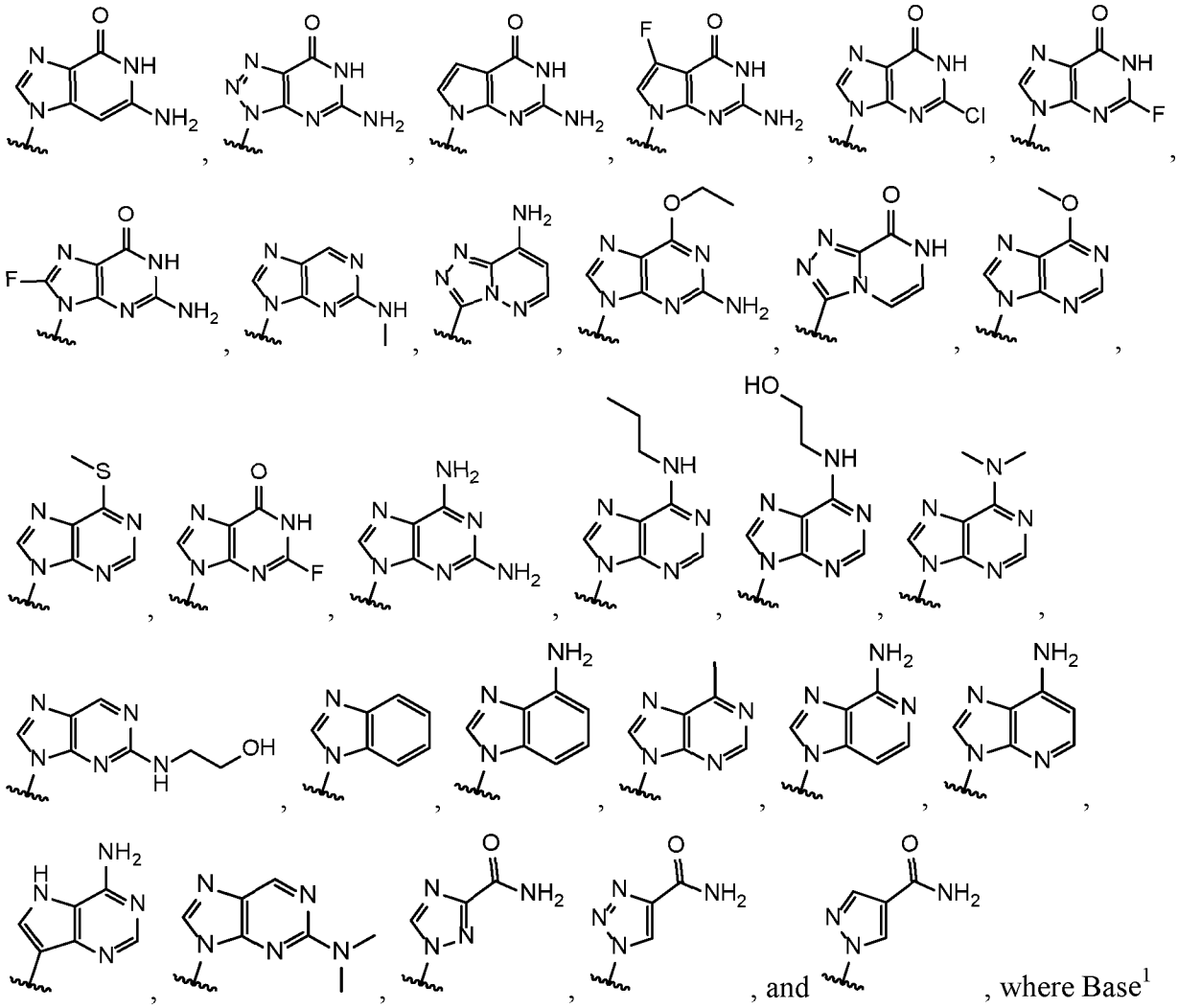
In a nineteenth aspect of the second embodiment, the compound of formula (I') is a compound of formula (I'a):



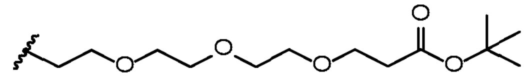
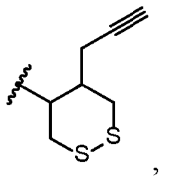
(I'a)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of

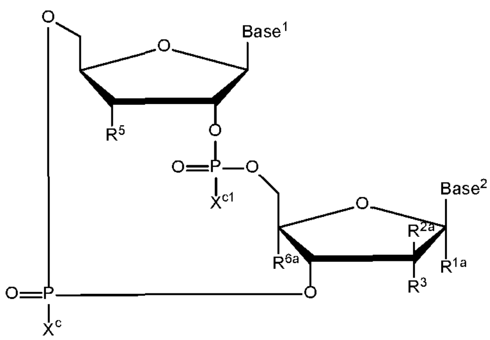




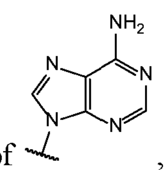
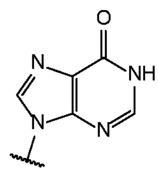
and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$; X^c and X^{c1} are each independently selected from the group consisting of OR^9, SR^9, and NR^9R^9; R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH; R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH; R^3 and R^5 are not both selected from the group consisting of: OH, R^5 C_1-C_6 alkyl substituted with OH, or C_1-C_6 haloalkyl substituted with OH; and each R^9 is independently selected from the

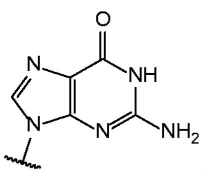
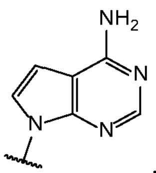
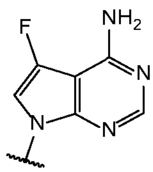
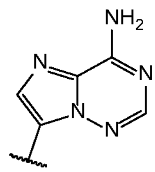
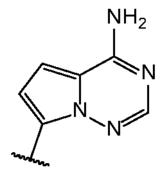
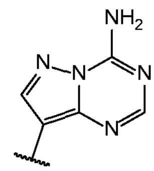
group consisting of H, C_2-C_3 alkyl,  , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl. In all instances of this aspect, all other groups are as provided in the general formula (I') of the second embodiment above.

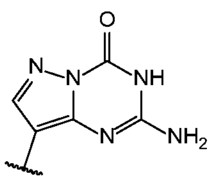
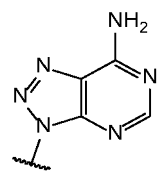
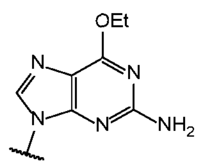
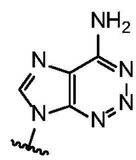
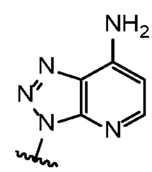
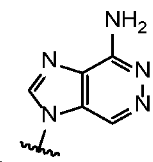
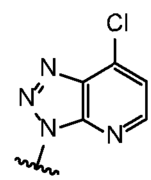
In a twentieth aspect of the second embodiment, the compound of formula (I') is a compound of formula (I'b):

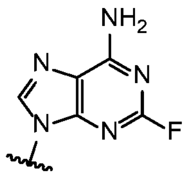
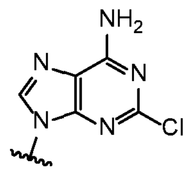
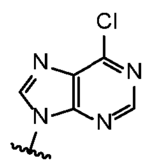
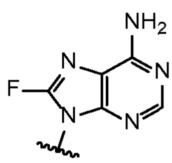
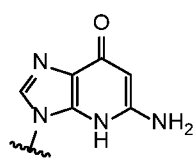
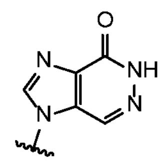


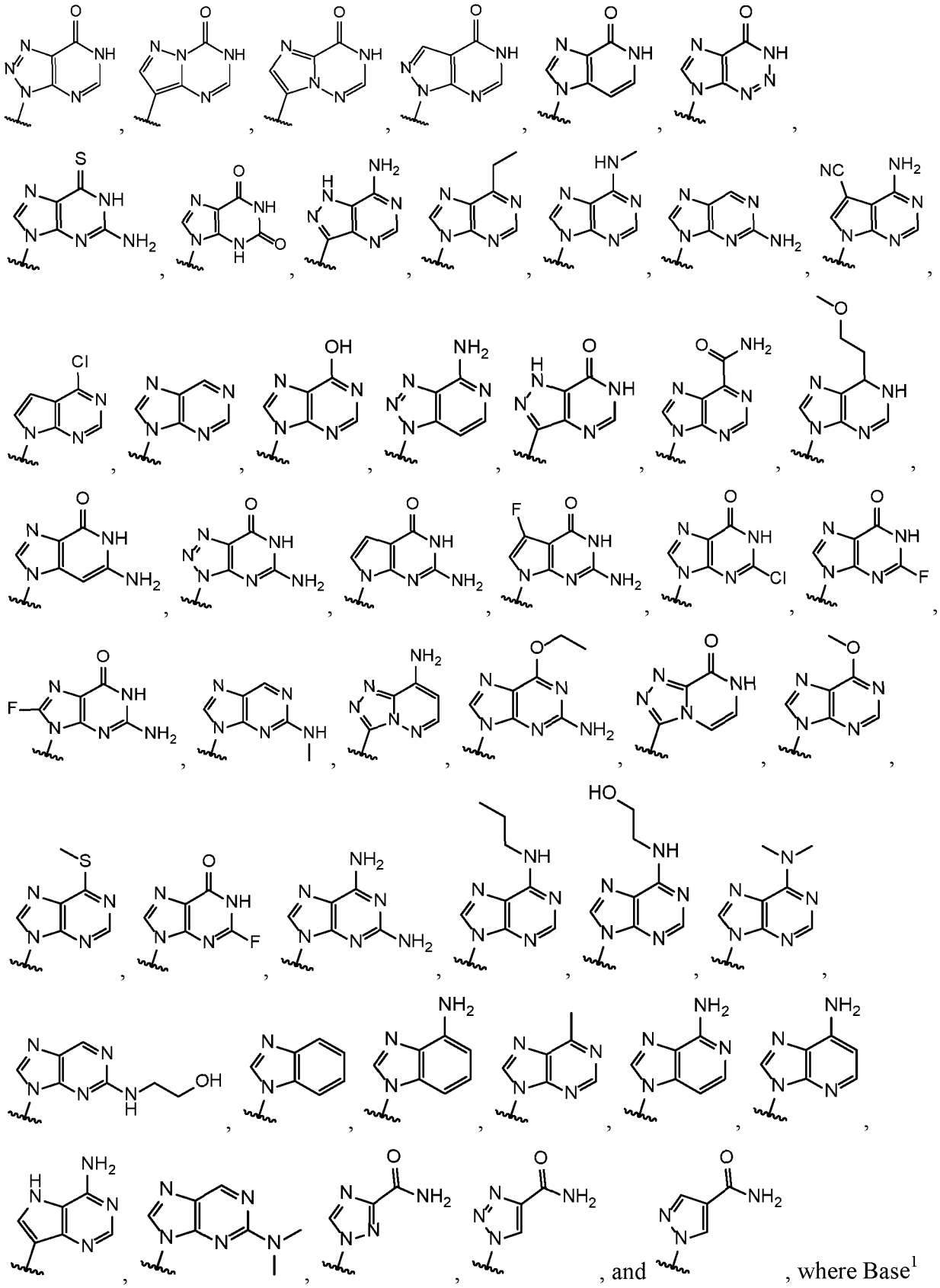
(I'b)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein Base[1] and Base[2] are each independently selected from the group consisting of  ,  ,

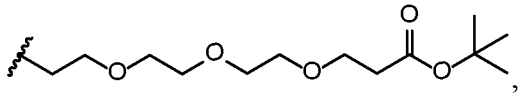
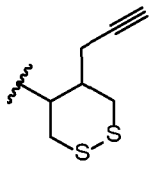
 ,  ,  ,  ,  ,  ,

 ,  ,  ,  ,  ,  ,  ,

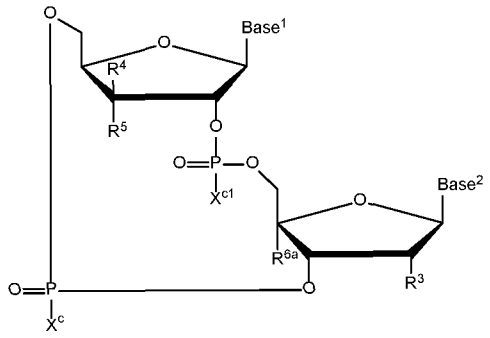
 ,  ,  ,  ,  ,  ,



and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6}

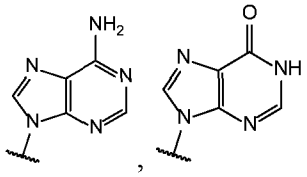
cycloalkyl, O(C_{1-3} alkyl), O(C_{3-6} cycloalkyl), S(C_{1-3} alkyl), S(C_{3-6} cycloalkyl), NH(C_{1-3} alkyl), NH(C_{3-6} cycloalkyl), N(C_{1-3} alkyl)$_2$, and N(C_{3-6} cycloalkyl)$_2$; X^c and X^{c1} are each independently selected from the group consisting of OR^9, SR^9, and NR^9R^9; R^{1a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^{1a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3; R^{2a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^{2a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH; R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH; R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH; R^3 and R^5 are not both selected from the group consisting of OH, C_1-C_6 alkyl substituted with OH, and C_1-C_6 haloalkyl substituted with OH; R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl; each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,  , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; and optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, and -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above.

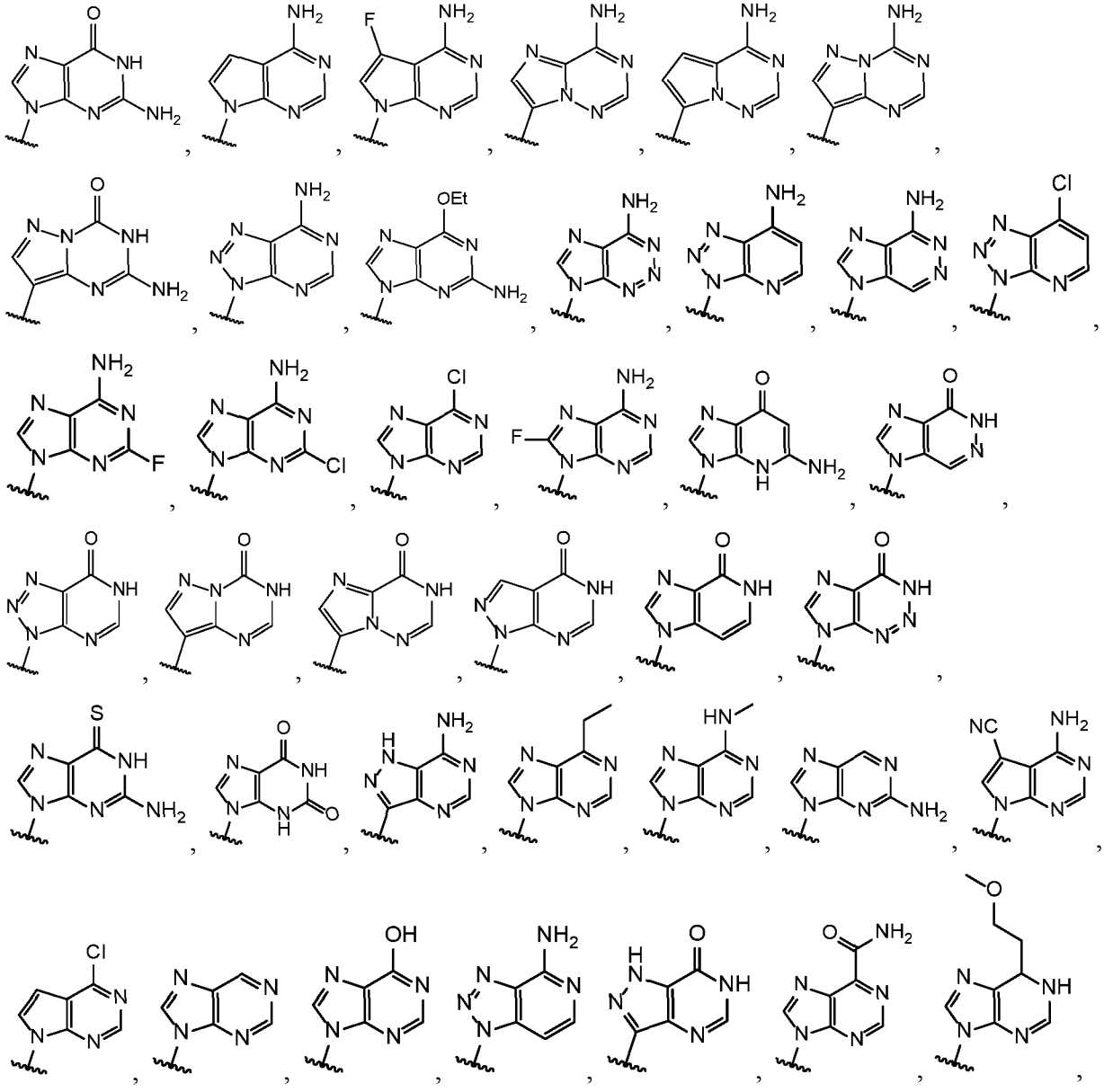
In a twenty-first aspect of the second embodiment, the compound of formula (I') is a compound of formula (I'c):

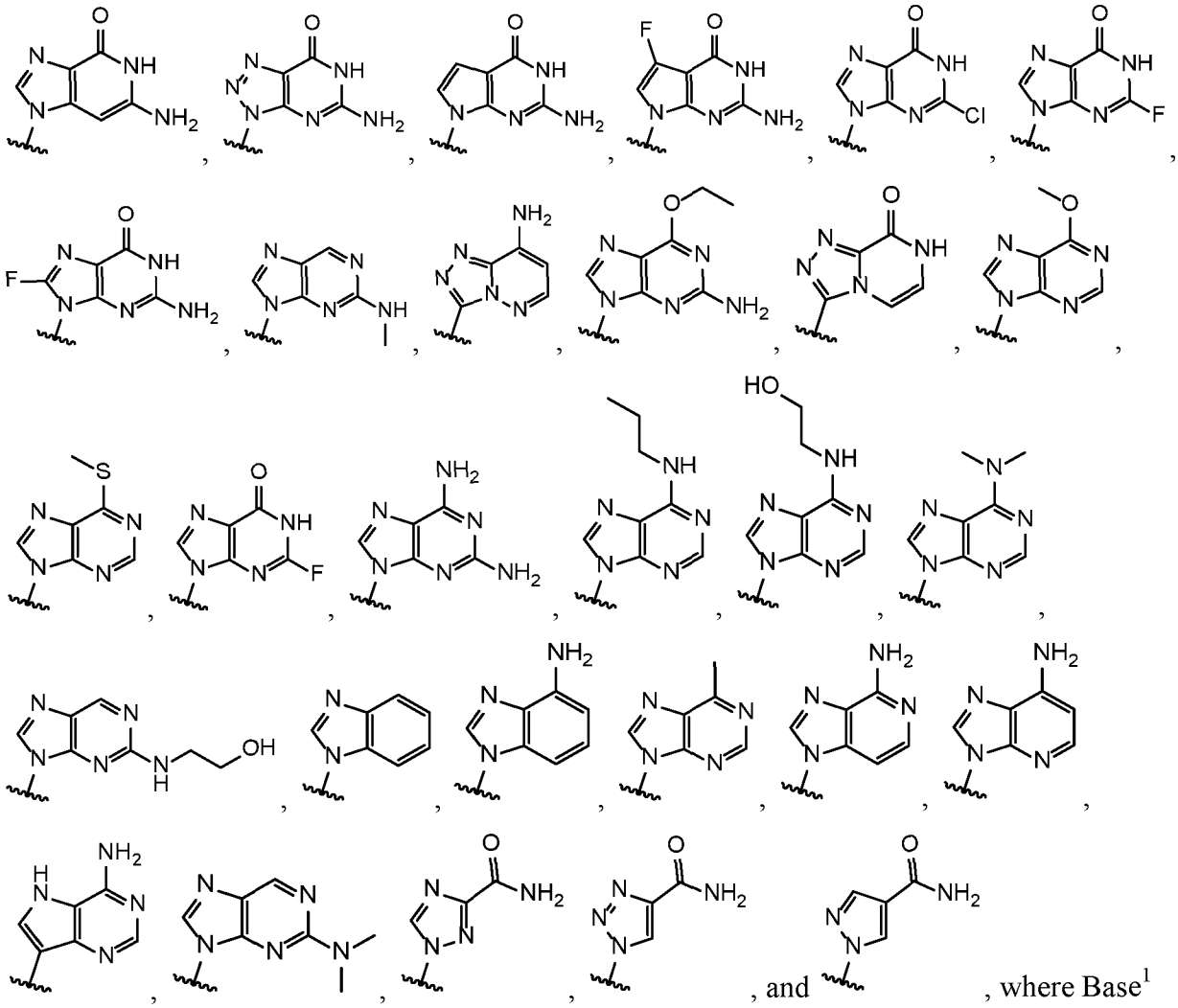


(I'c)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of

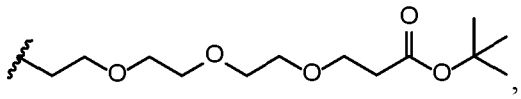
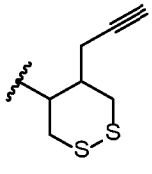






and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$; X^c and X^{c1} are each independently selected from the group consisting of OR^9, SR^9, and NR^9R^9; R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH; R^4 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^4 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH; R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH; R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^{6a} C_1-C_6 alkyl or C_1-C_6

haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH; each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,

 , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; and optionally R^4 and R^5 are connected by C_1-C_6 alkylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position. In this aspect, all other groups are as provided in the general formula (I') of the second embodiment above.

A twenty-second aspect of the second embodiment relates to a pharmaceutical composition, said pharmaceutically acceptable composition comprising (a) a compound according to any one of general formula (I') of the second embodiment above or in the first through twenty-first aspects described above or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof; and (b) a pharmaceutically acceptable carrier.

A twenty-third aspect of the second embodiment relates to methods of inducing an immune response in a subject, comprising administering a therapeutically effective amount of a compound according to any one of general formula (I') of the second embodiment above or in the first through twenty-first aspects described above or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof to the subject.

A twenty-fourth aspect of the second embodiment relates to methods of inducing an immune response in a subject, comprising administering a therapeutically effective amount of a composition according to the twenty-second aspect described above or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof to the subject.

A twenty-fifth aspect of the second embodiment relates to methods of inducing a STING-dependent type I interferon production in a subject, comprising administering a therapeutically effective amount of a compound according to any one of general (I') of the second embodiment above or in the first through twenty-first aspects described above or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof to the subject.

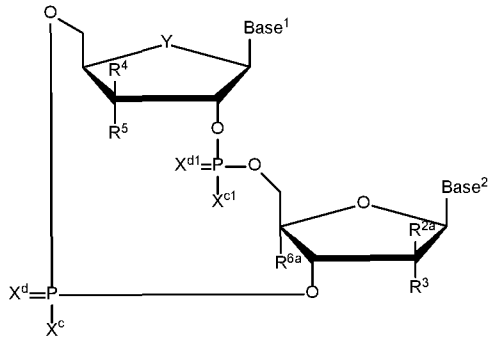
A twenty-sixth aspect of the second embodiment relates to methods of inducing a STING-dependent type I interferon production in a subject, comprising administering a

therapeutically effective amount of a composition according to the twenty-second aspect described above to the subject.

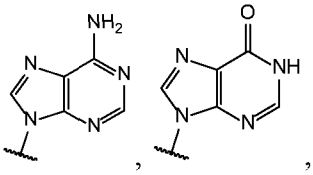
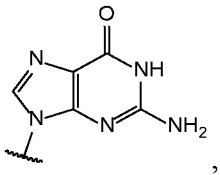
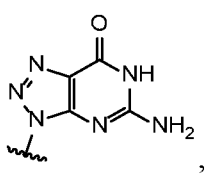
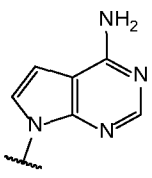
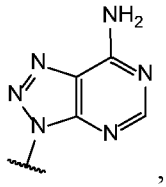
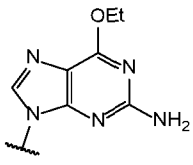
A twenty-seventh aspect of the second embodiment relates to methods of inducing a STING-dependent cytokine production in a subject, comprising administering a therapeutically effective amount of a compound according to any one of general (I') of the second embodiment above or in the first through twenty-first aspects described above or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof to the subject.

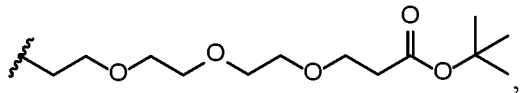
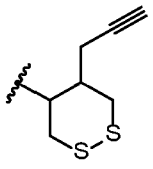
A thirty-third aspect of the second embodiment relates to methods of inducing a STING-dependent cytokine production in a subject, comprising administering a therapeutically effective amount of a composition according to the twenty-second aspect described above to the subject.

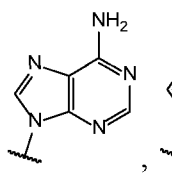
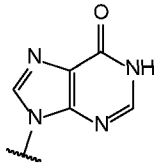
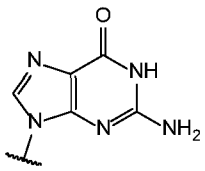
A third embodiment of the disclosure relates to cyclic di-nucleotide compounds of general formula (I''):

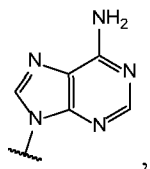
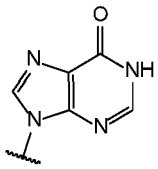
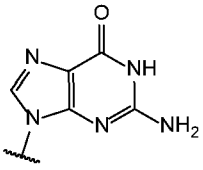
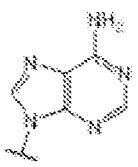
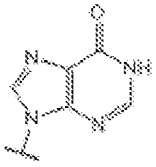
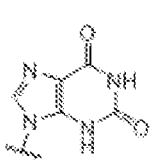
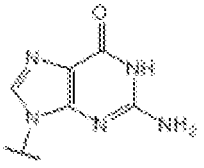


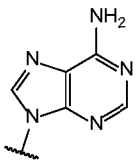
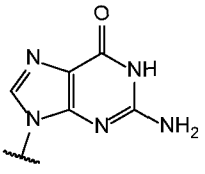
(I'')

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of  , ,  ,  ,  ,  , and  ; Y is selected from the group consisting of -O- and -S-; X^c and X^{c1} are each independently selected from the group consisting of OR^9 and SR^9; X^d and X^{d1} are each independently selected from the group consisting of O and S; R^{2a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6

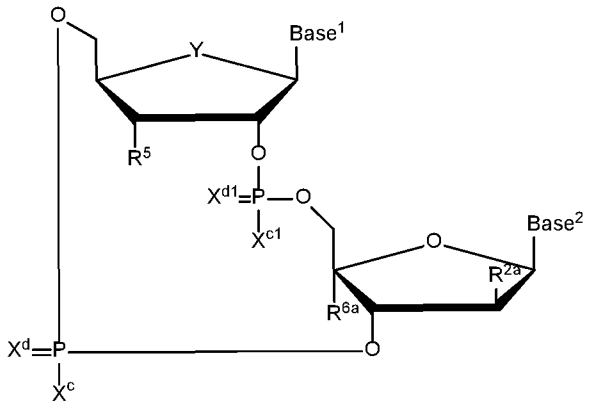
haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; R^4 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; each R^9 is independently selected from the group consisting of H, C_1-C_{20} alkyl,  , and  , where each R^9 C_1-C_{20} alkyl is optionally substituted by 0 to 3 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; and optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position.

In specific aspects of this embodiment, when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH or SH, X^d and X^{d1} are each O, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group consisting of  ,  , and  , R^5 and R^3 are not both selected from the group consisting of H, F and OH. That is, when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH or SH, X^d and X^{d1} are each O, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group

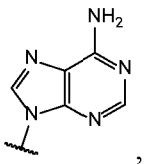
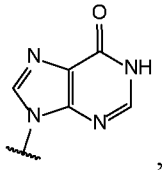
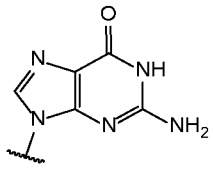
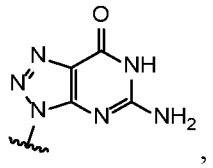
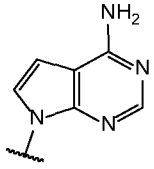
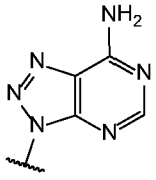
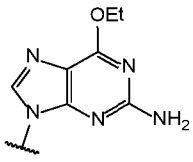
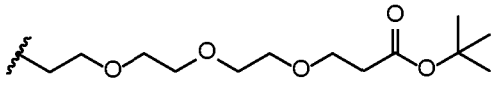
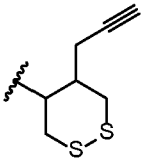
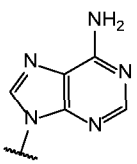
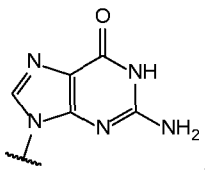
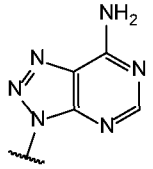
consisting of  ,  , and  , either only one of R^5 and R^3 is selected from the group consisting of H, F, and OH, or neither R^5 and R^3 is selected from the group consisting of H, F, and OH. In further specific instances of this aspect, when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH, X^d and X^{d1} are each O or S, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group consisting of  ,  ,  and  , R^5 and R^3 are not both selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, where said C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I and OH.

In further specific aspects of this embodiment, when $Base^1$ and $Base^2$ are each selected from the group consisting of  and  , and R^{2a} is F and R^5 is F, at least one of X^c and X^{c1} is SR^9.

In a first aspect of the third embodiment, the compound of formula (I") is a compound of formula (I"a):



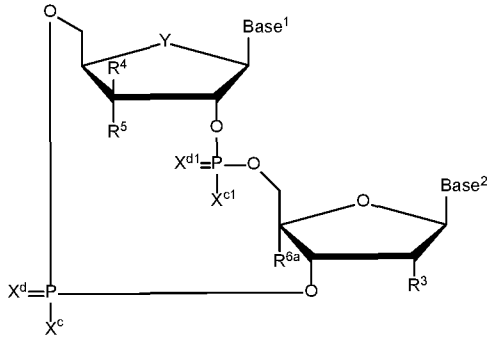
(I"a)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  ,  ,  ,  ,  , and  ; Y is selected from the group consisting of -O- and -S-; X^c and X^{c1} are each independently selected from the group consisting of OR^9 and SR^9; X^d and X^{d1} are each independently selected from the group consisting of O and S; R^{2a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; and each R^9 is independently selected from the group consisting of H, C_1-C_{20} alkyl,  , and  , where each R^9 C_1-C_{20} alkyl is optionally substituted by 0 to 3 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl. In instances of this aspect, $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  , and  ; Y is selected from the group consisting of -O- and -S-; X^c and X^{c1} are each independently selected from the group consisting of OR^9 and SR^9; X^d and X^{d1} are each independently selected from the group consisting of O and S; R^{2a} is F; R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl,

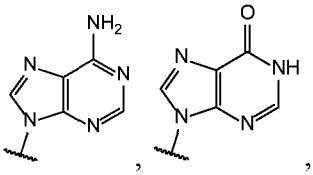
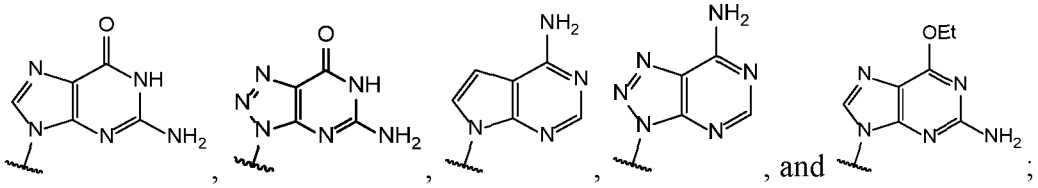
C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; and each R^9 is independently H.

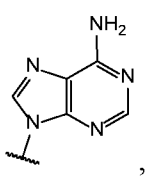
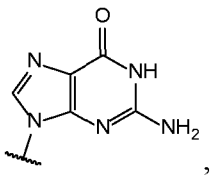
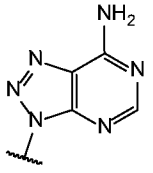
In a second aspect of the third embodiment, the compound of formula (I") is a compound wherein R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position.

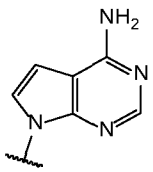
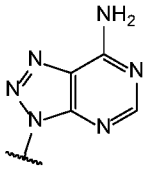
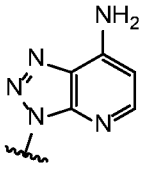
In a third aspect of the third embodiment, the compound of formula (I") is a compound of formula (I"b):



(I"b)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  , and ; Y is selected from the group consisting of -O- and -S-; X^c and X^{c1} are each independently selected from the group consisting of OR^9 and SR^9; X^d and X^{d1} are each independently selected from the group consisting of O and S; R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; R^4 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3,

C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; each R^9 is independently H; and R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position. In instances of this aspect, $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  , and  .

In a fourth aspect of the third embodiment, the compound of formula (I") is a compound wherein at least one of $Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  , and  .

A fifth aspect of the third embodiment relates to a pharmaceutical composition, said pharmaceutical composition comprising (a) a compound according to any one of general formula (I") of the third embodiment or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof; and (b) a pharmaceutically acceptable carrier.

A sixth aspect of the third embodiment relates to methods of inducing an immune response in a subject, comprising administering a therapeutically effective amount of a compound according to general formula (I") of the third embodiment above or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof to the subject.

A seventh aspect of the third embodiment relates to methods of inducing an immune response in a subject, comprising administering a therapeutically effective amount of a composition according to the fifth aspect described above to the subject.

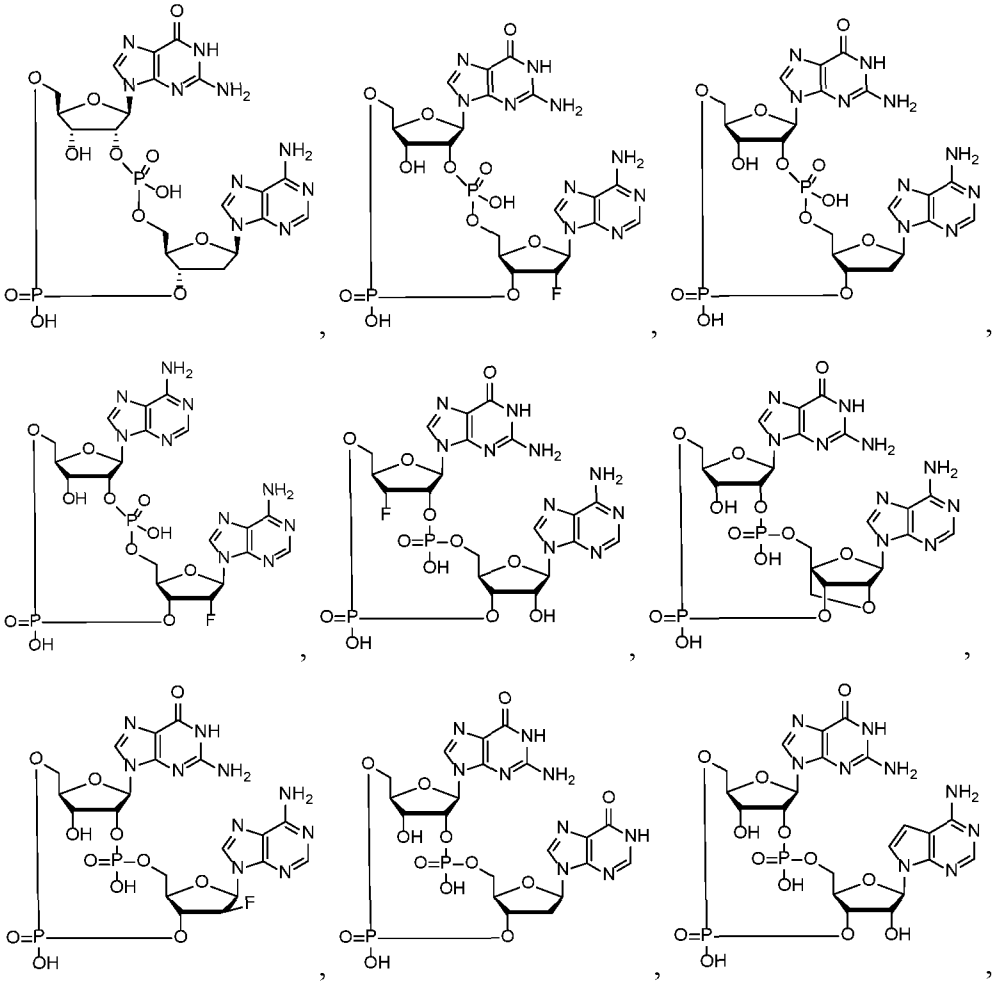
An eighth aspect of the third embodiment relates to methods of inducing a STING-dependent type I interferon production in a subject, comprising administering a therapeutically effective amount of a compound according general formula (I") of the third embodiment above or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof to the subject.

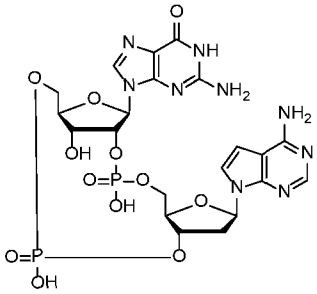
A ninth aspect of the third embodiment relates to methods of inducing a STING-dependent type I interferon production in a subject, comprising administering a therapeutically effective amount of a composition according to the fifth aspect described above to the subject.

A tenth aspect of the third embodiment relates to methods of inducing a STING-dependent cytokine production in a subject, comprising administering a therapeutically effective amount of a compound according to general formula (I") of the third embodiment above or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof to the subject.

An eleventh aspect of the third embodiment relates to methods of inducing a STING-dependent cytokine production in a subject, comprising administering a therapeutically effective amount of a composition according to the fifth aspect described above to the subject.

In an additional embodiment, the compound is selected from the group consisting of

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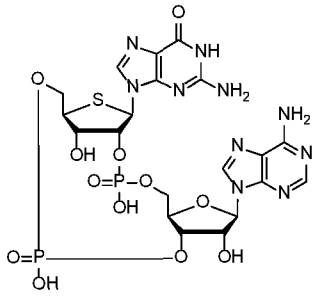
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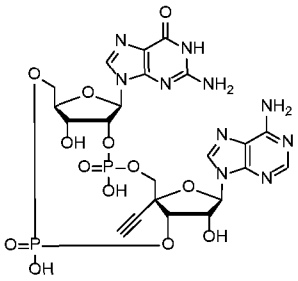
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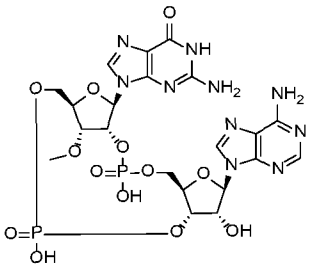
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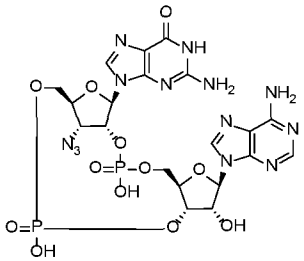
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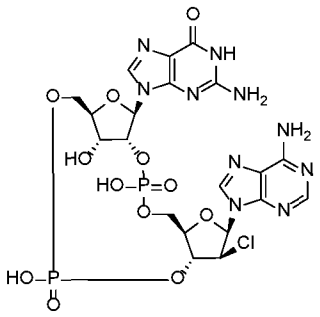
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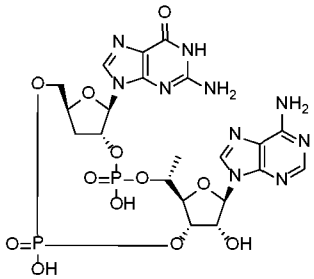
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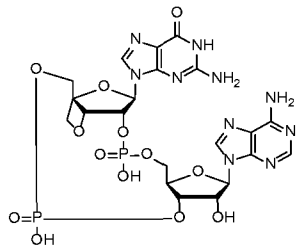
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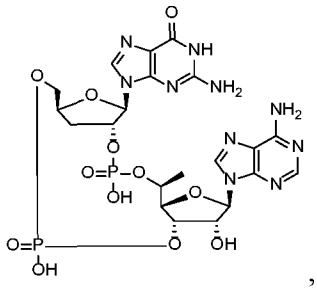
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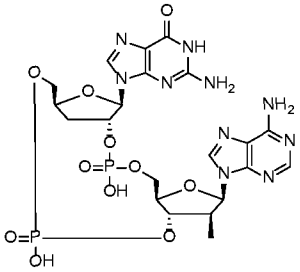
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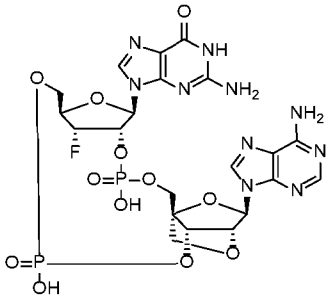
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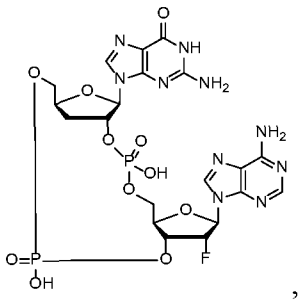
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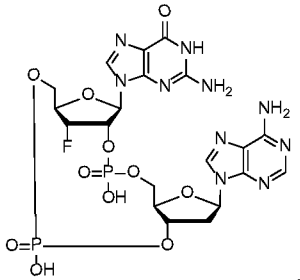
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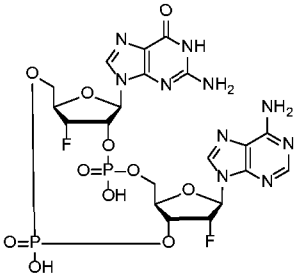
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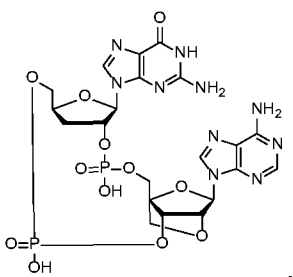
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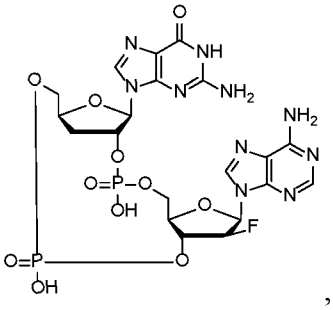
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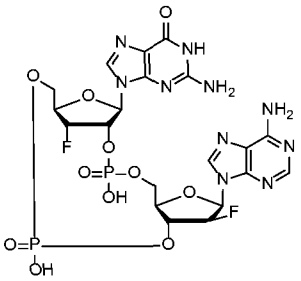
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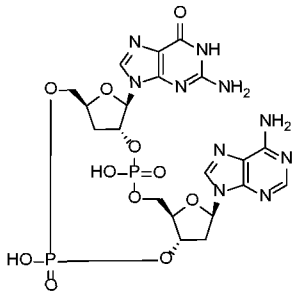
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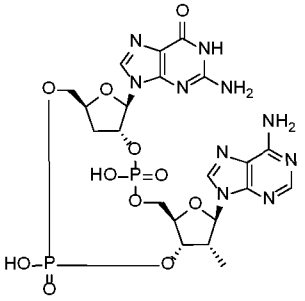
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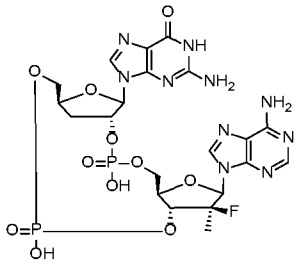
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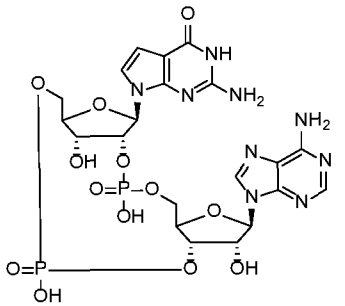
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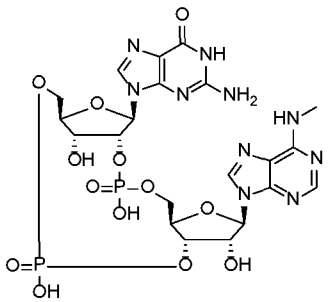
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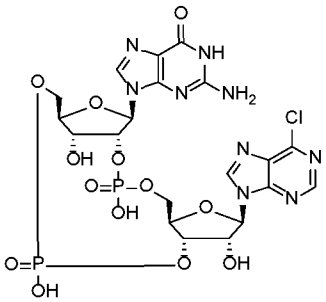
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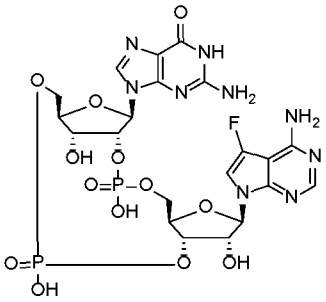
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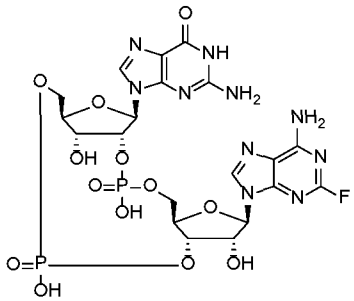
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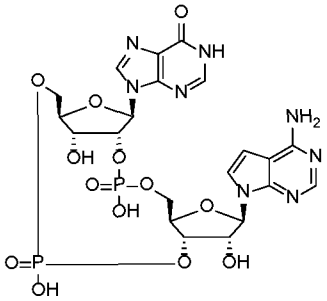
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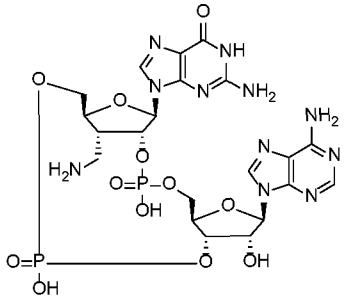
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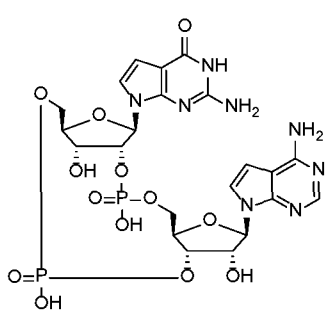
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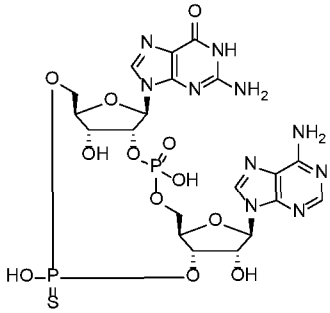
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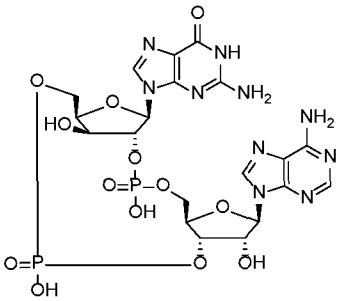
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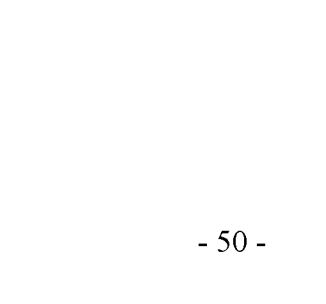
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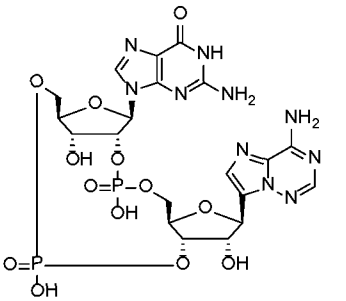
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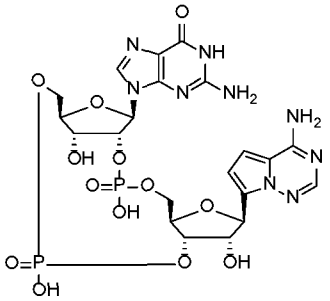
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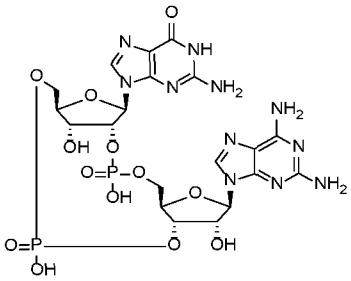
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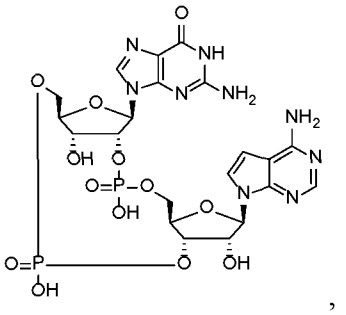
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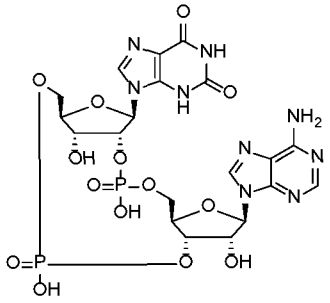
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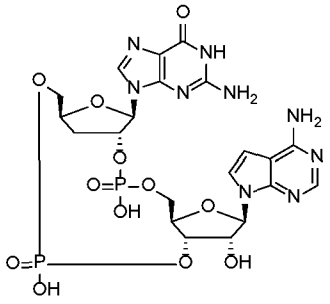
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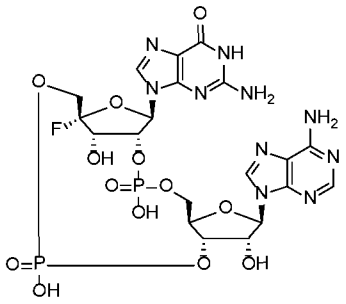
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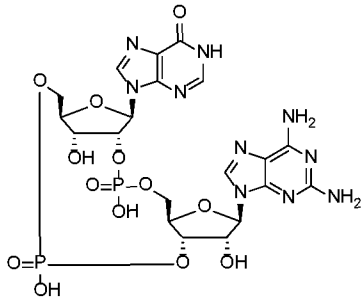
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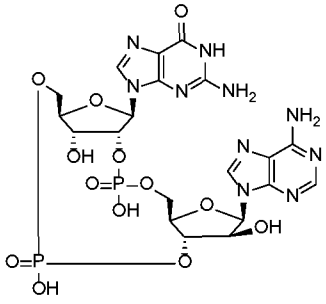
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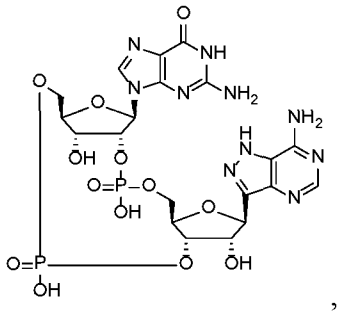
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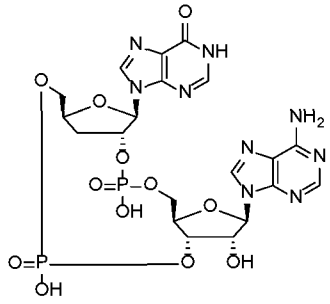
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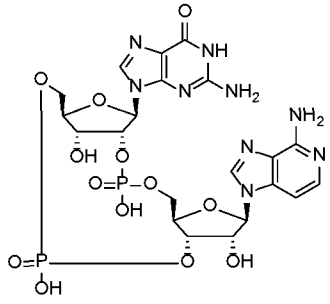
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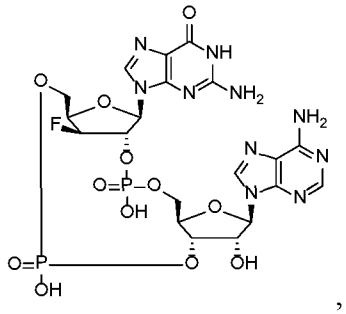
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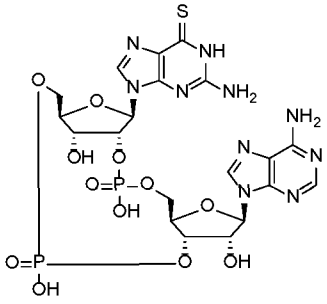
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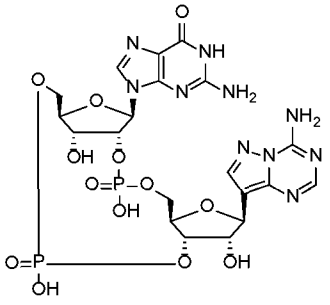
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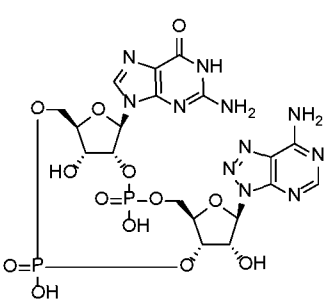
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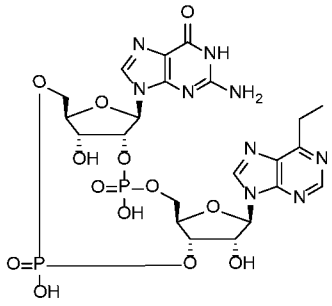
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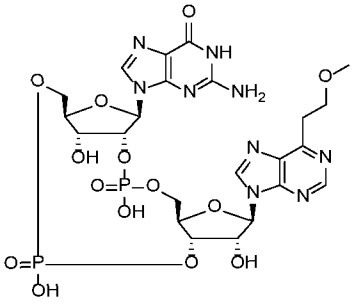
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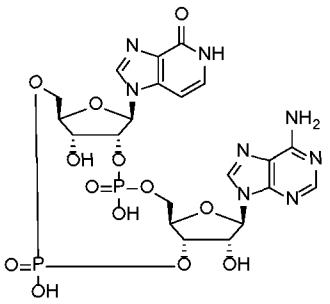
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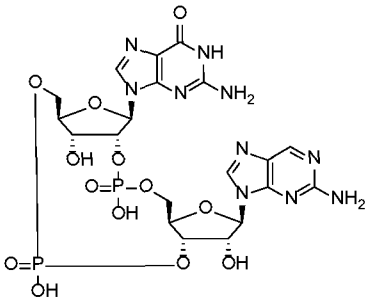
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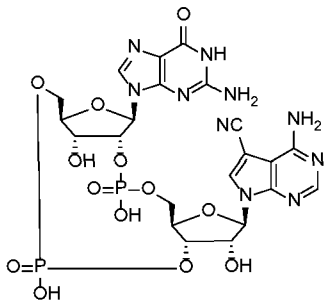
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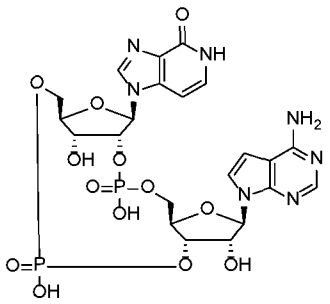
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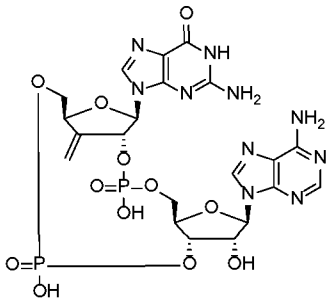
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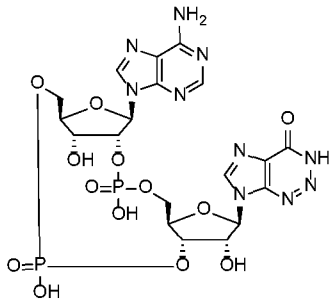
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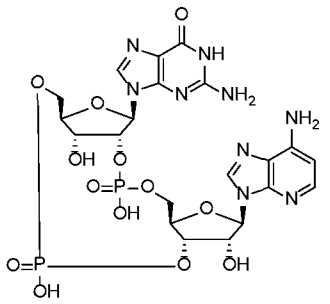
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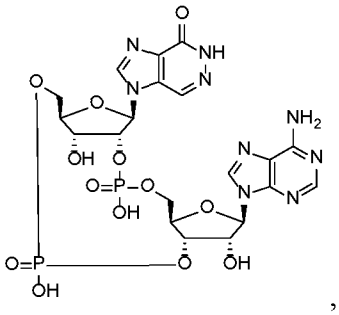
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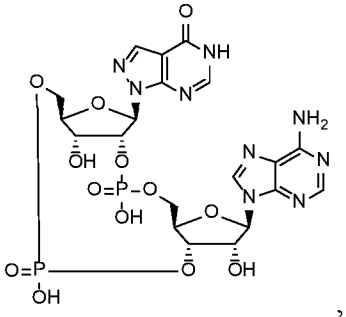
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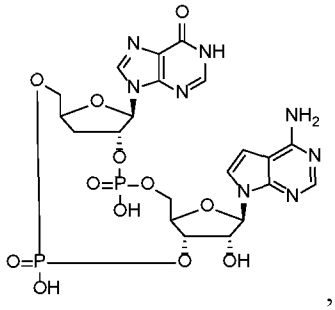
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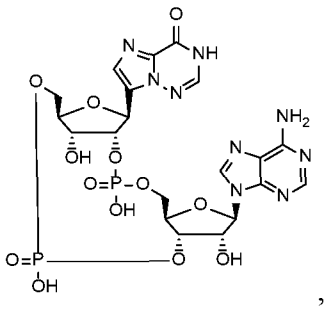
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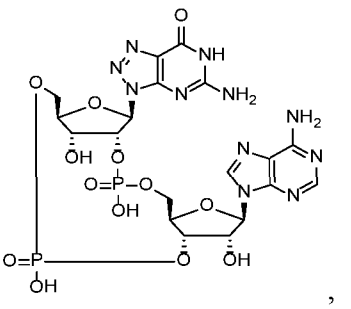
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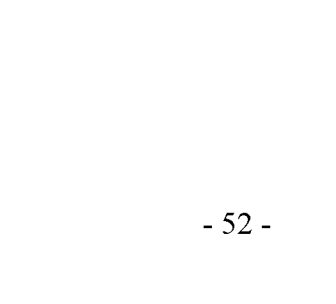
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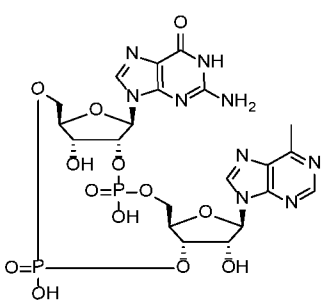
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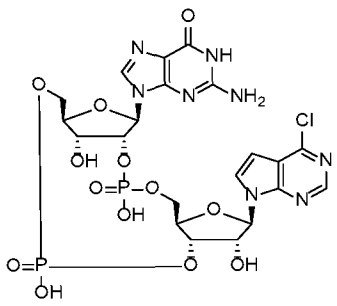
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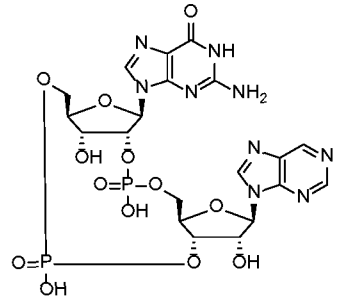
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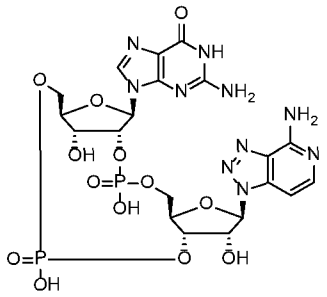
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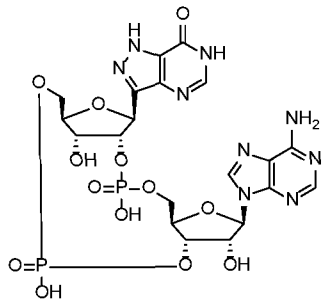
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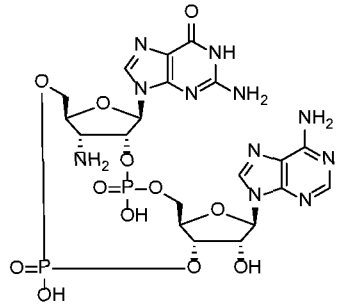
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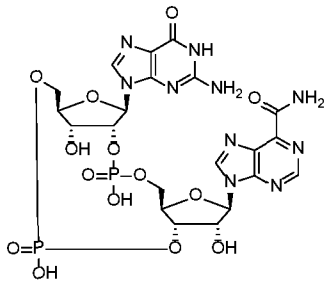
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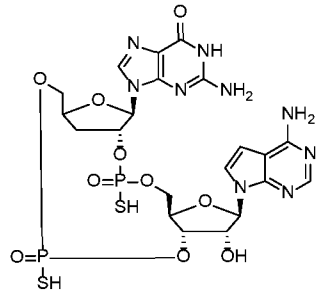
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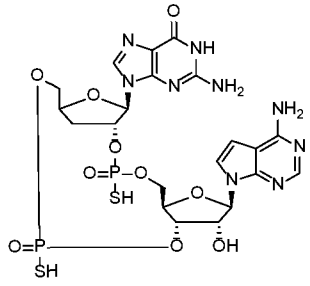
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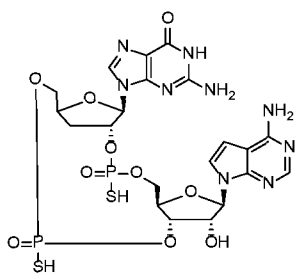
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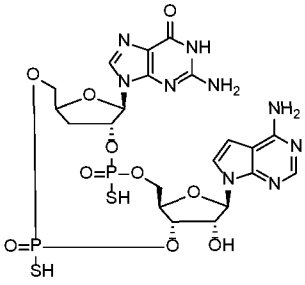
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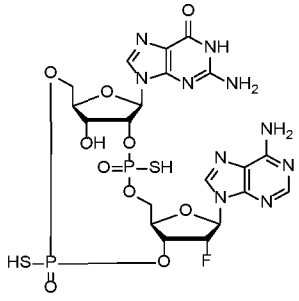
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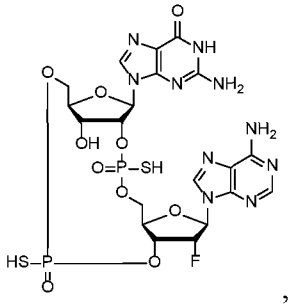
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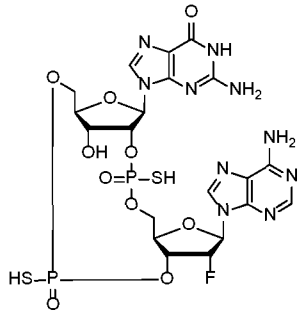
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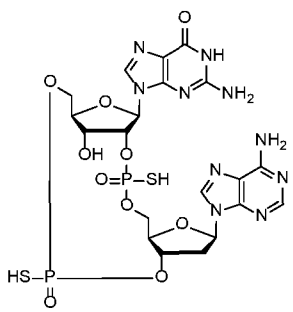
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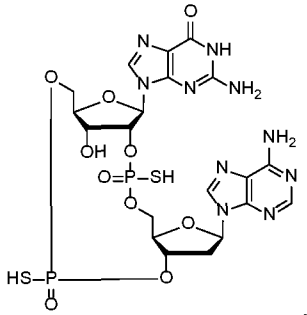
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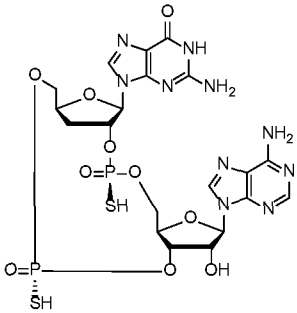
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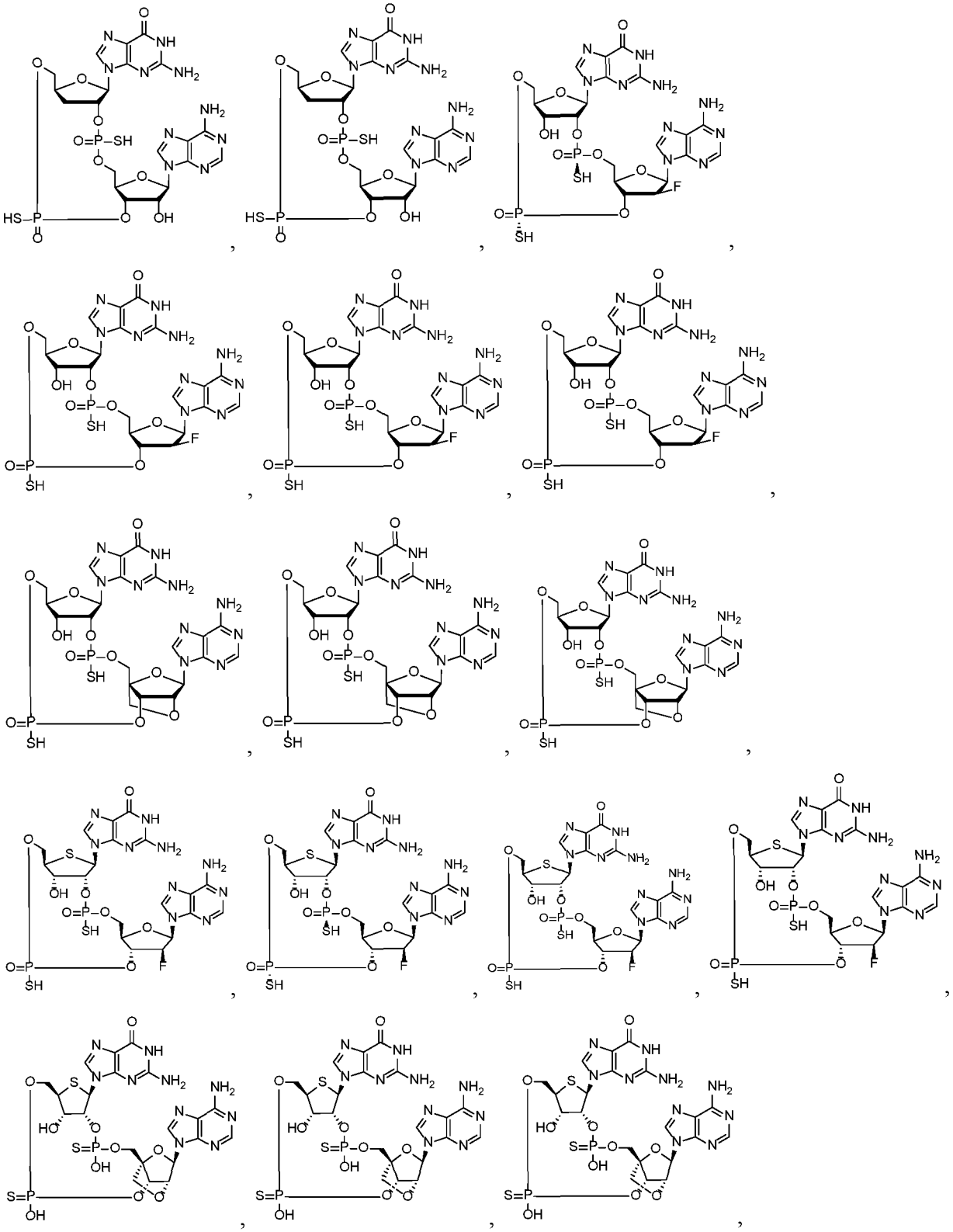

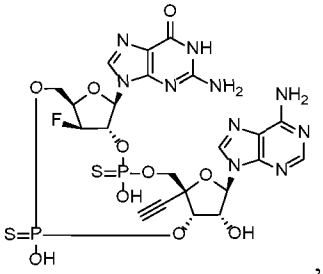
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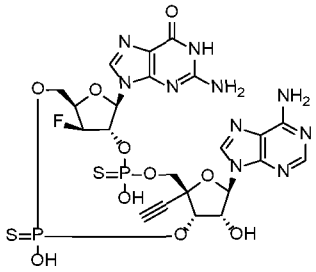
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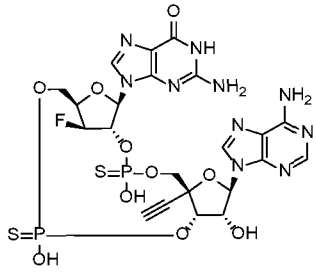
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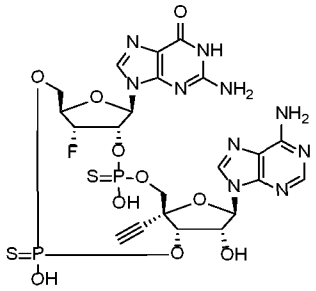
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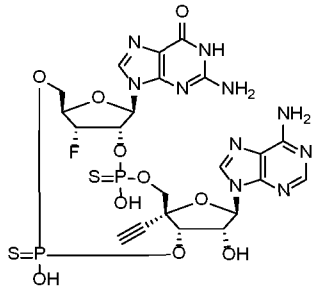
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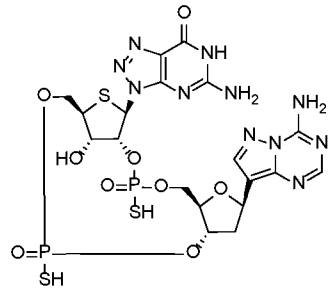
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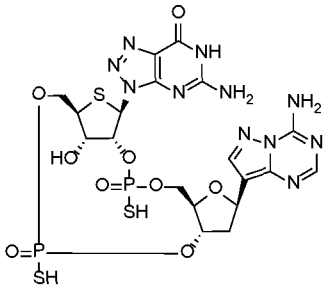
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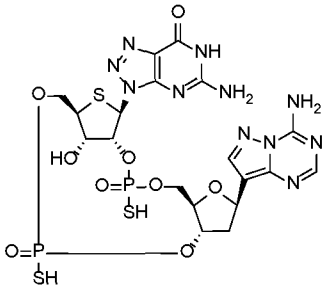
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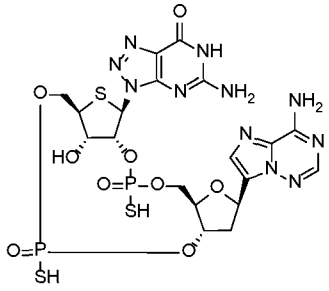
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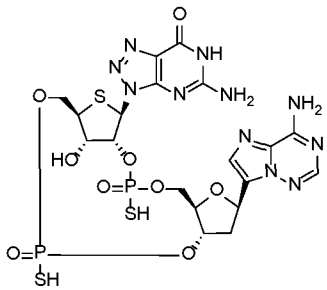
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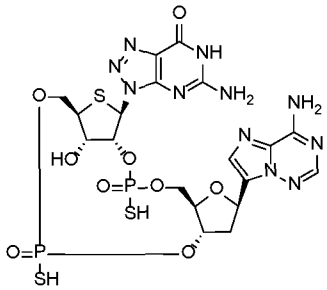
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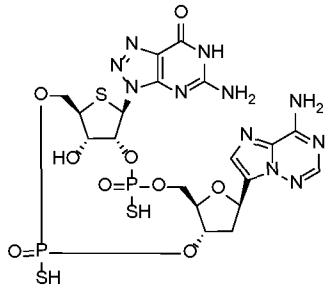
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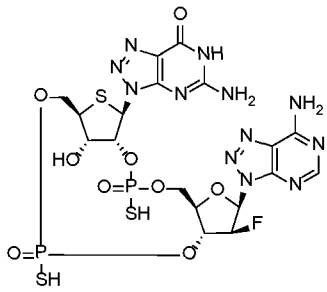
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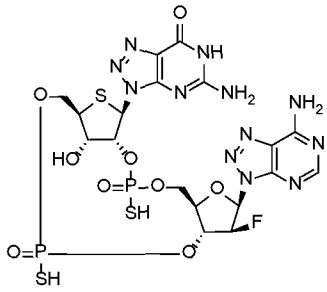
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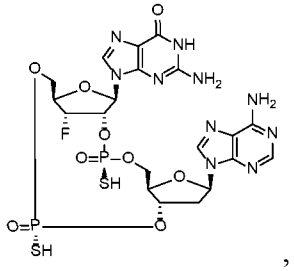
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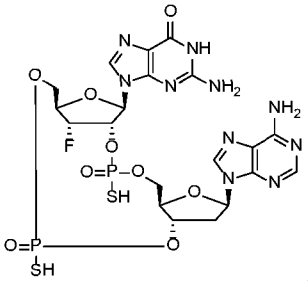
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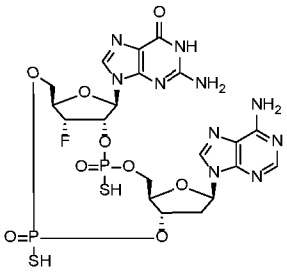
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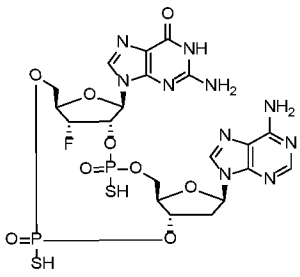
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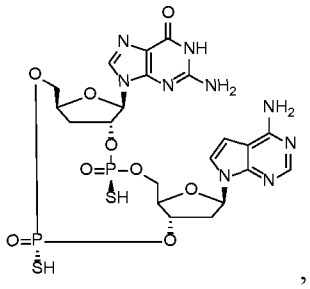
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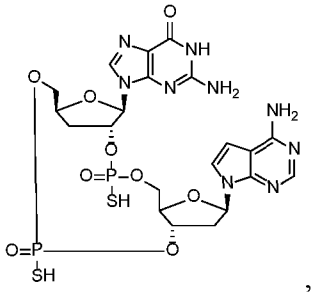
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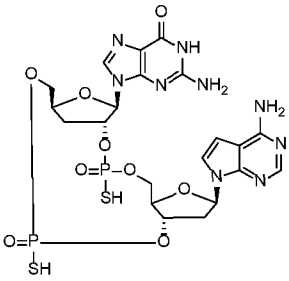
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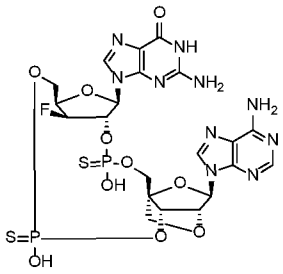
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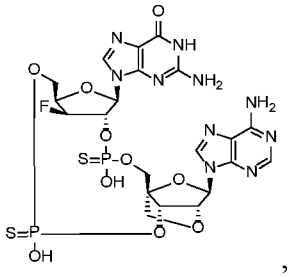
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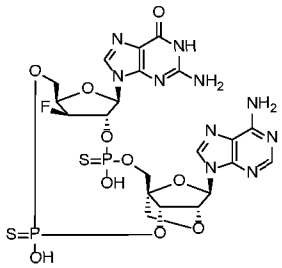
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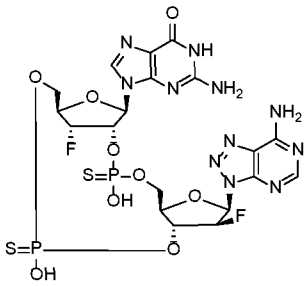
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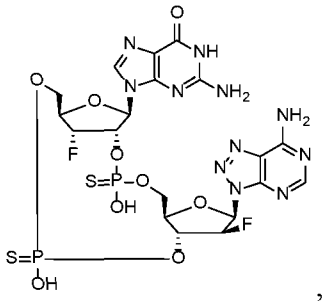
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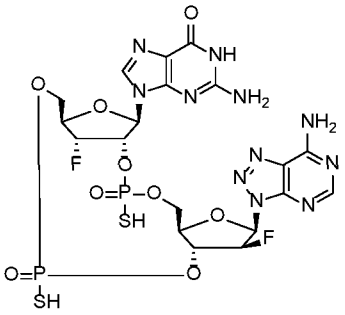
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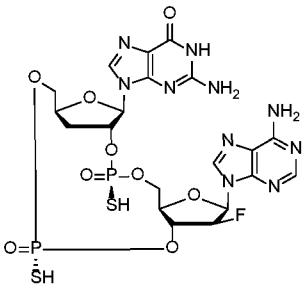
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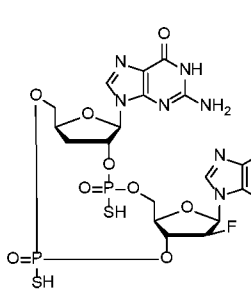
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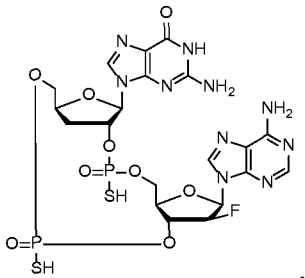
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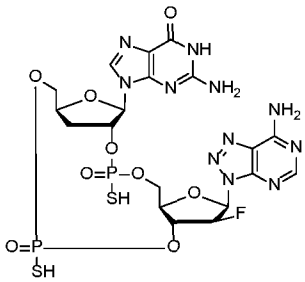
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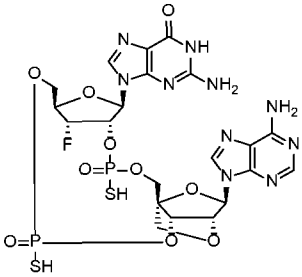
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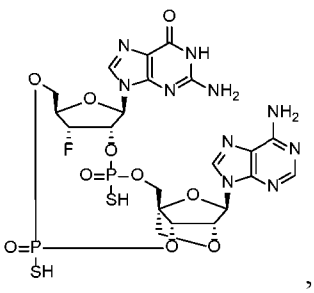
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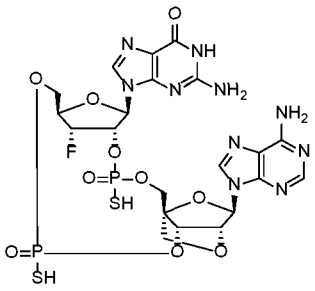
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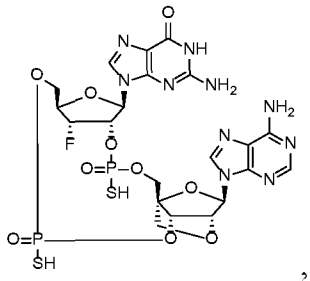
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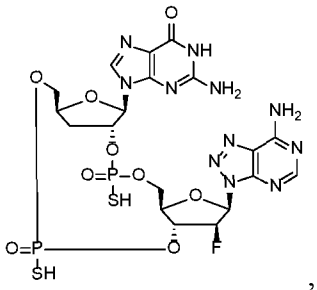
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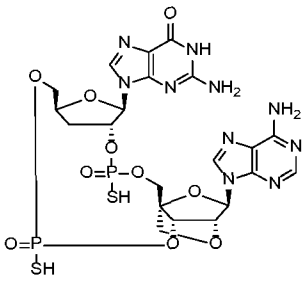
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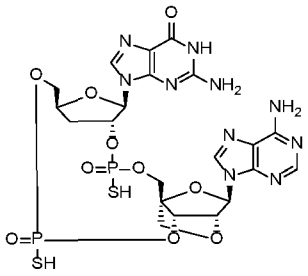
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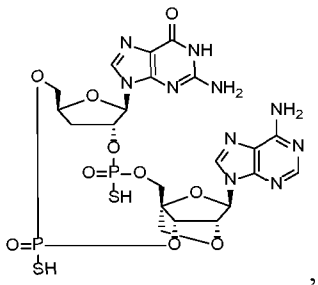
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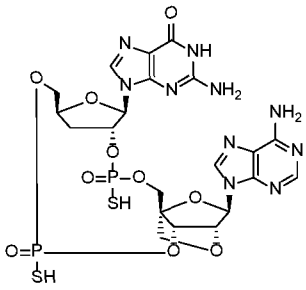
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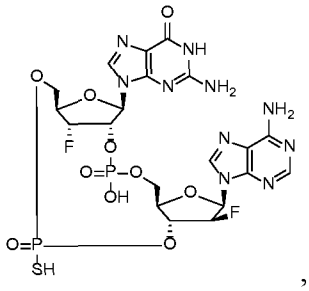
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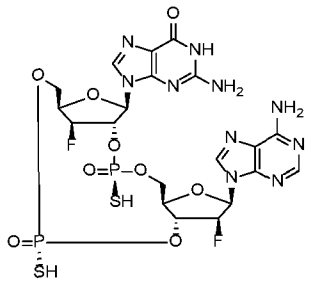
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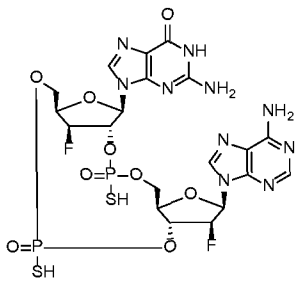
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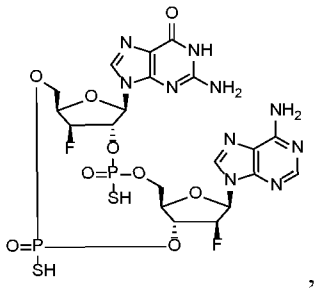
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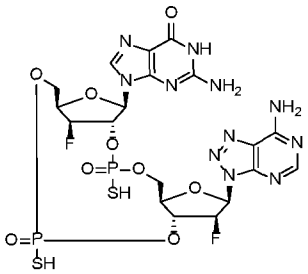
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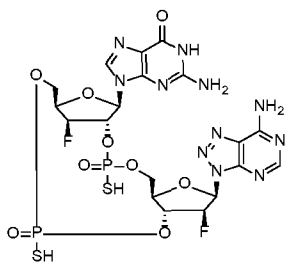
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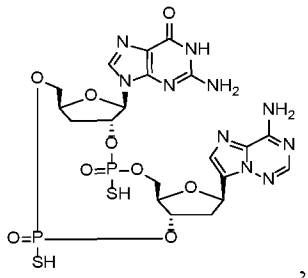
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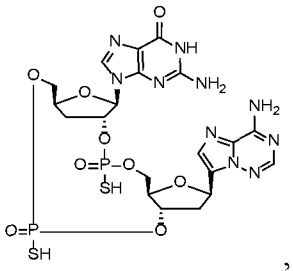
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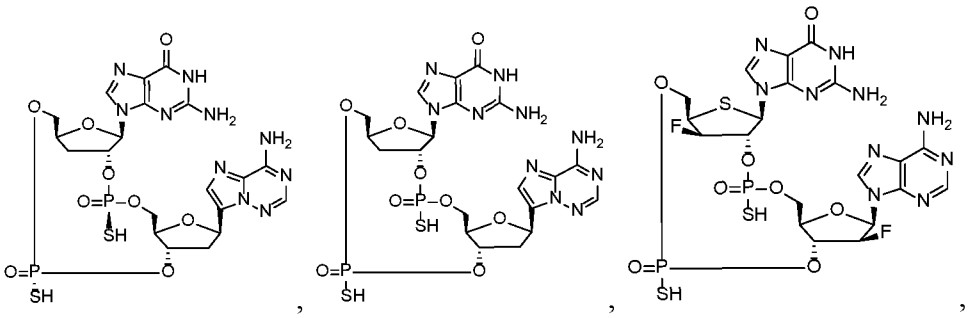
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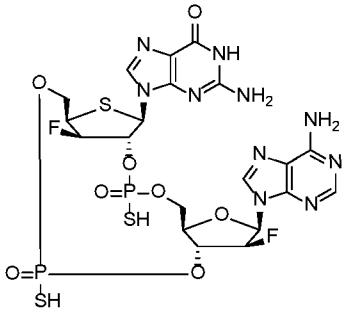
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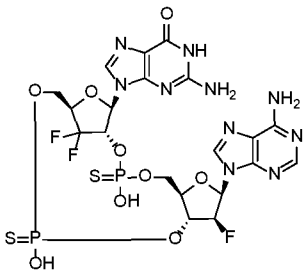
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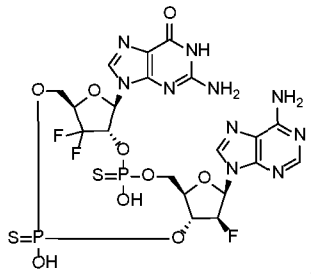
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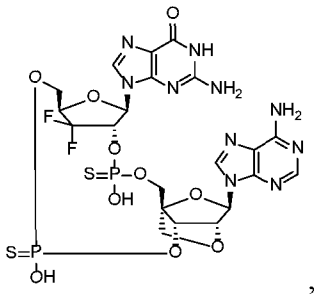
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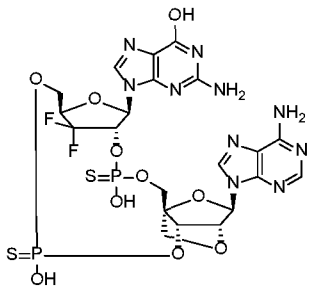
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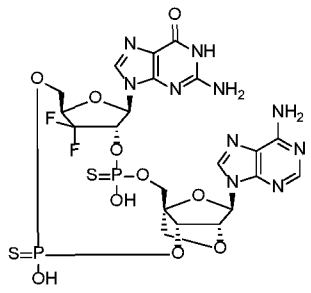
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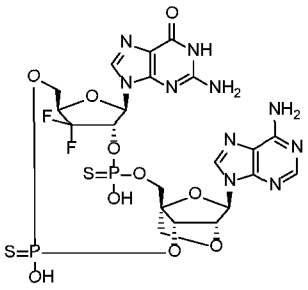
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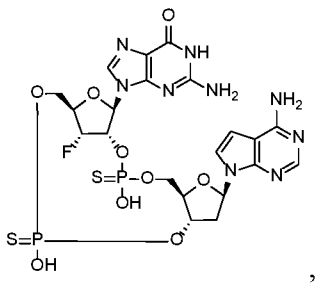
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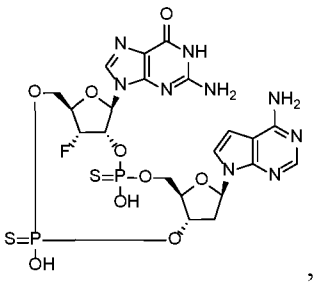
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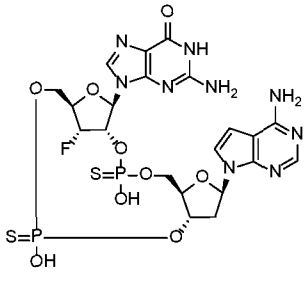
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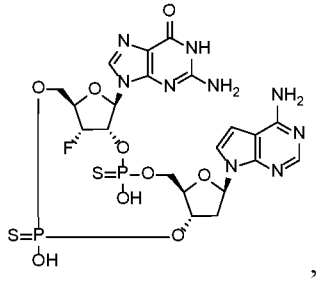
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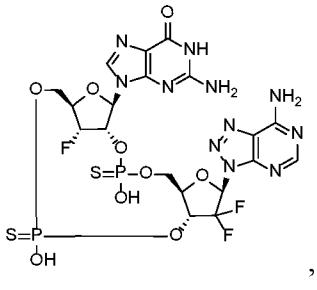
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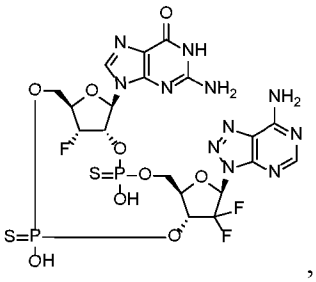
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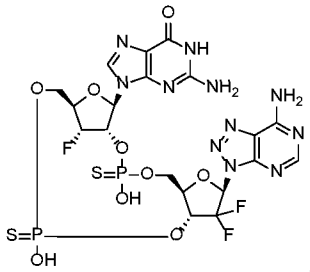
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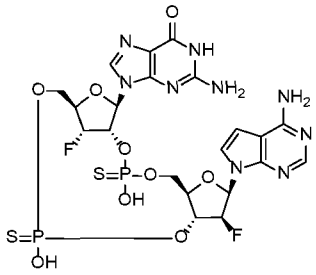
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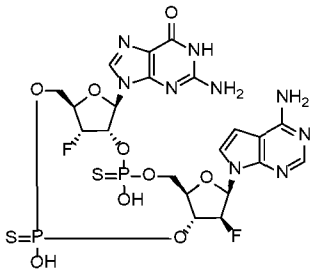
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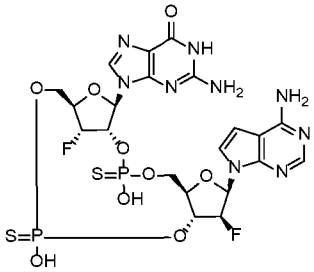
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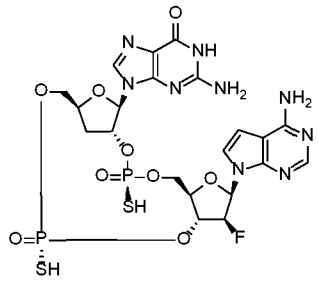
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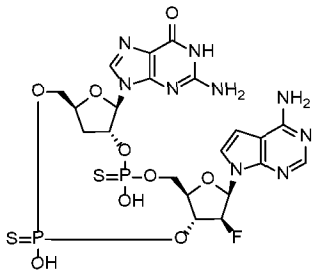
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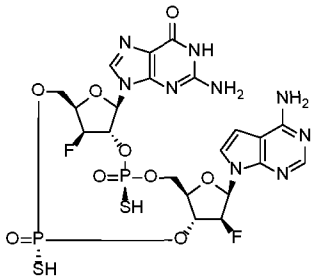
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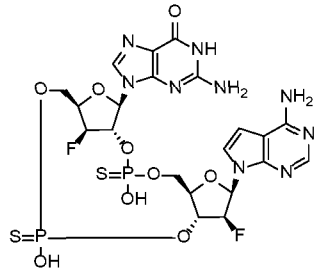
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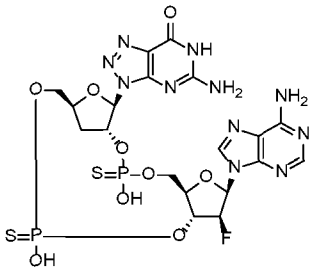
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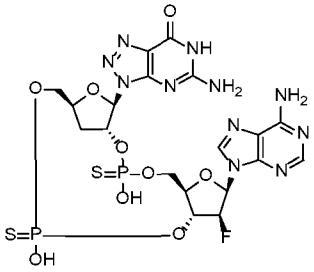
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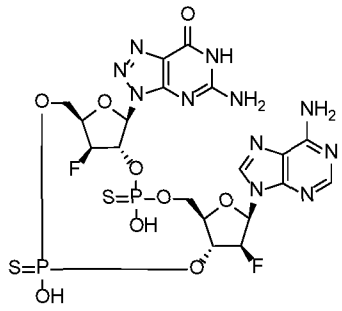
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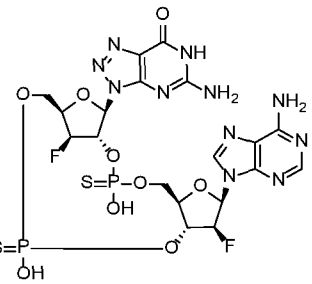
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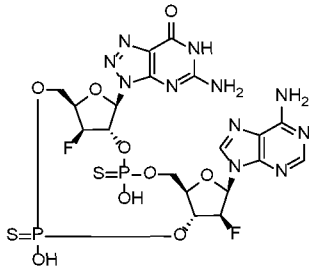
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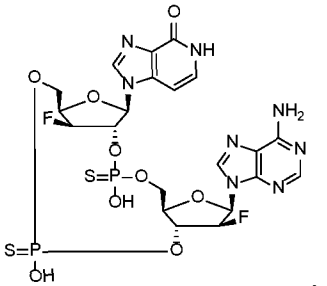
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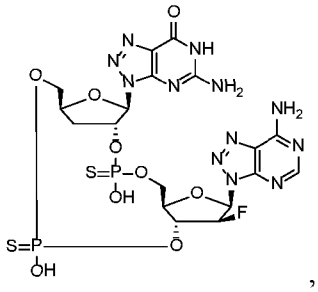
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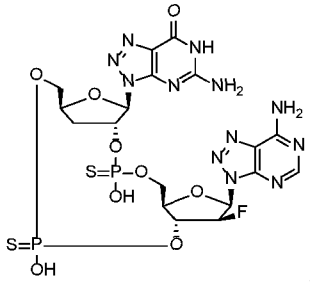
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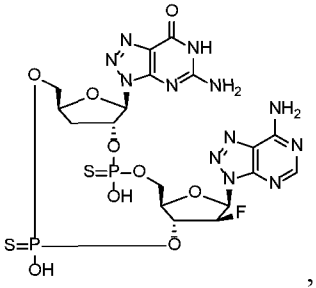
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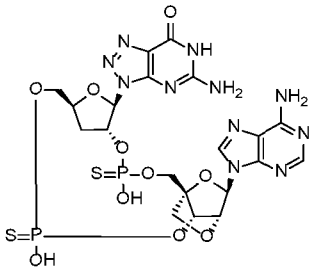
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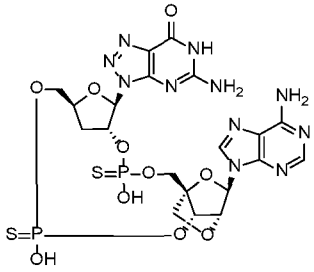
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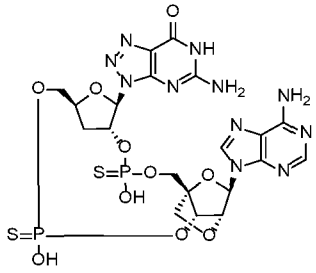
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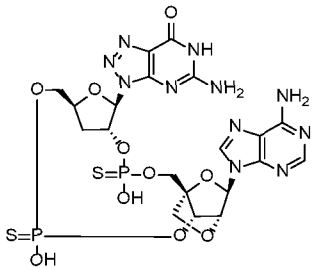
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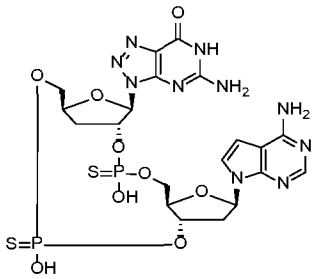
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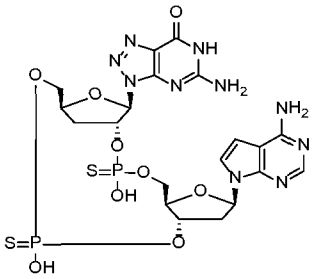
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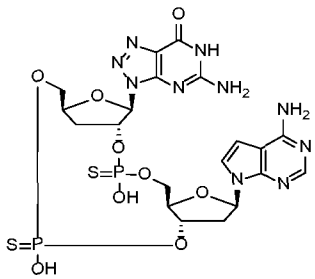
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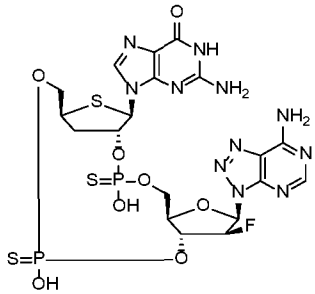
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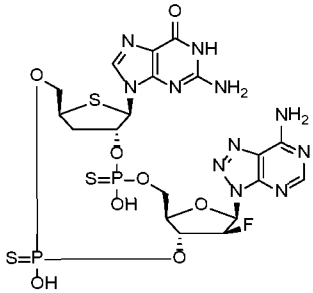
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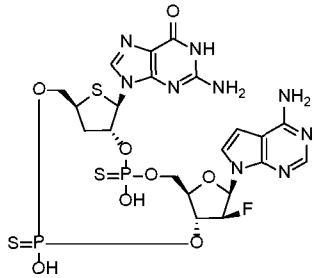
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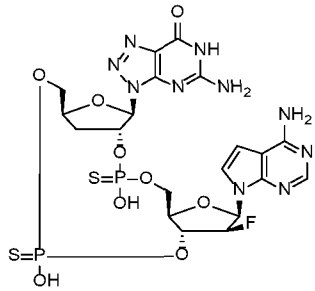
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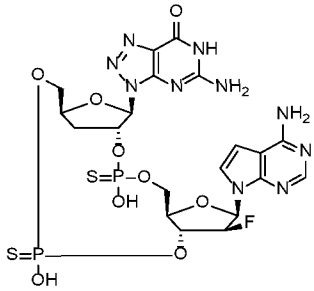
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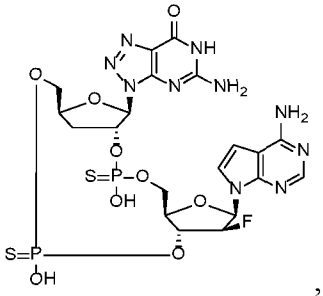
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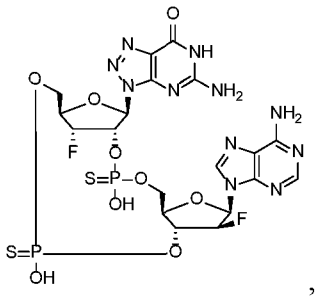
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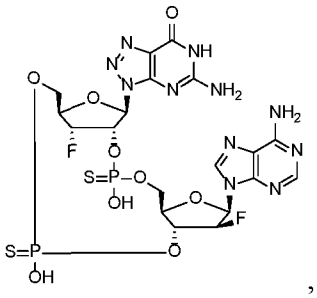
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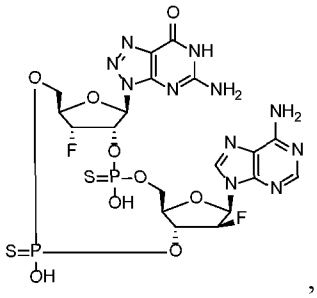
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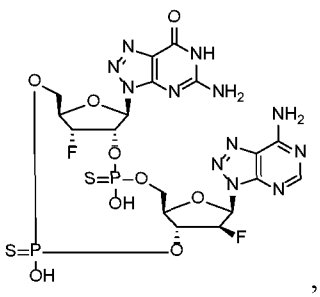
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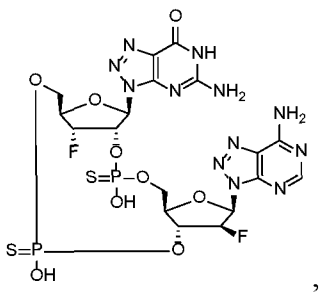
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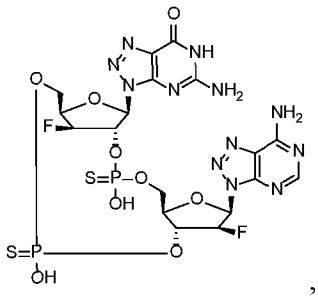
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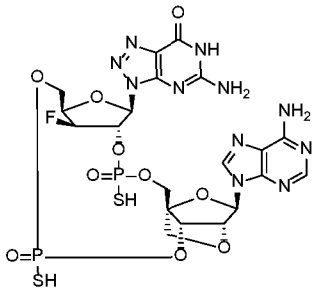
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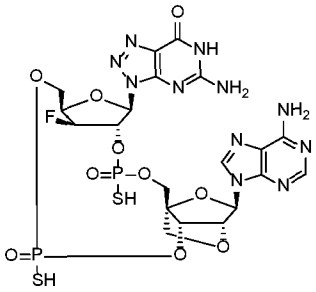
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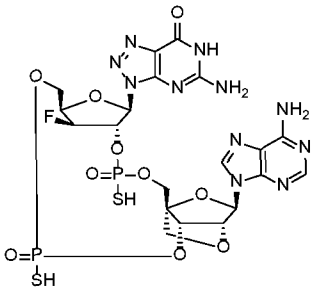
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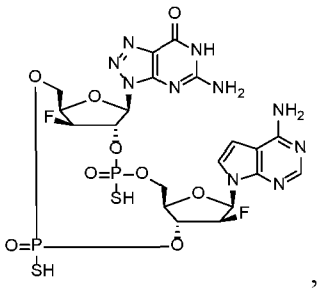
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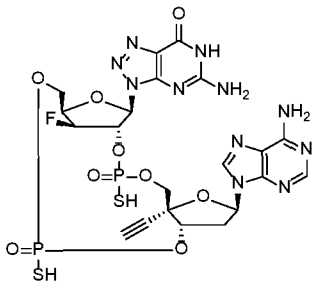
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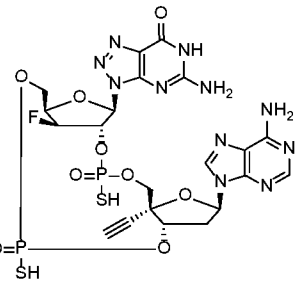
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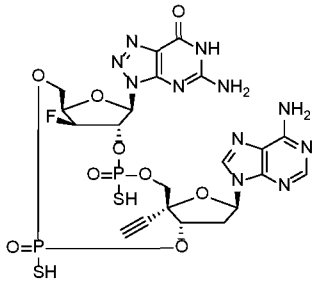
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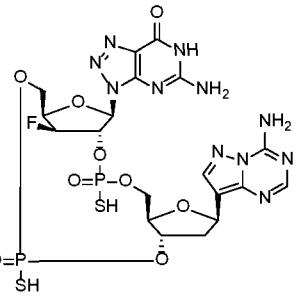
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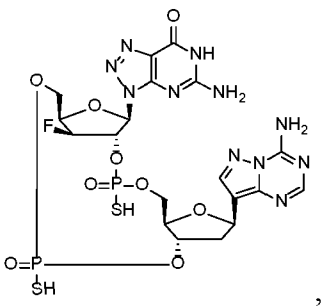
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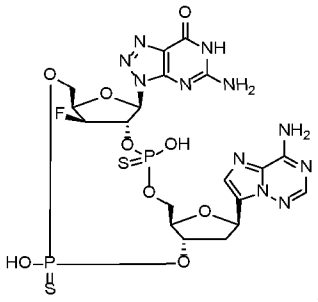
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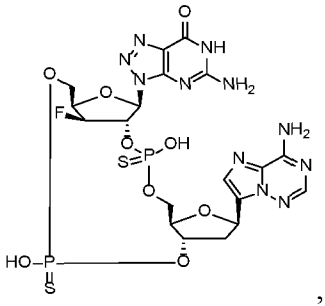
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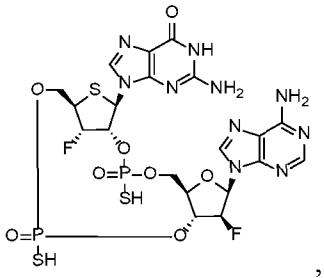
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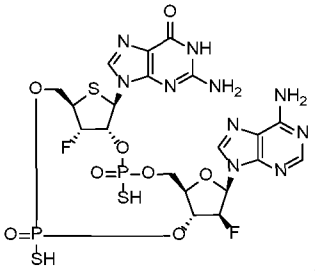
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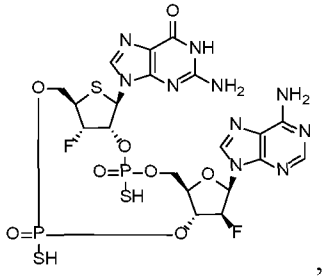
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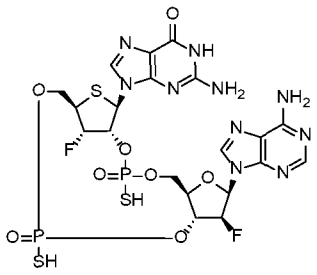
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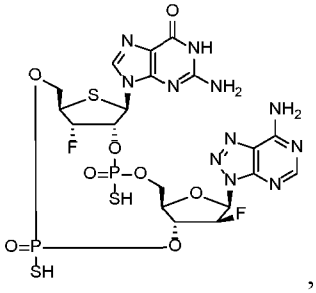
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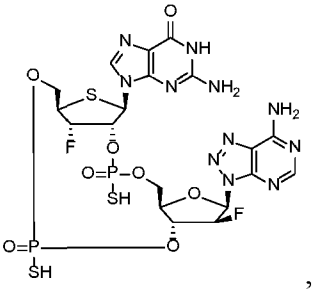
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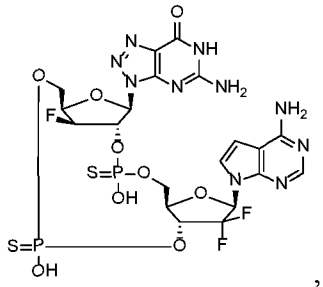
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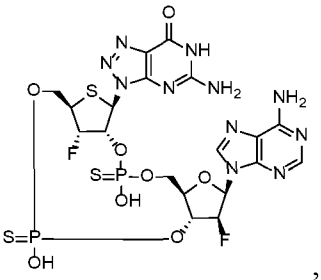
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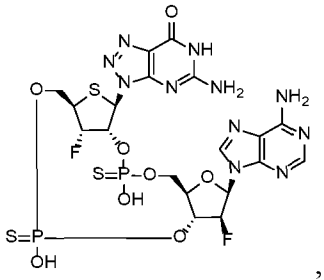
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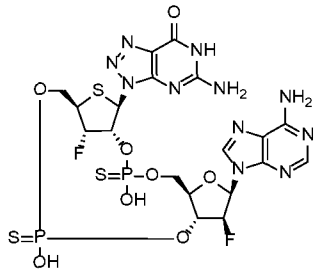
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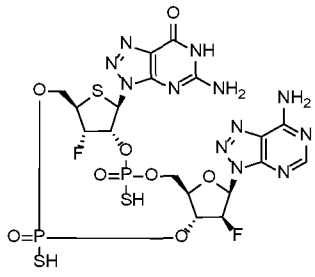
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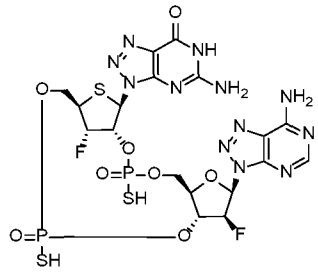
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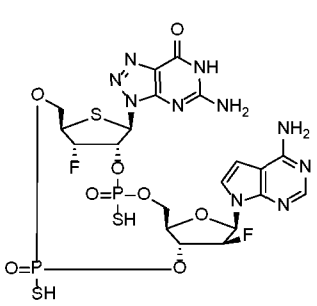
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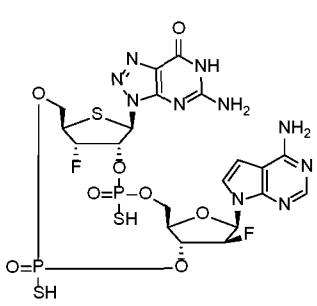
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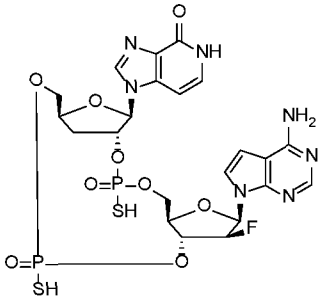
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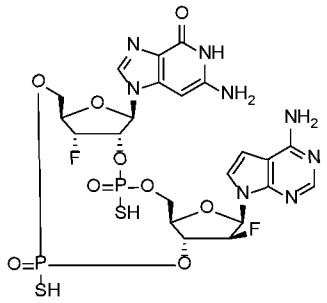
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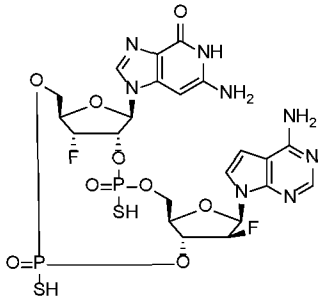
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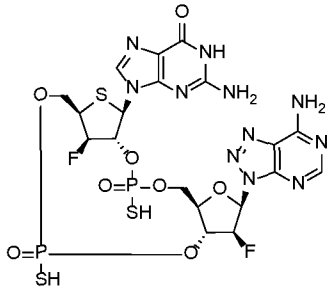
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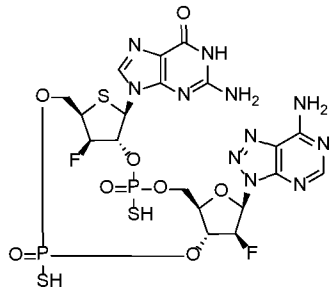
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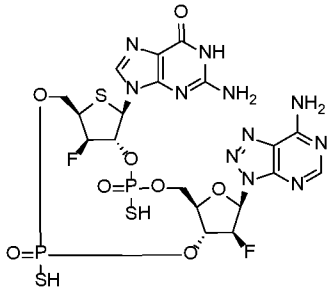
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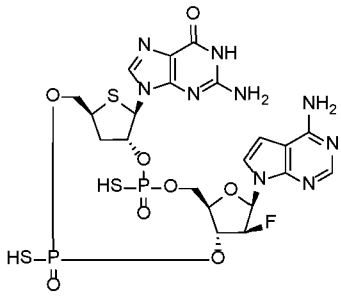
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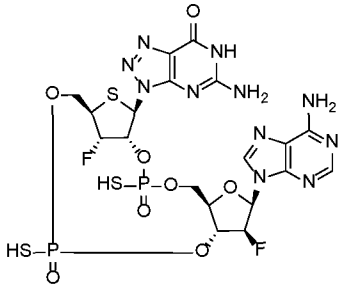
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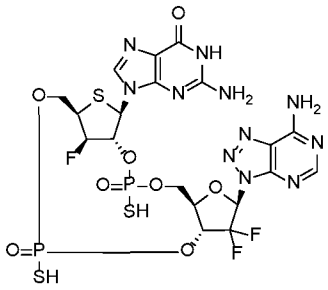
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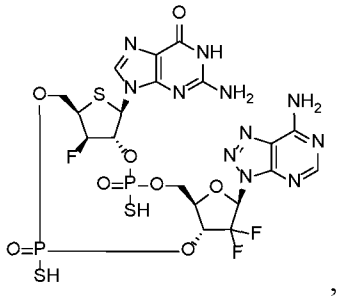
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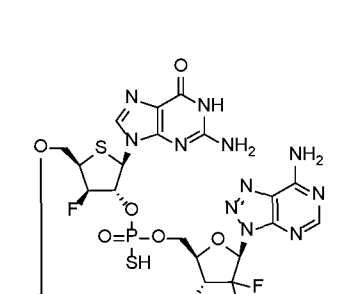
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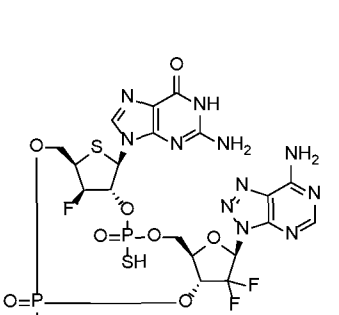
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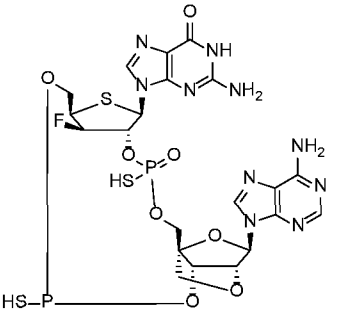
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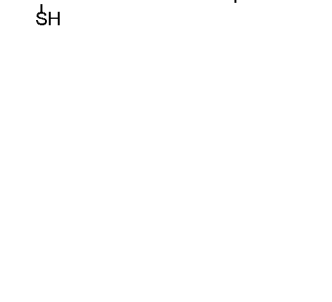
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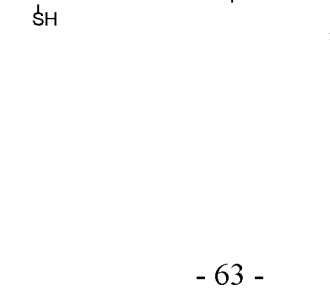
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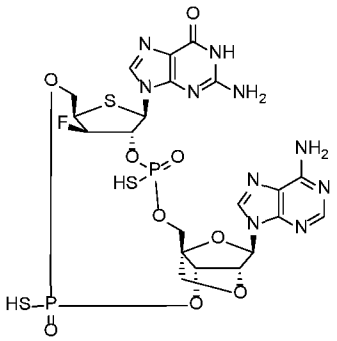
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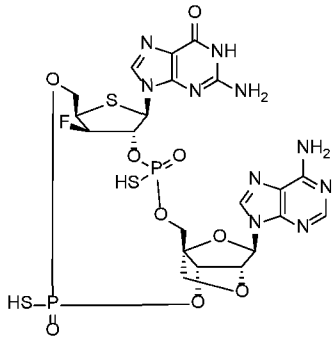
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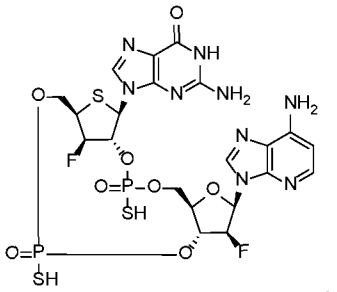
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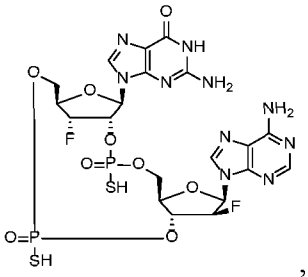
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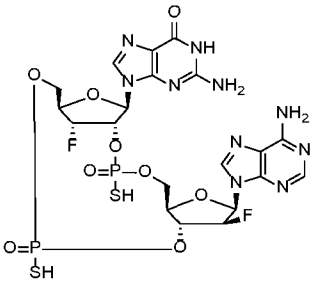
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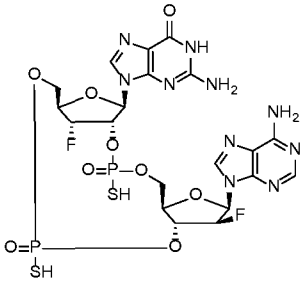
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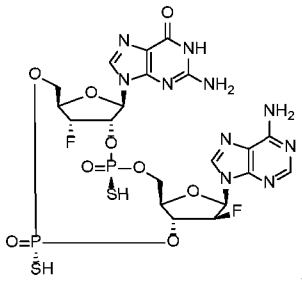
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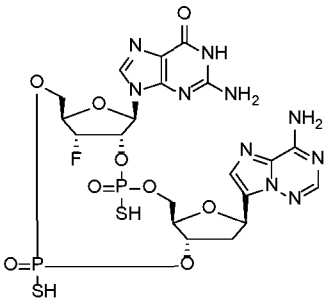
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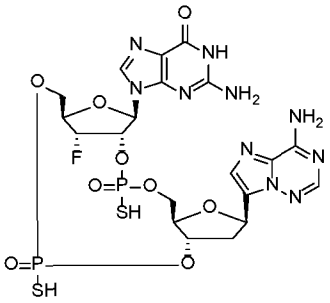
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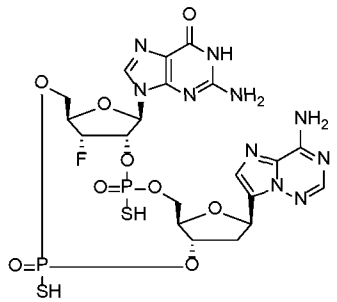
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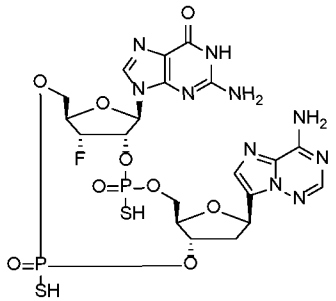
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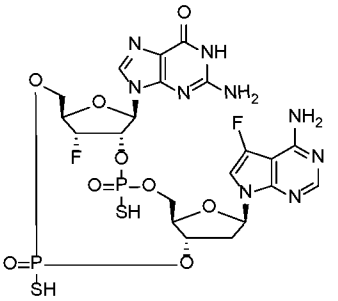
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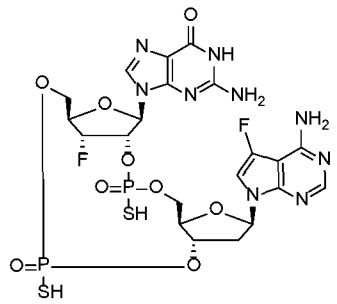
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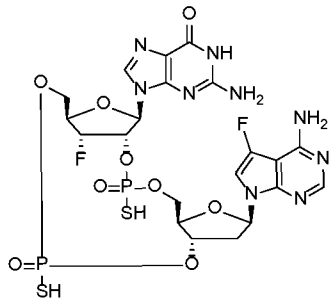
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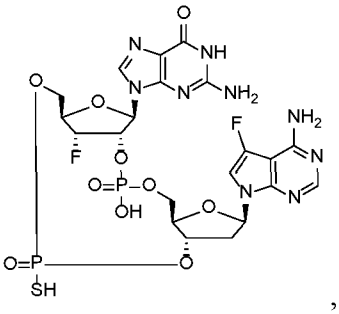
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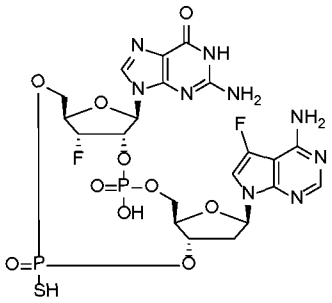
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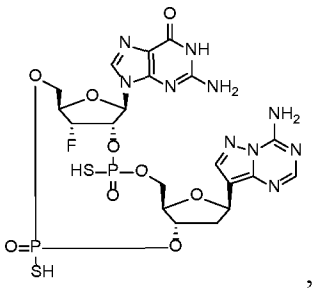
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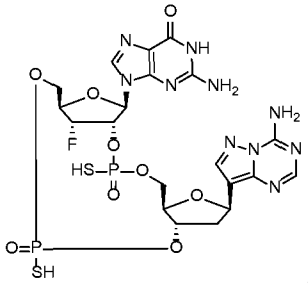
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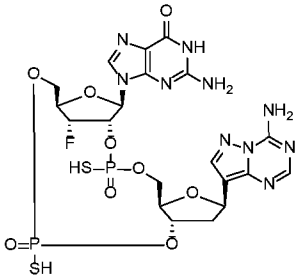
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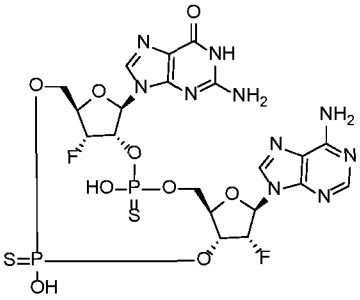
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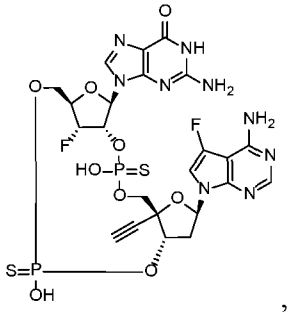
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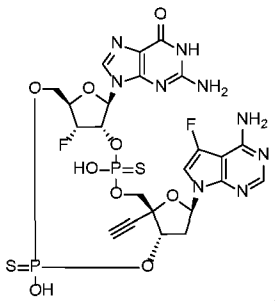
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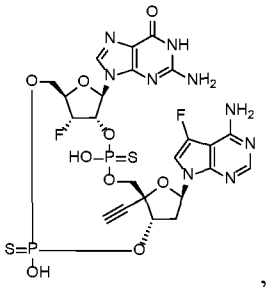
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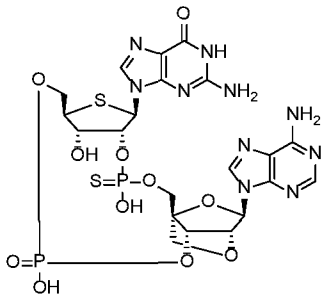
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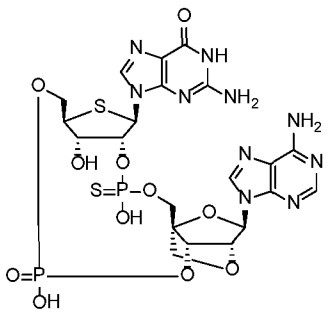
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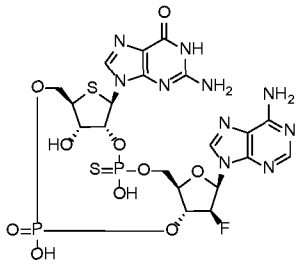
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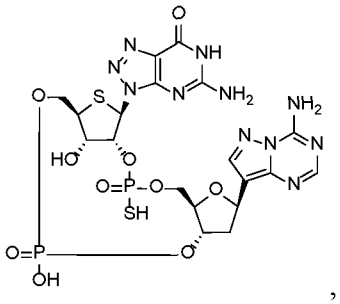
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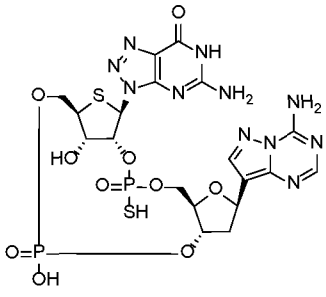
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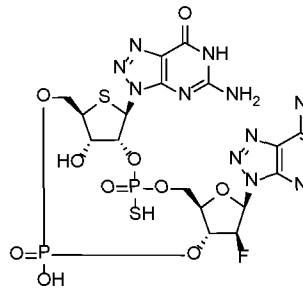
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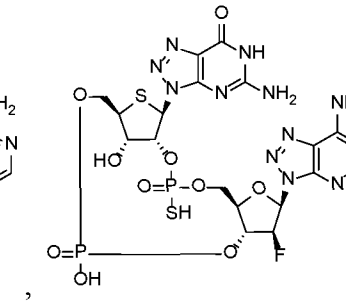
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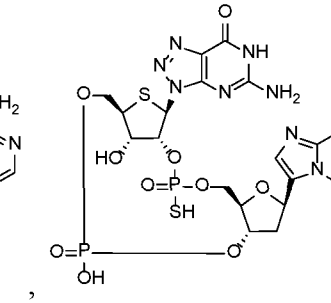
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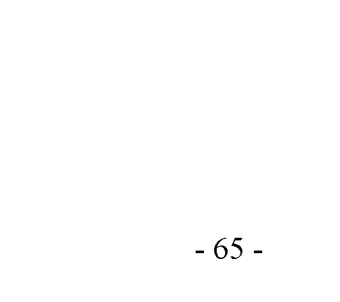
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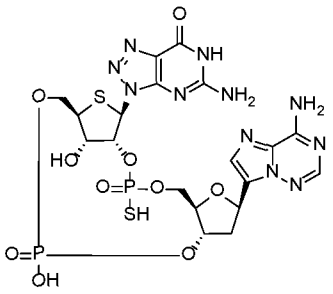
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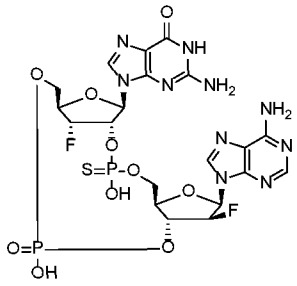
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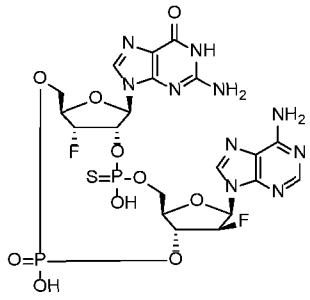
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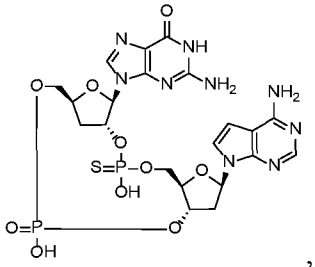
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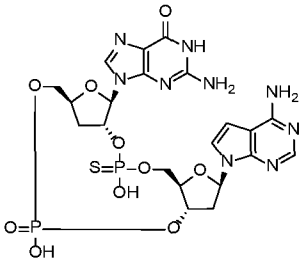
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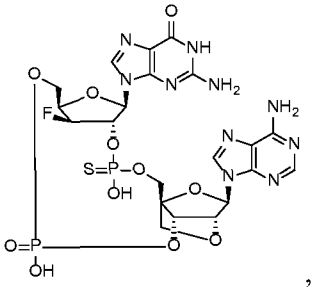
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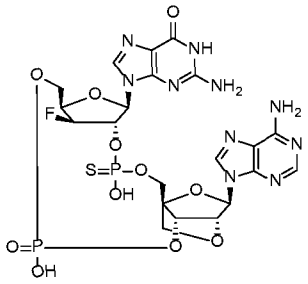
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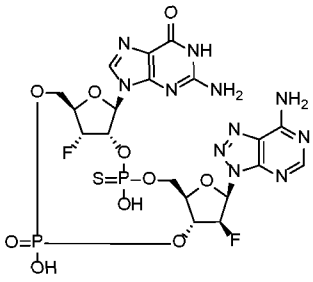
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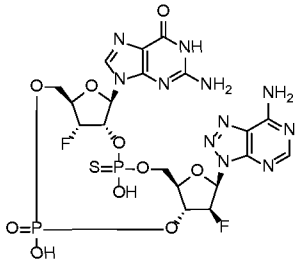
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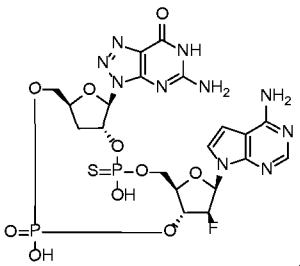
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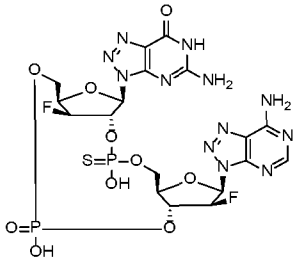
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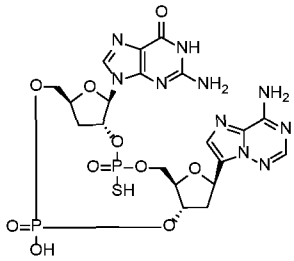
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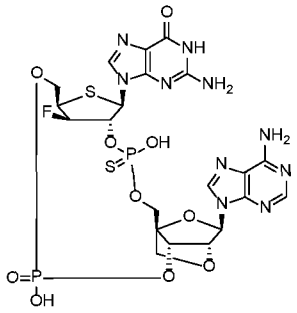
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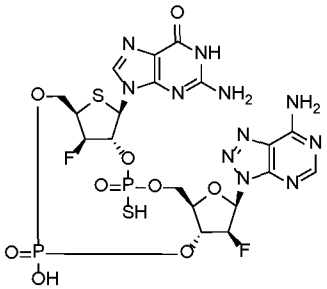
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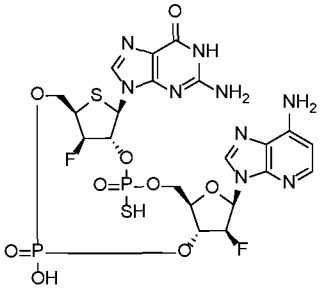
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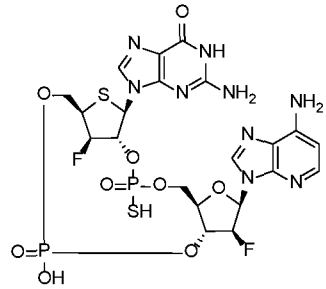
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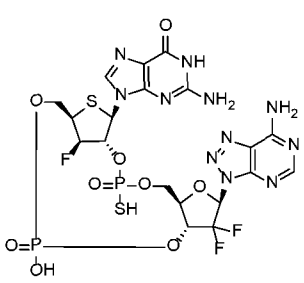
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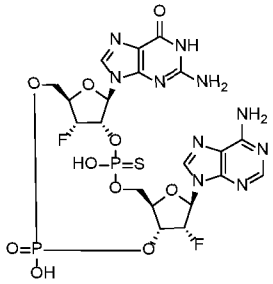
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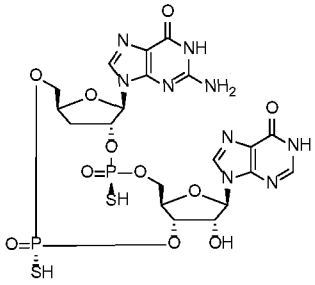
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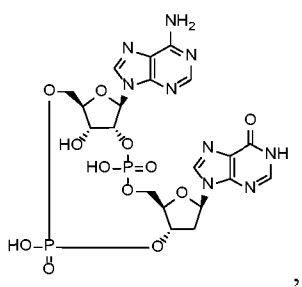
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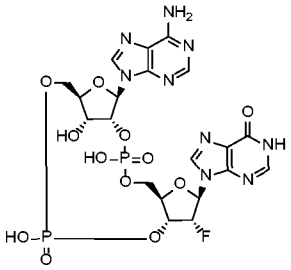
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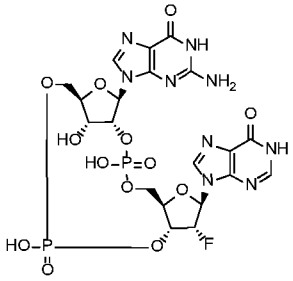
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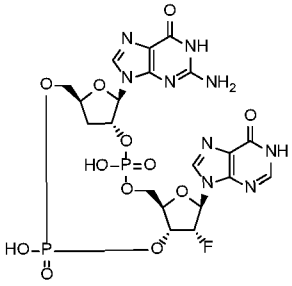
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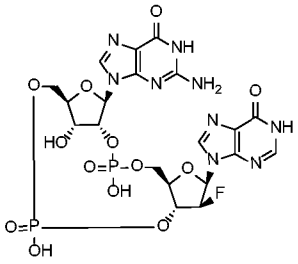
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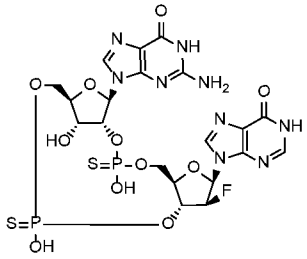
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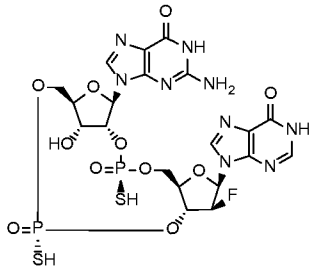
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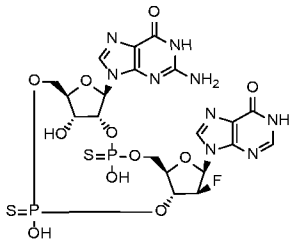
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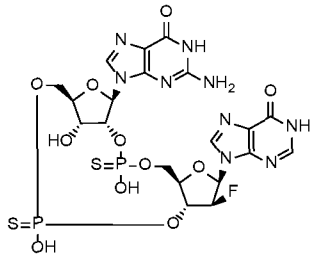
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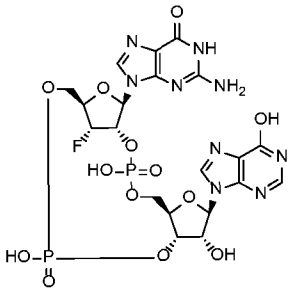
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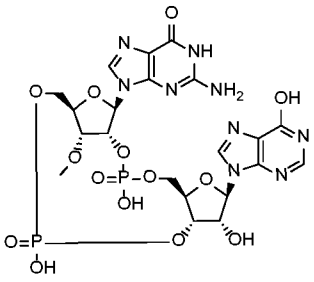
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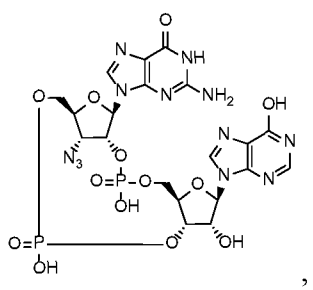
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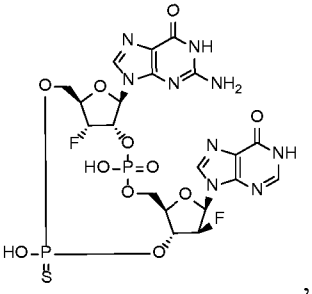
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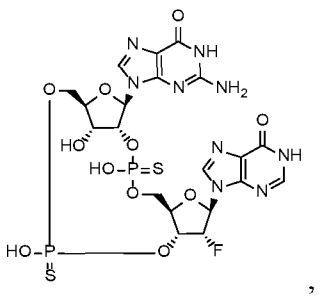
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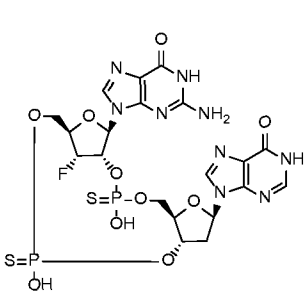
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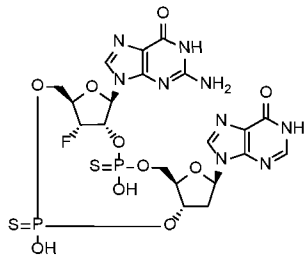
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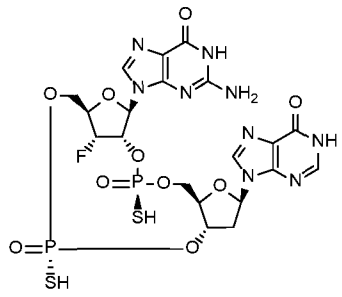
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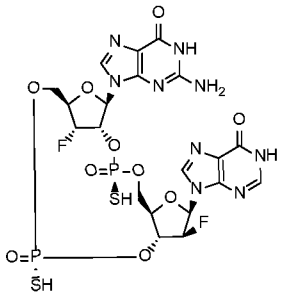
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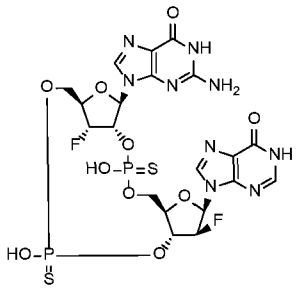
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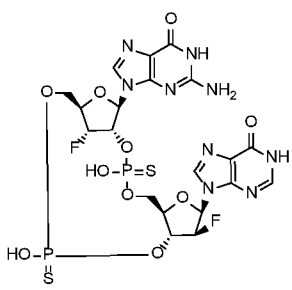
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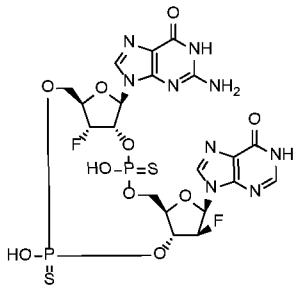
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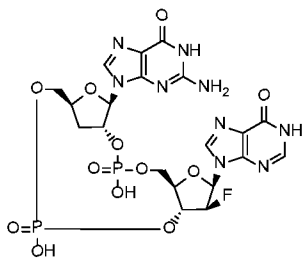
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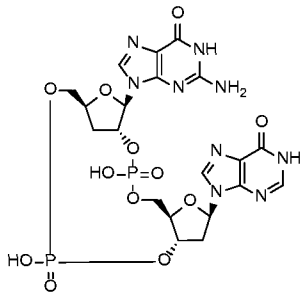
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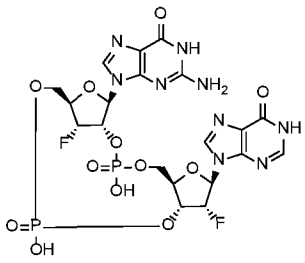
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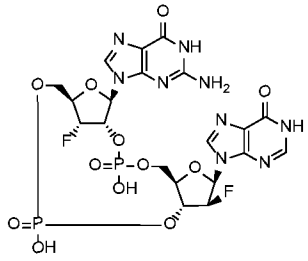
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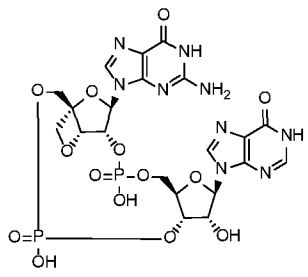
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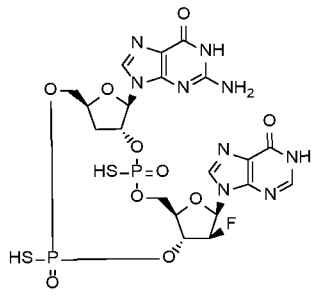
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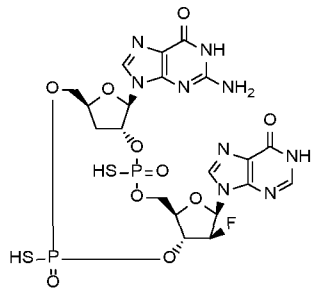
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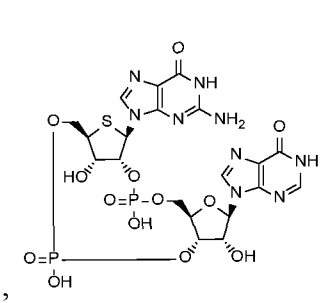
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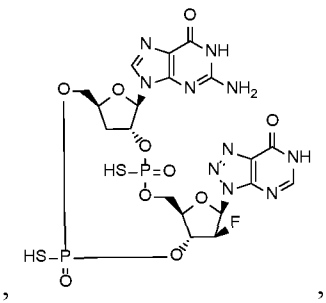
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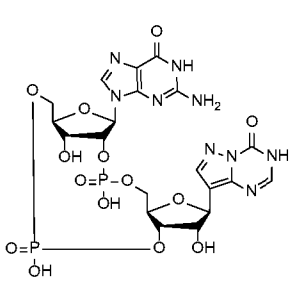
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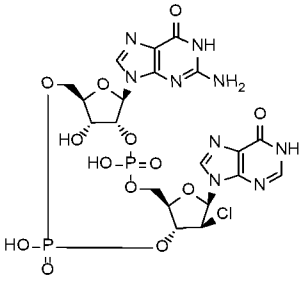
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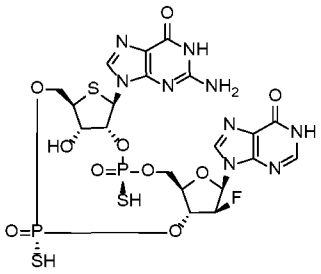
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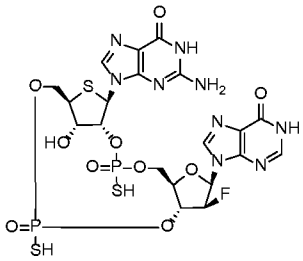
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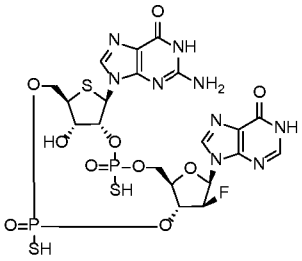
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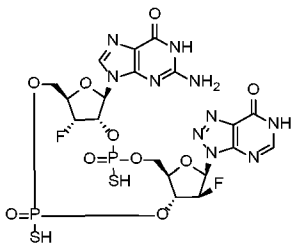
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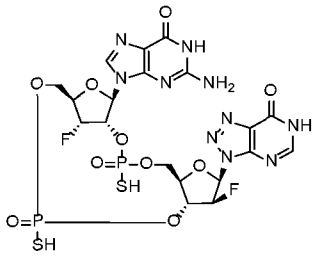
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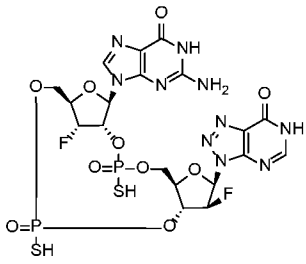
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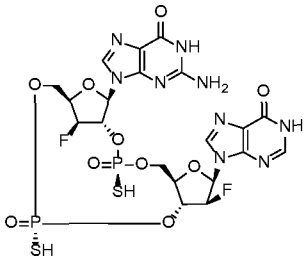
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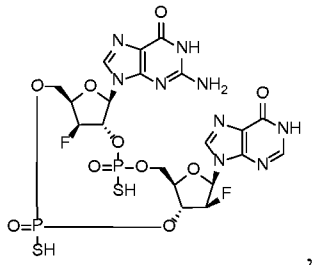
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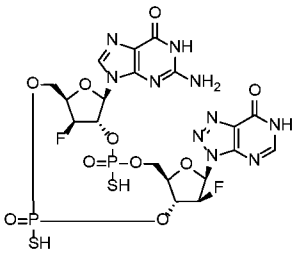
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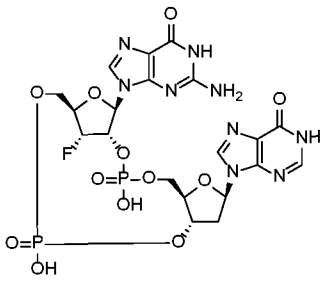
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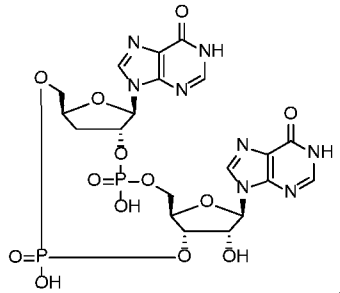
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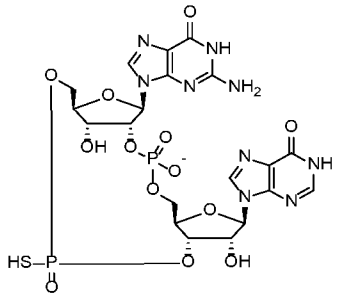
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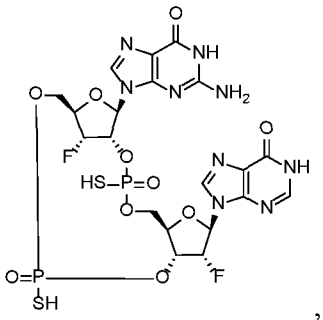
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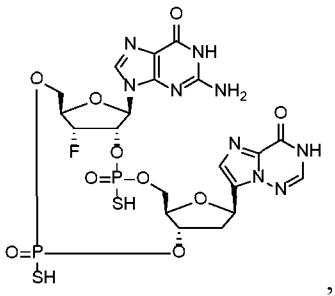
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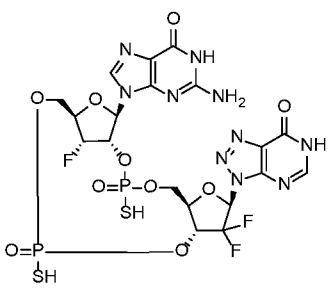
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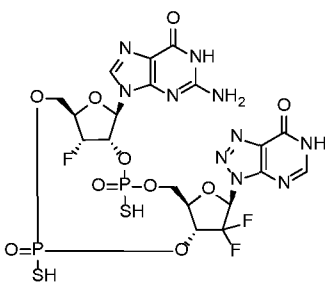
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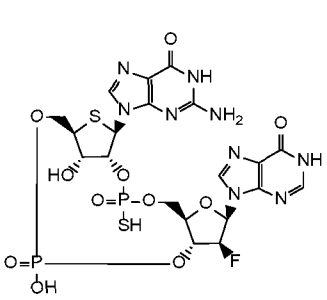
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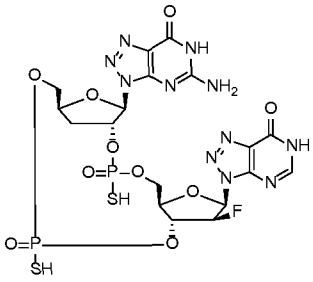
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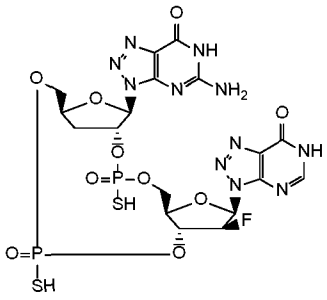
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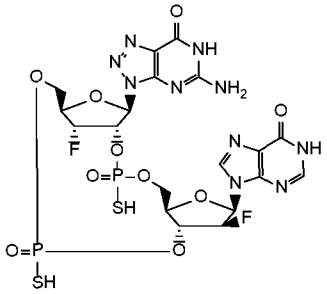
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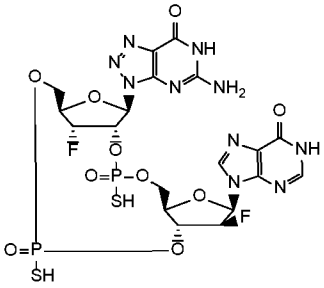
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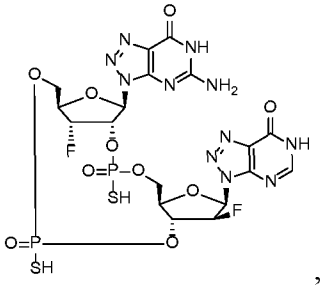
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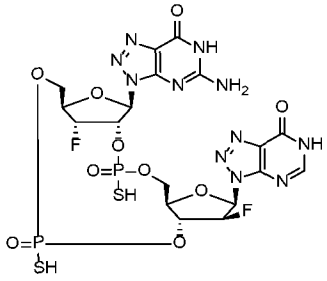
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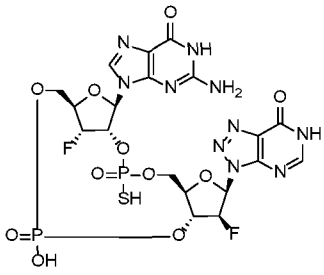
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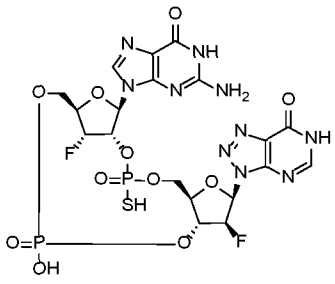
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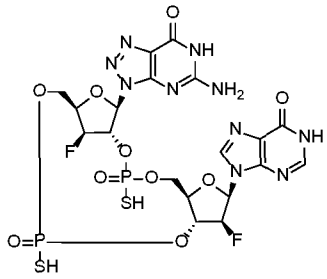
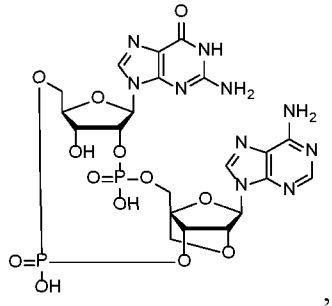

, and pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof. In aspects of this embodiment, the compound is selected from the group consisting of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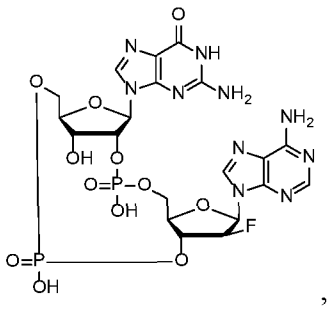
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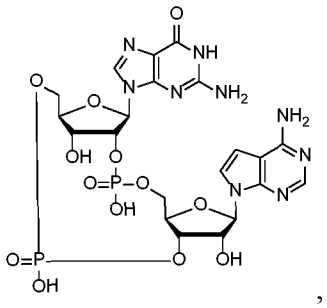
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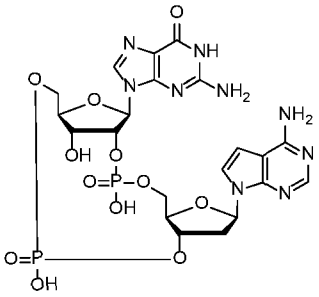
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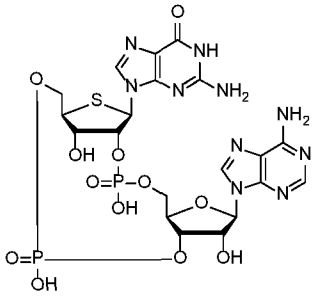
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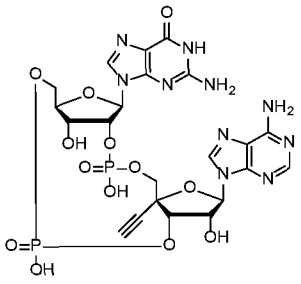
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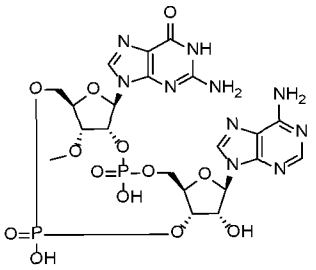
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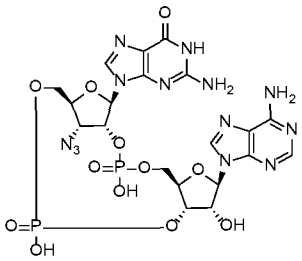
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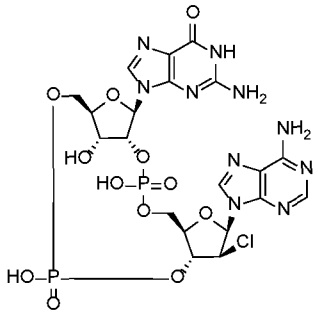
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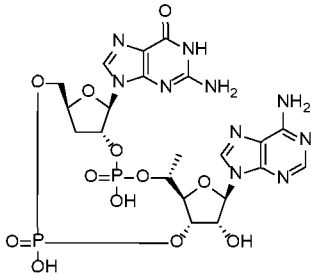
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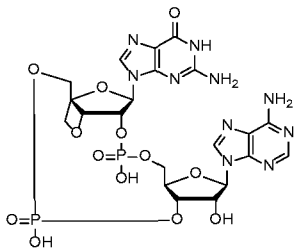
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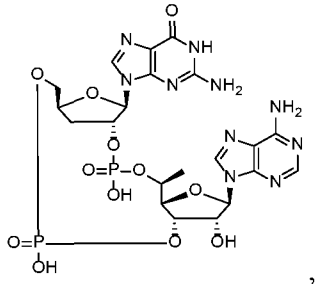
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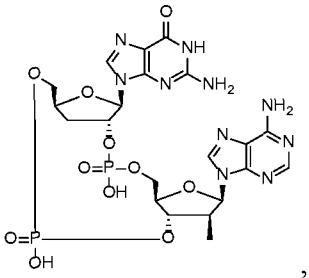
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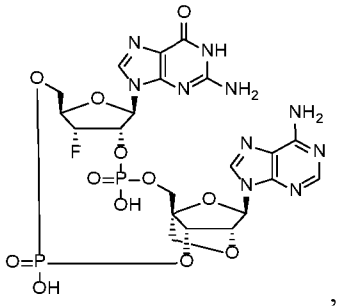
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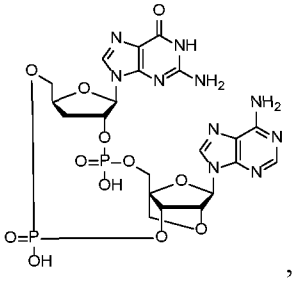
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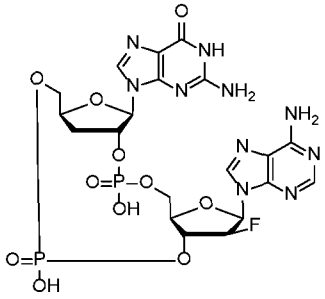
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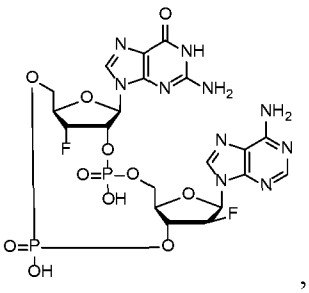
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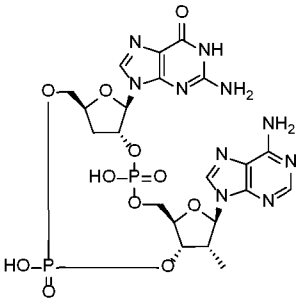
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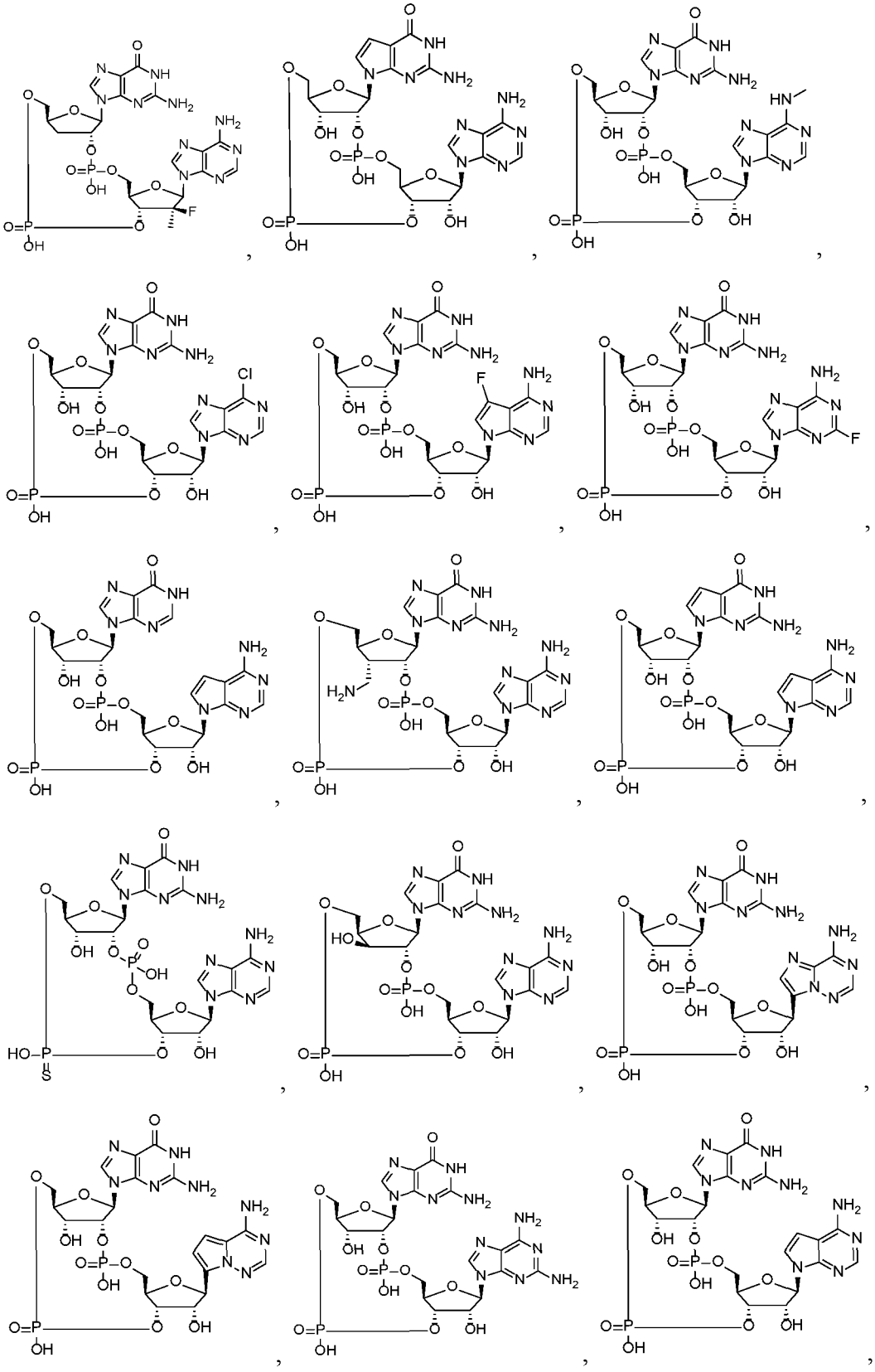

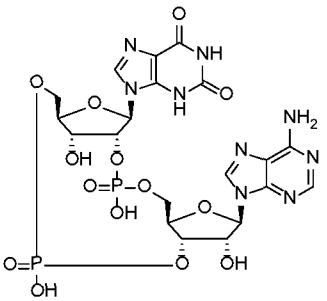
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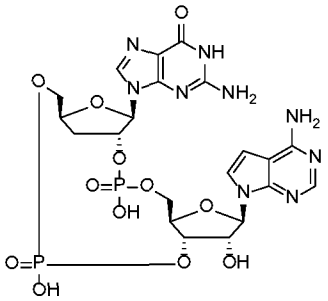
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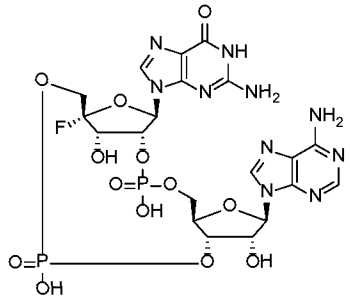
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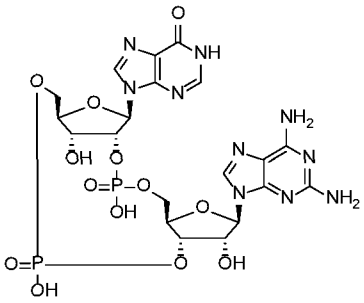
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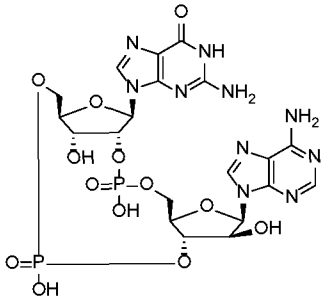
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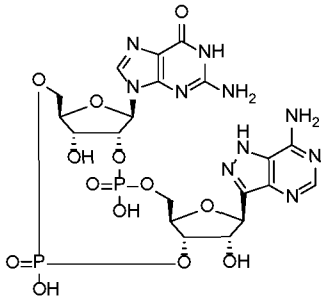
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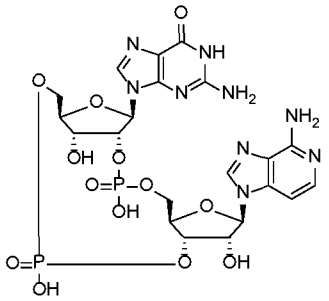
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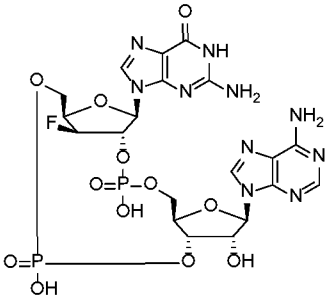
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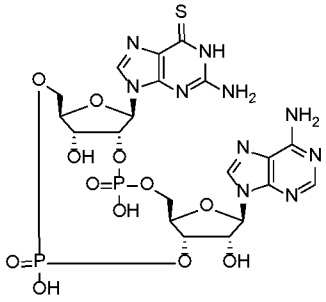
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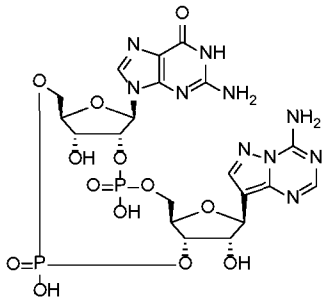
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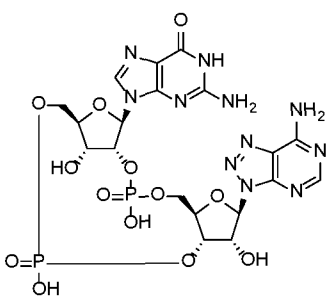
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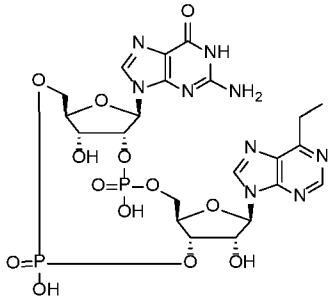
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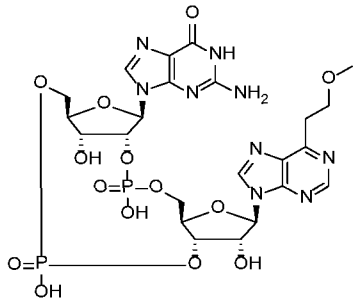
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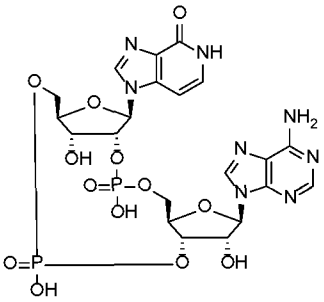
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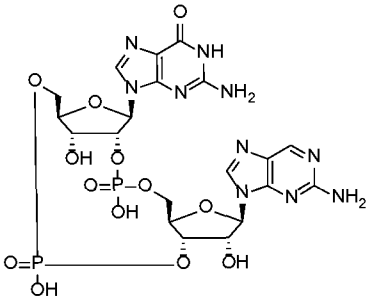
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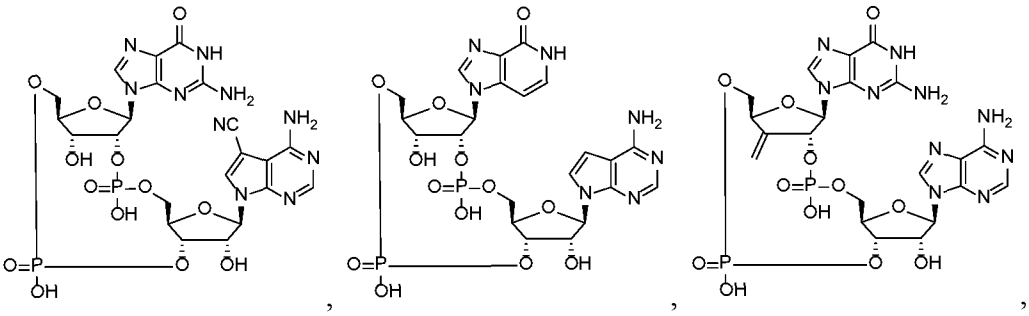
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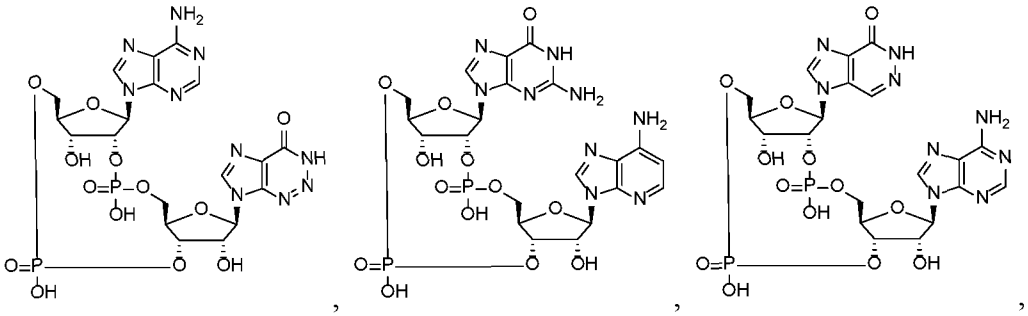
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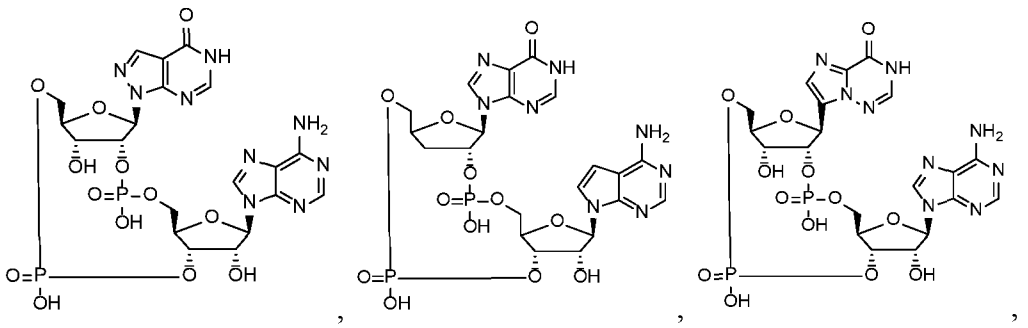
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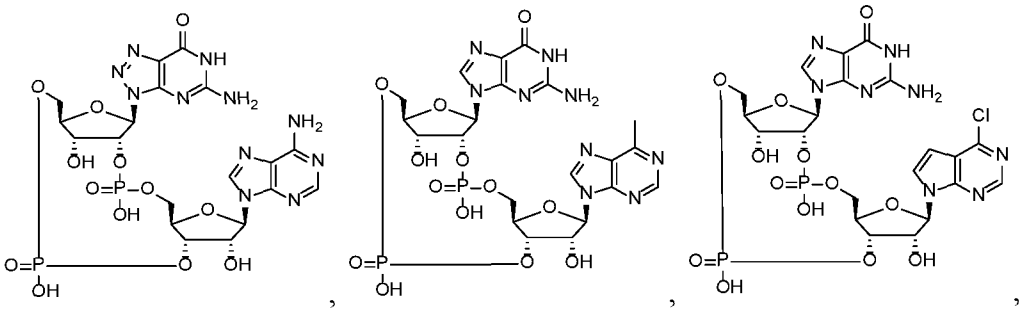
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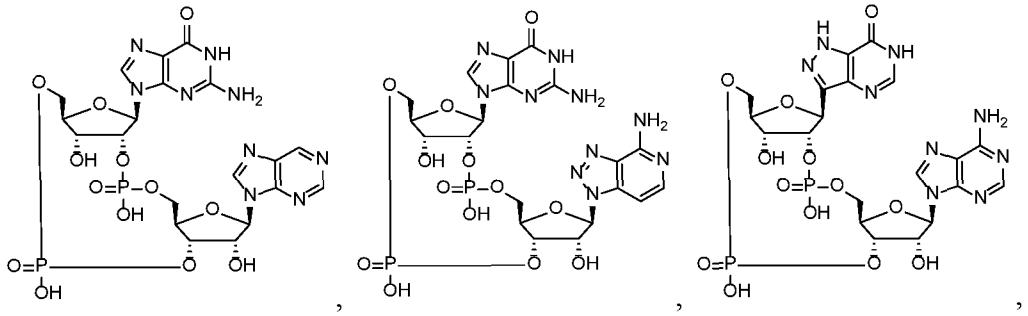
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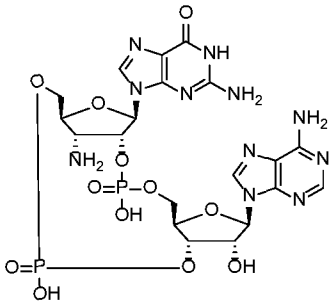
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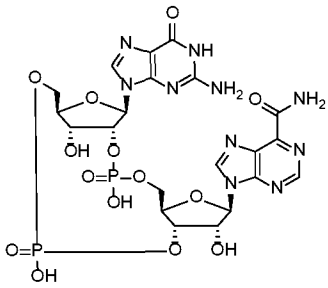
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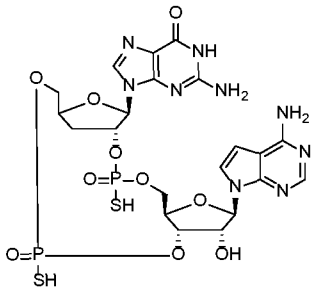
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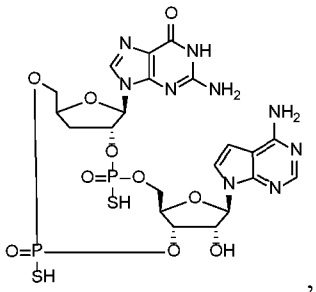
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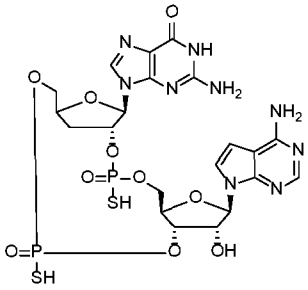
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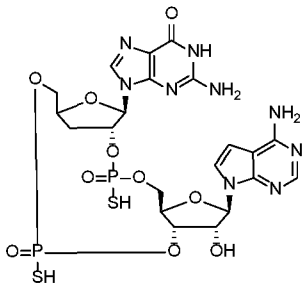
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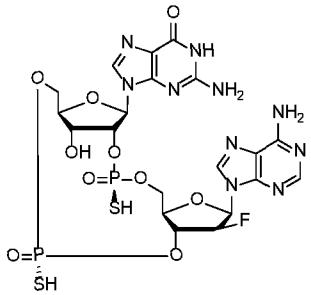
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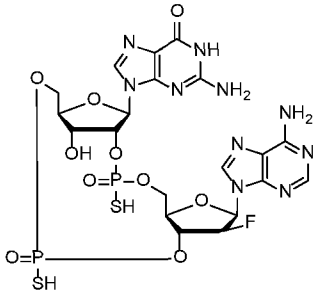
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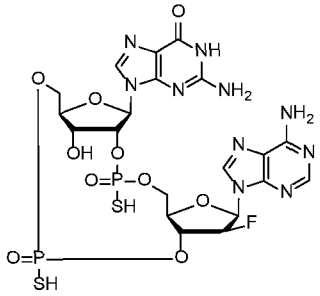
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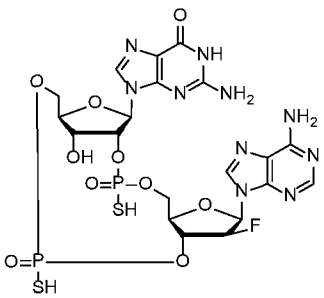
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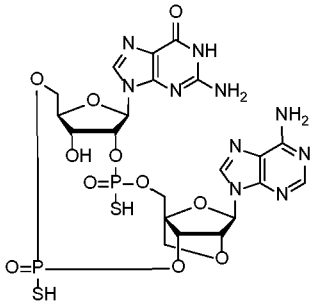
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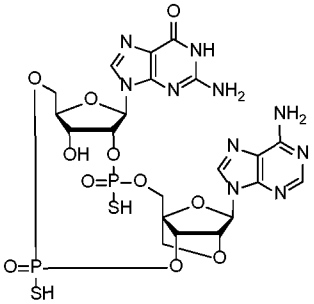
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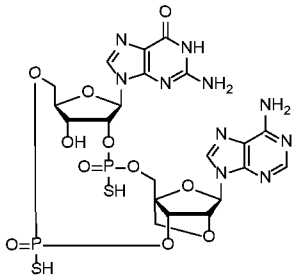
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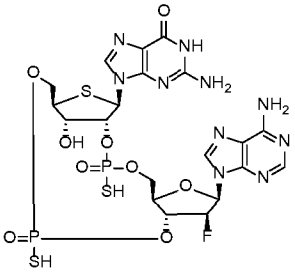
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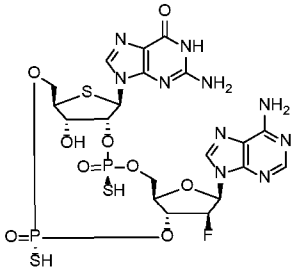
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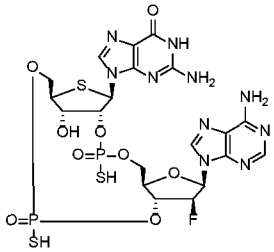
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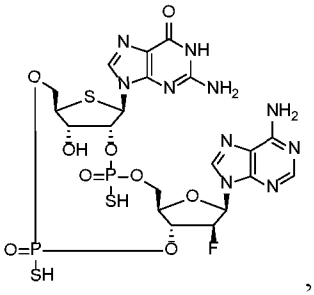
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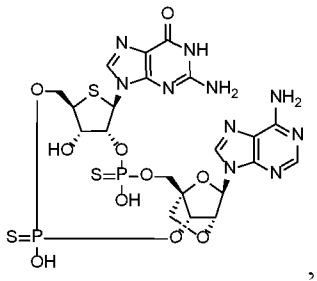
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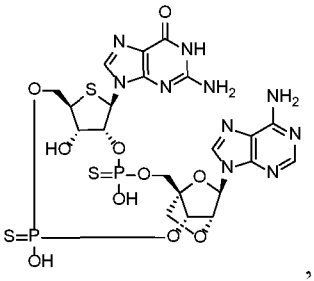
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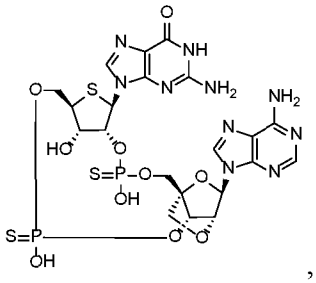
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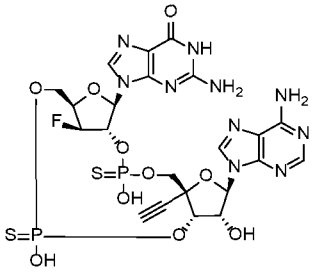
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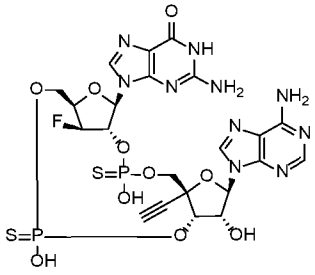
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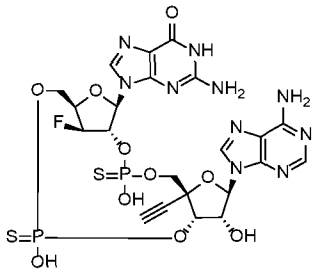
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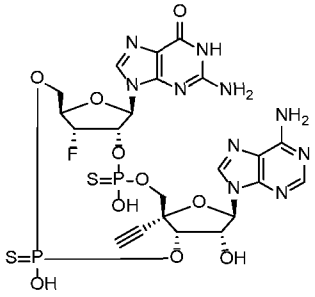
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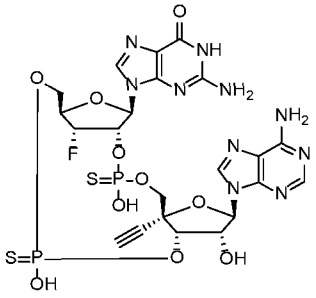
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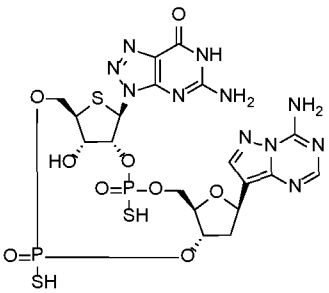
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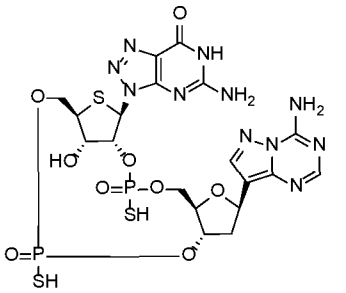
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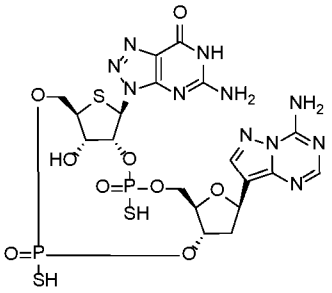
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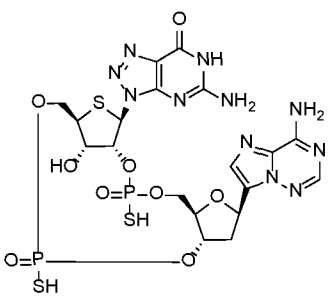
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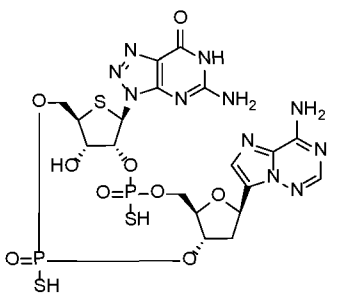
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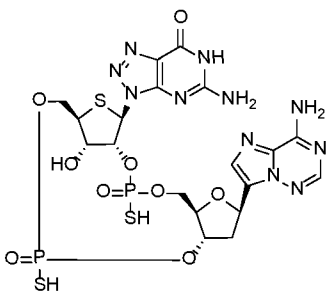
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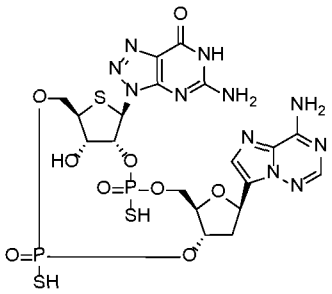
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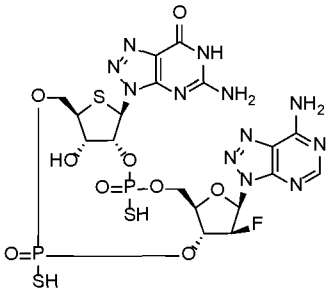
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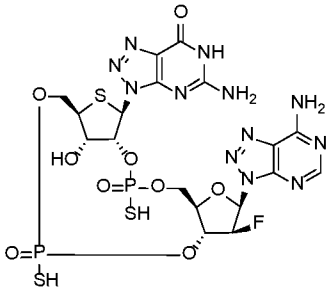
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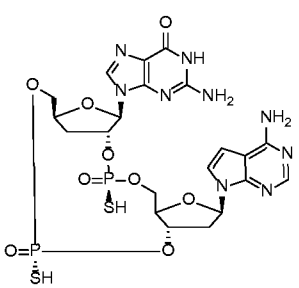
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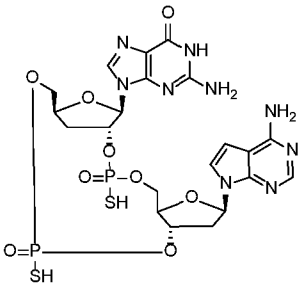
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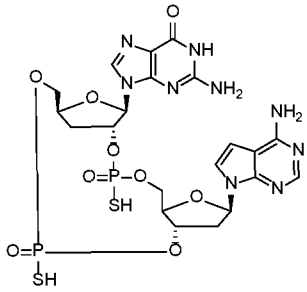
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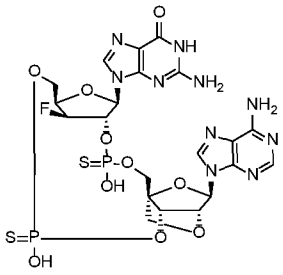
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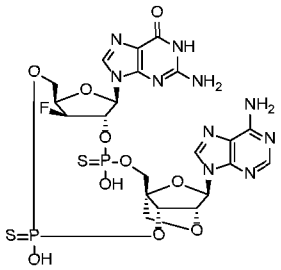
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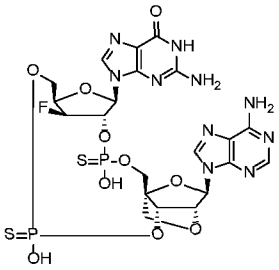
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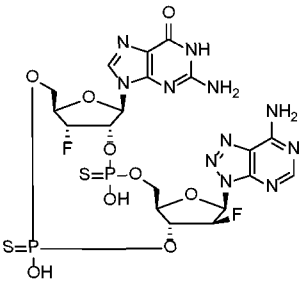
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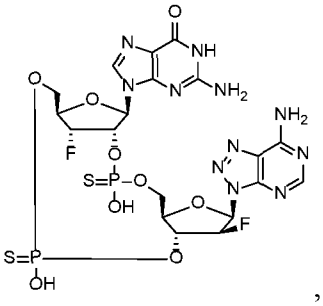
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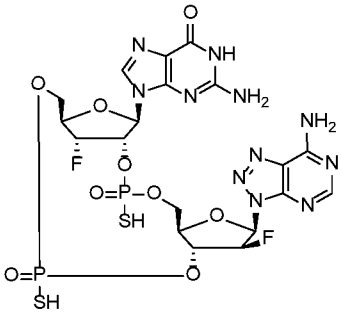
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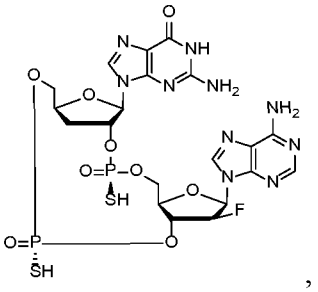
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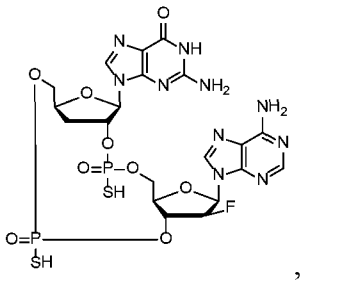
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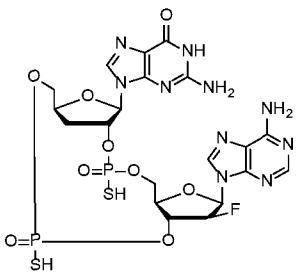
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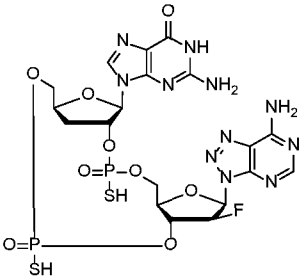
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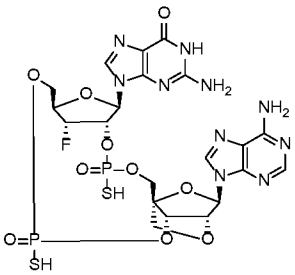
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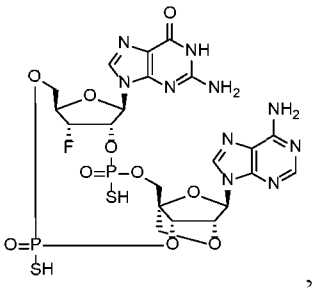
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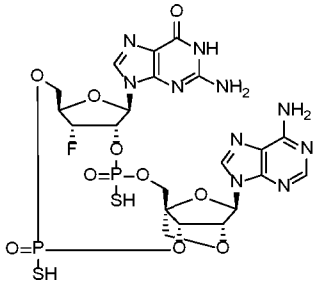
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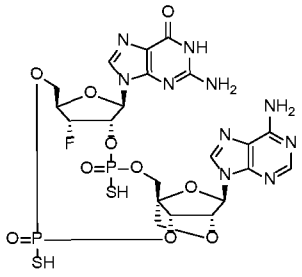
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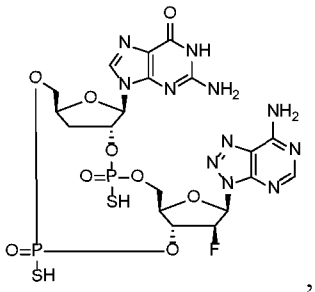
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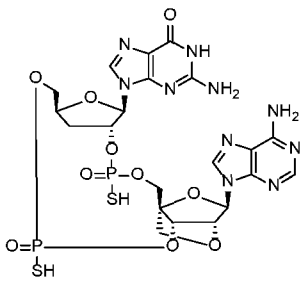
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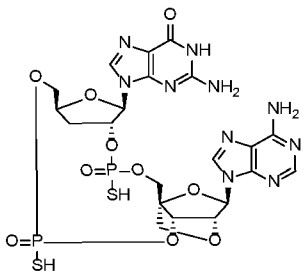
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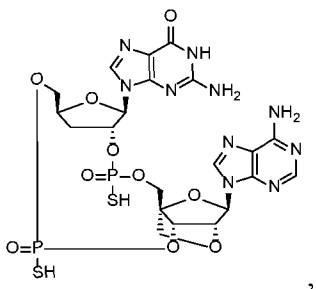
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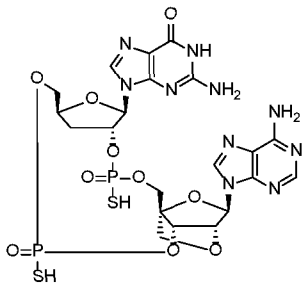
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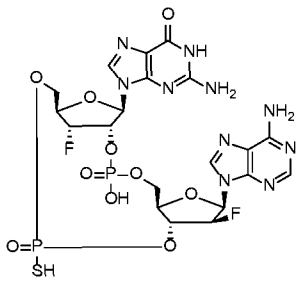
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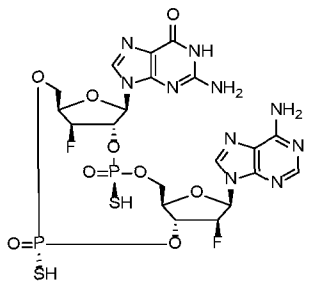
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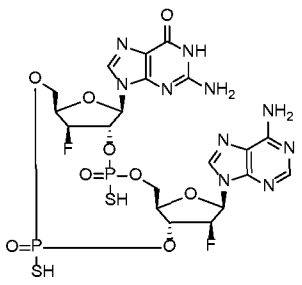
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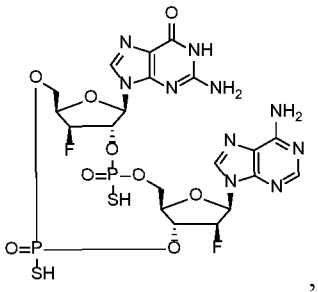
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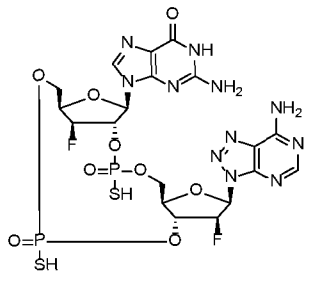
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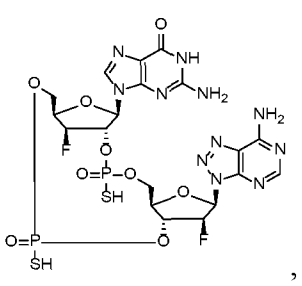
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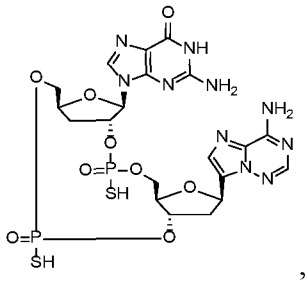
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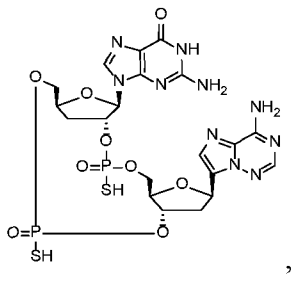
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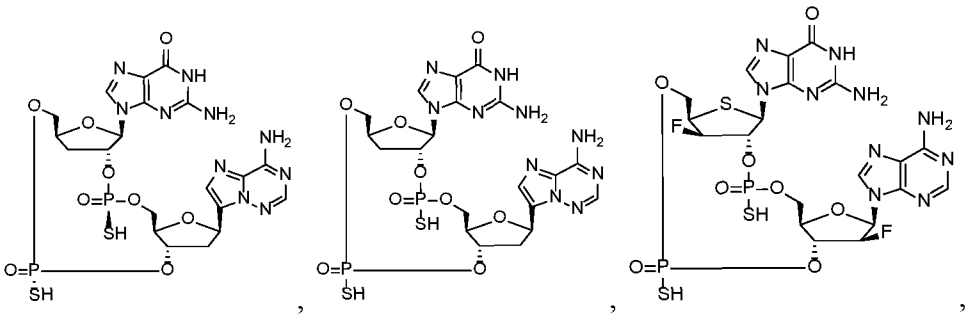
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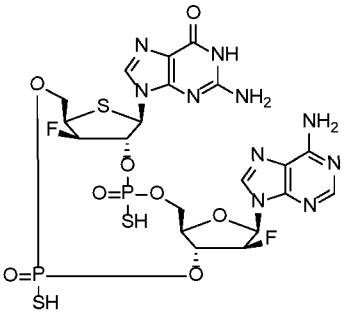
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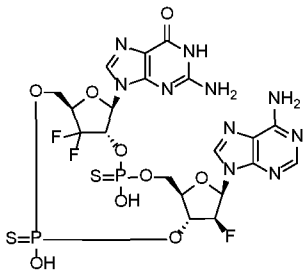
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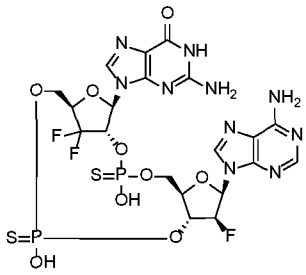
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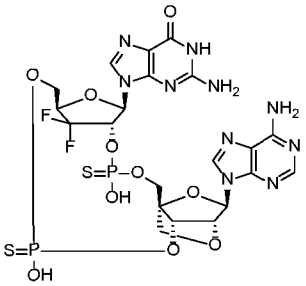
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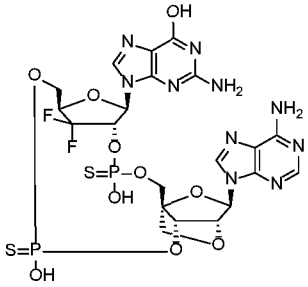
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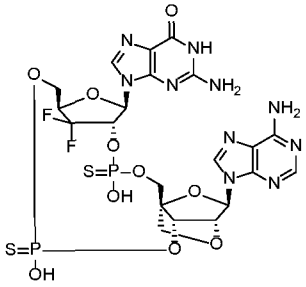
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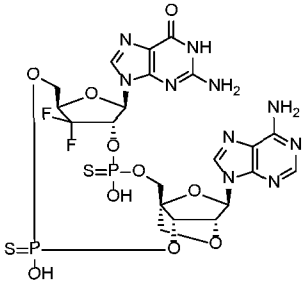
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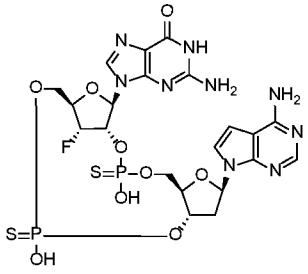
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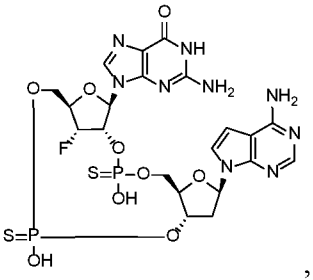
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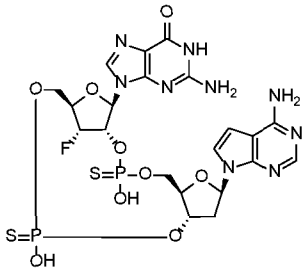
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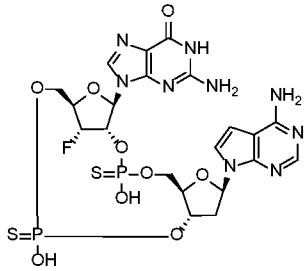
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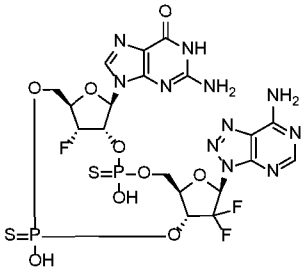
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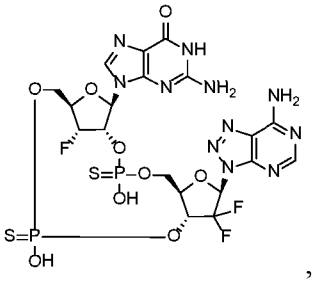
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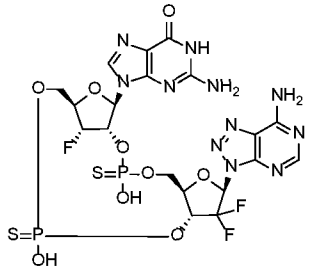
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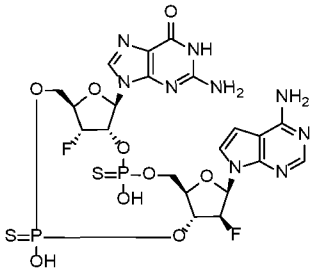
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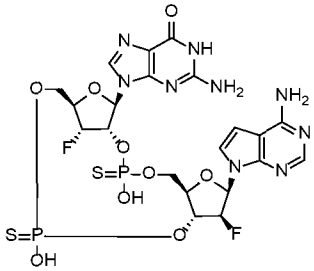
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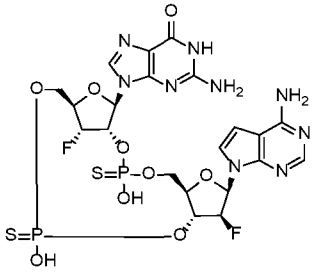
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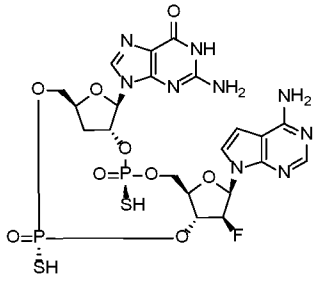
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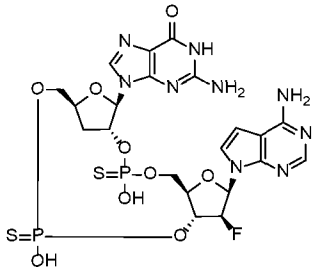
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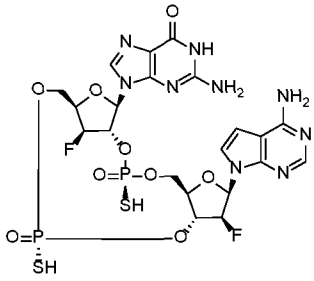
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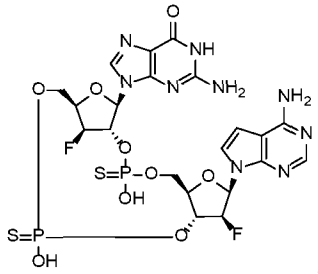
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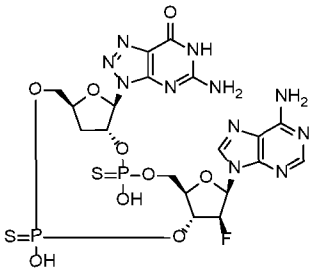
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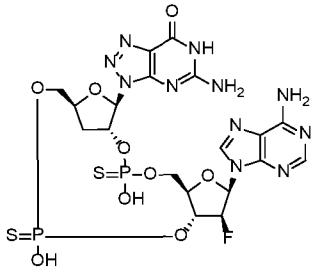
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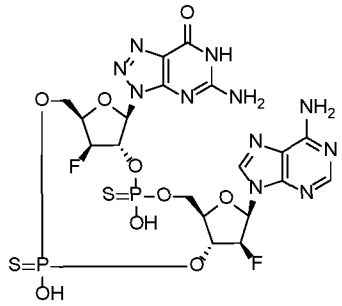
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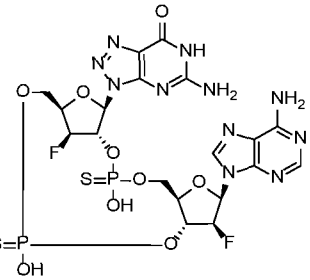
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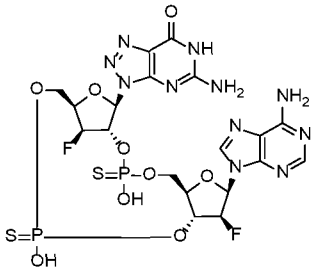
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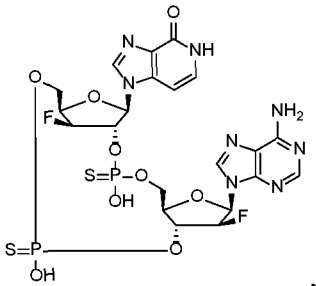
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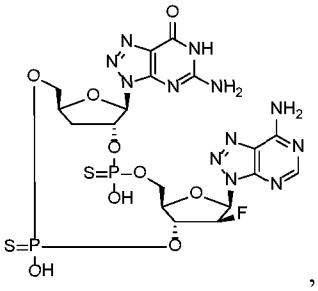
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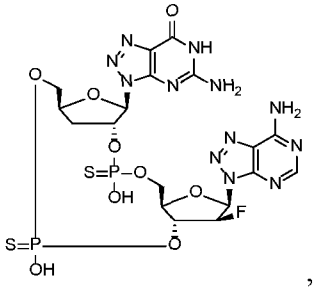
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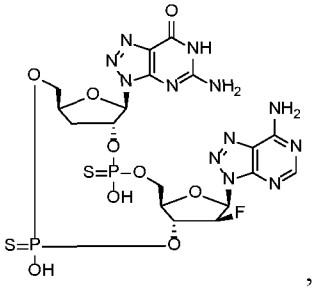
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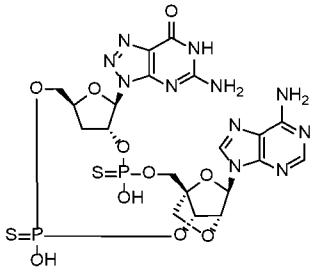
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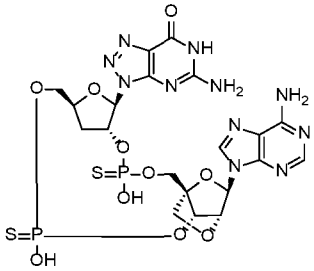
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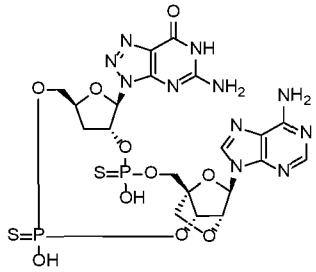
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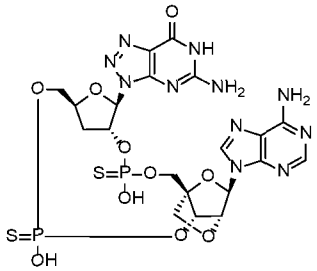
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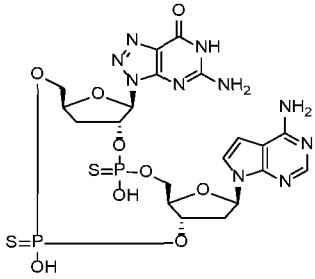
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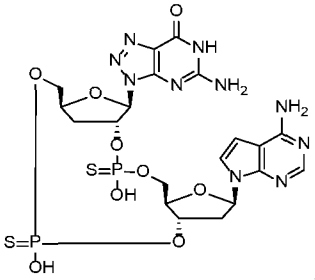
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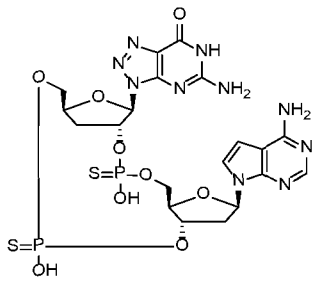
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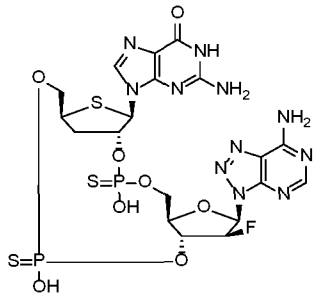
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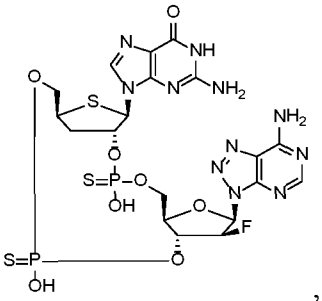
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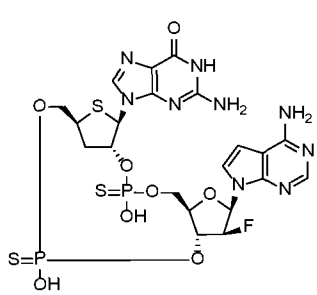
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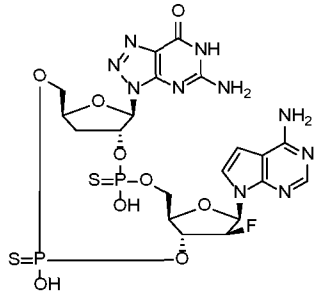
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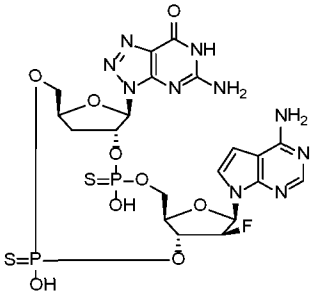
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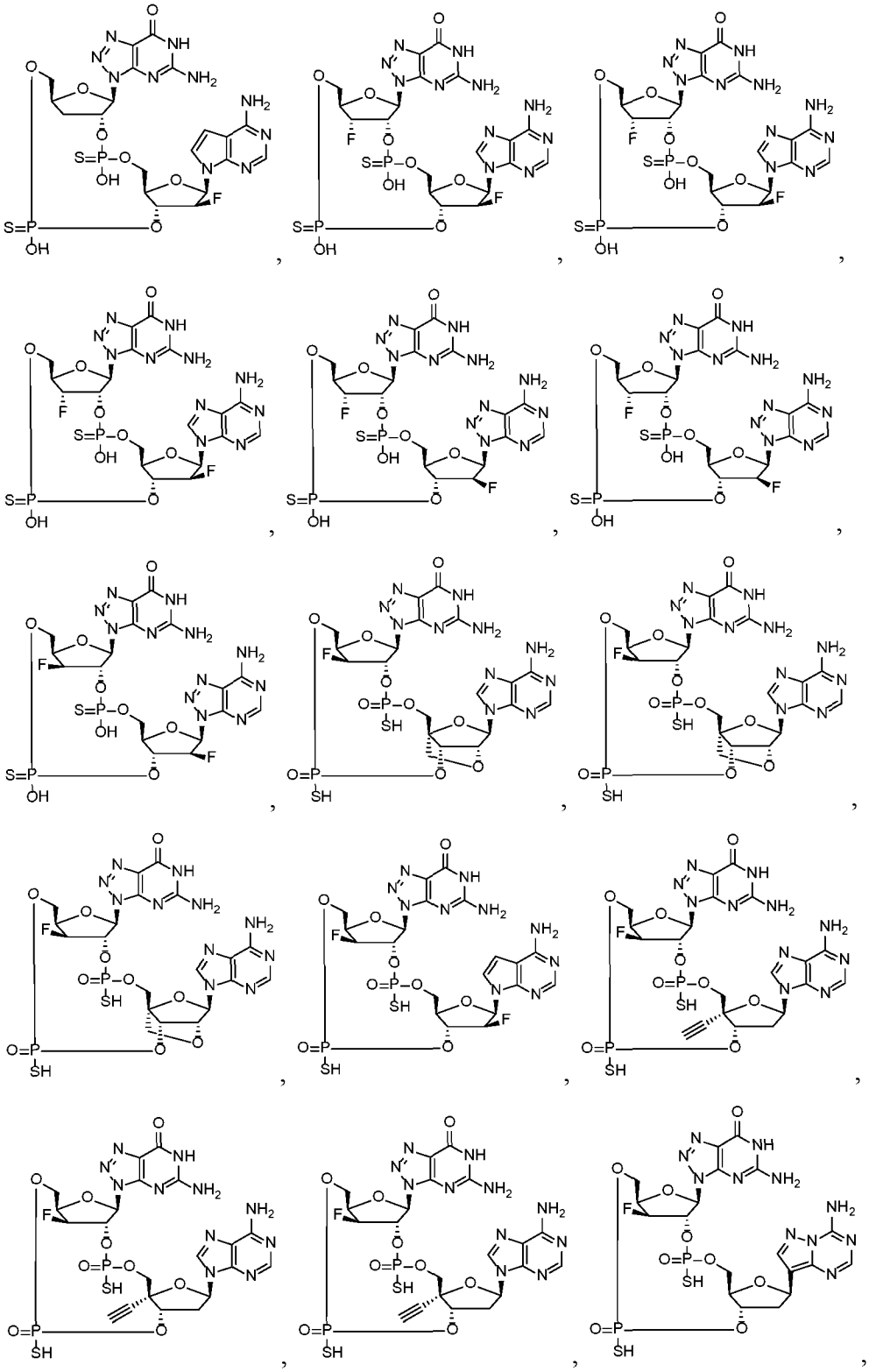

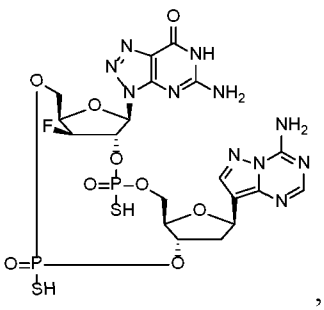
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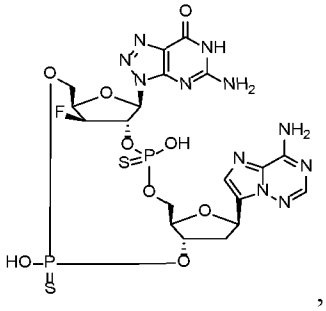
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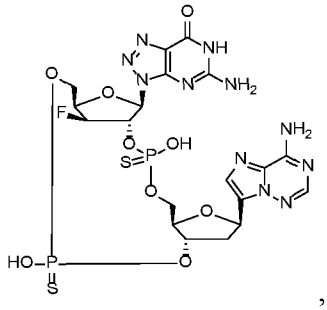
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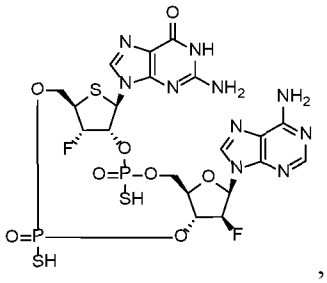
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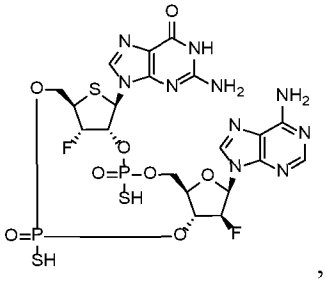
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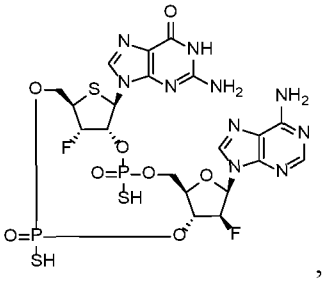
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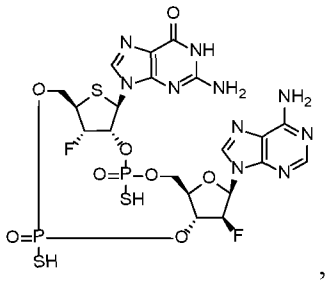
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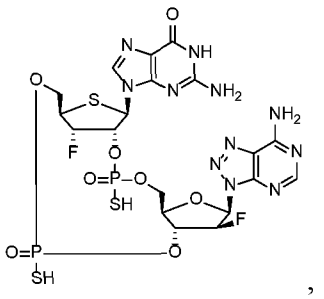
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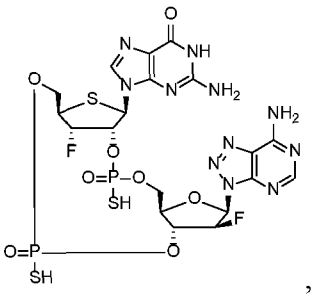
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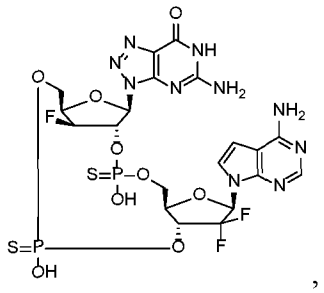
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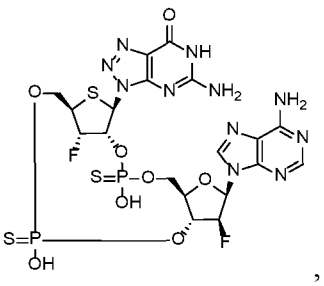
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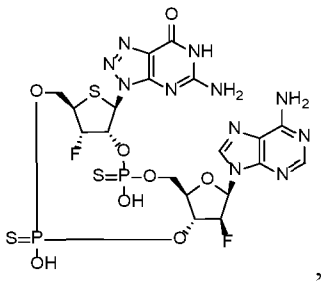
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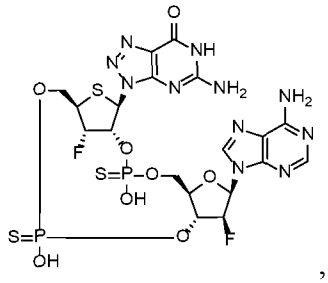
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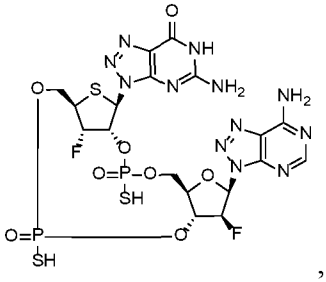
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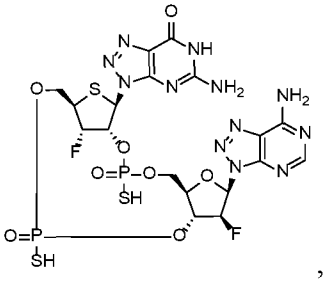
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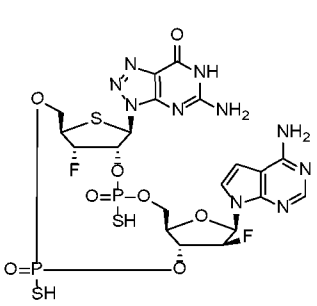
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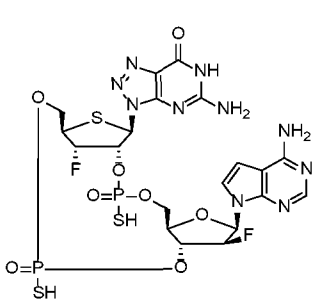
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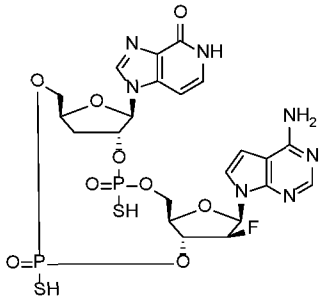
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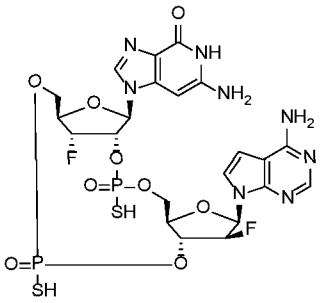
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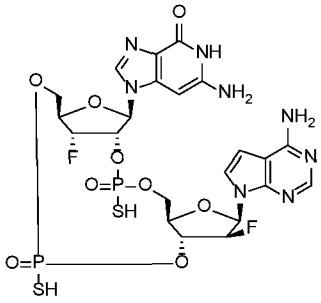
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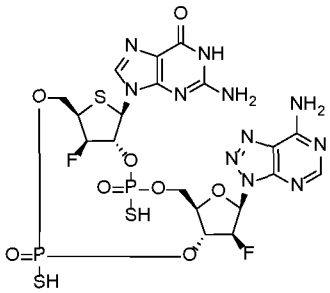
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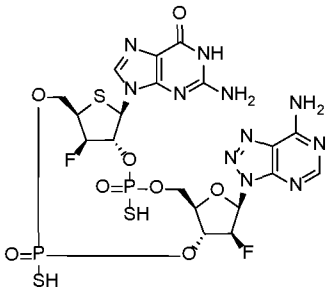
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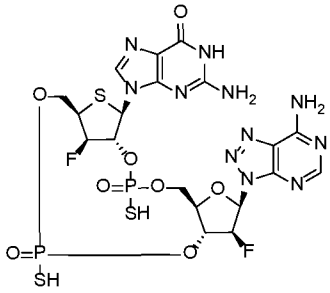
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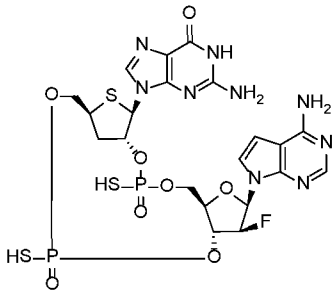
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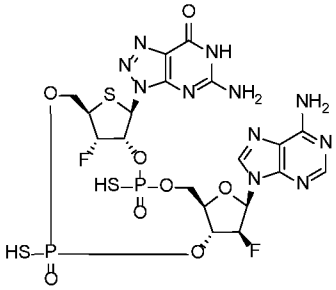
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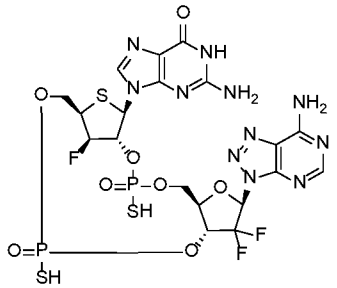
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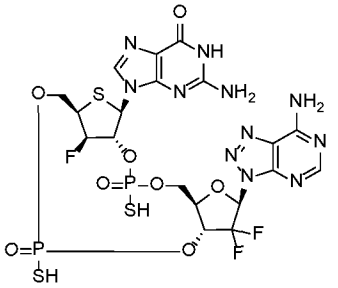
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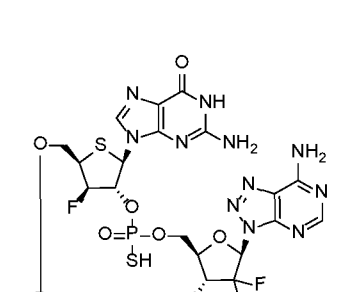
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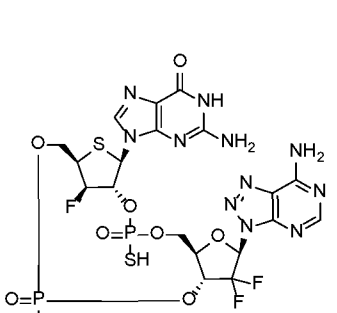
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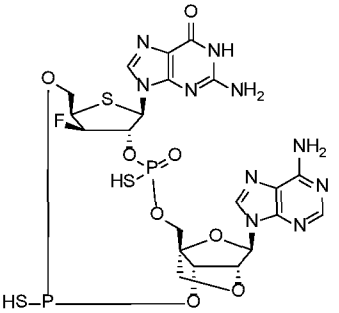
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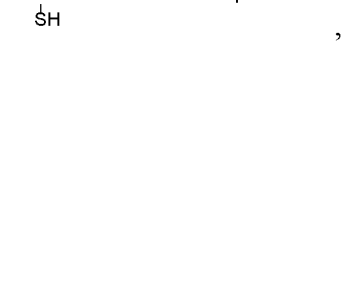
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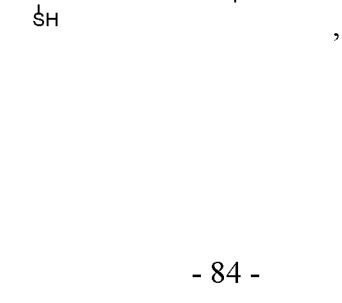
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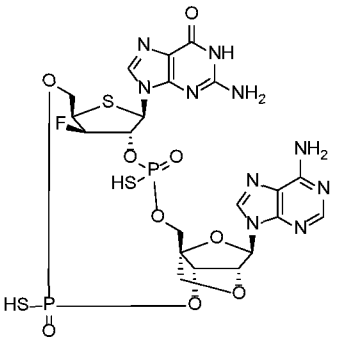
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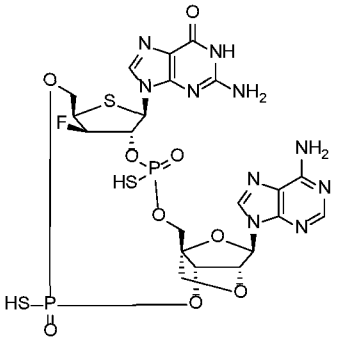
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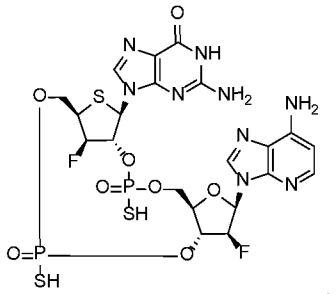
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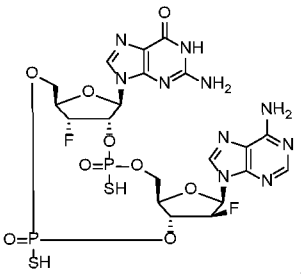
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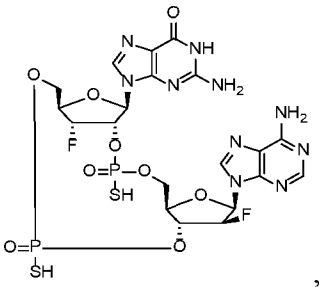
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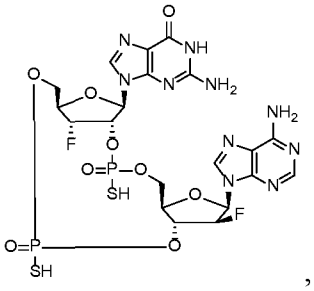
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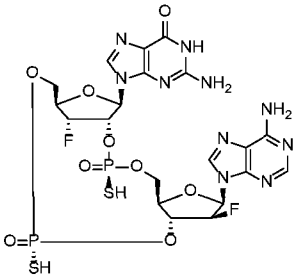
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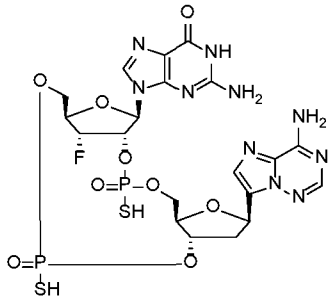
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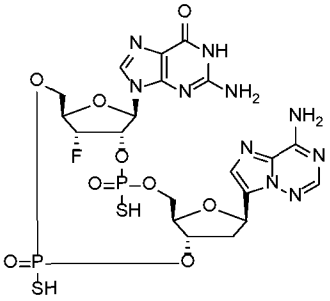
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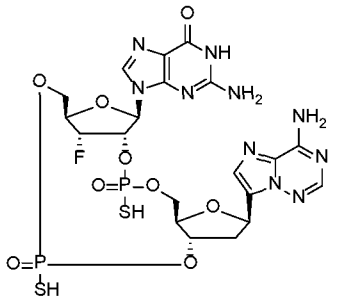
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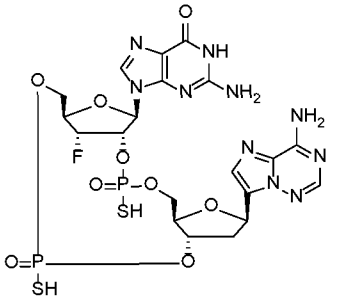
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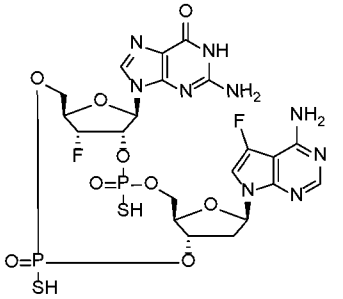
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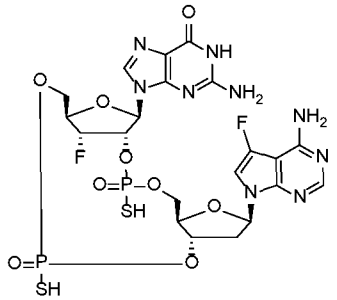
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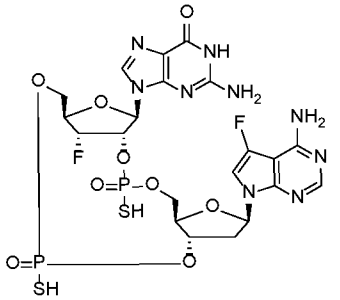
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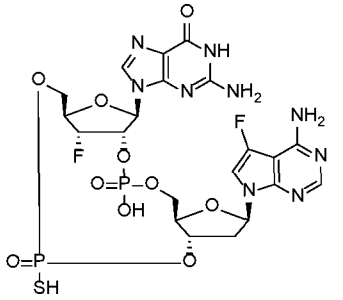
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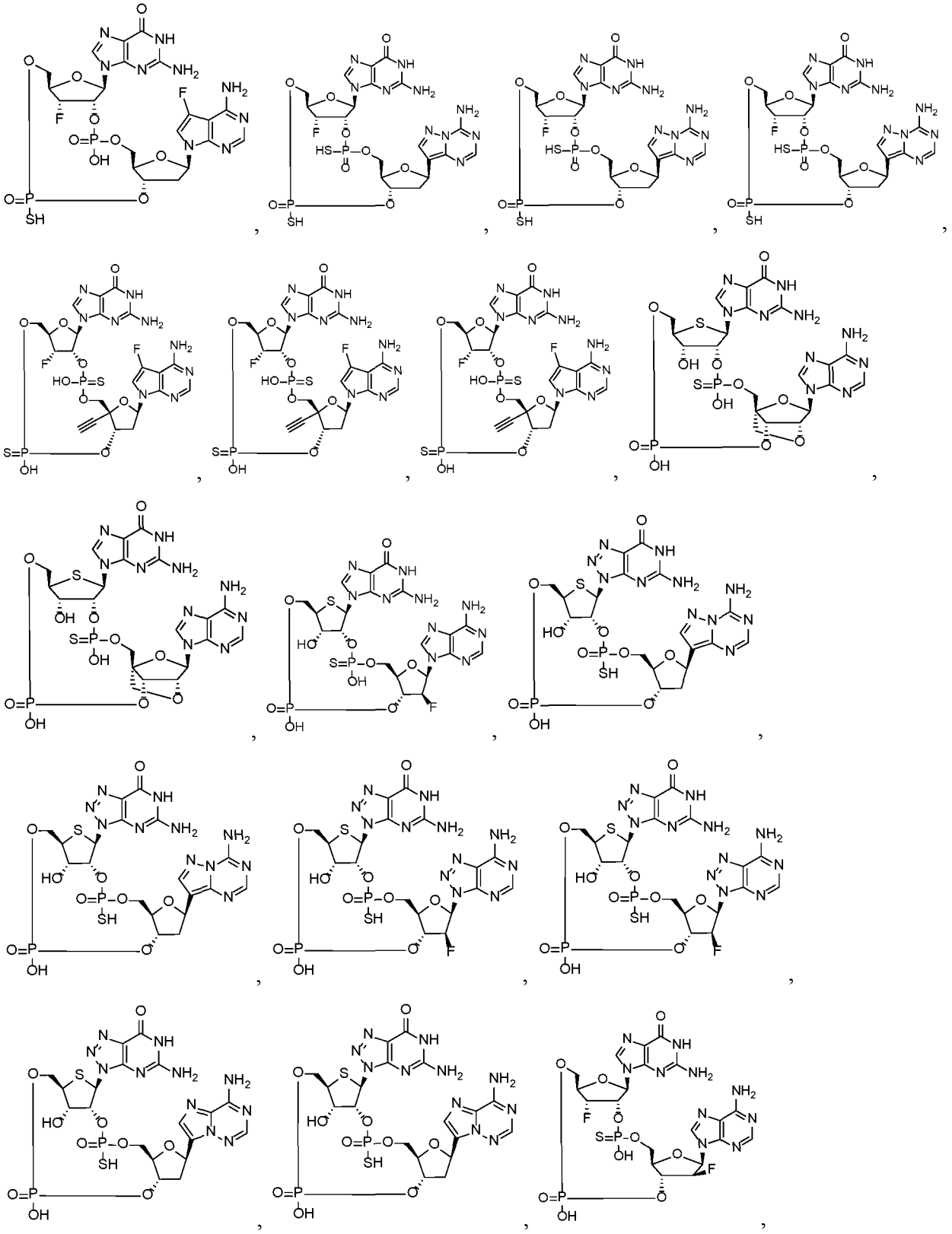
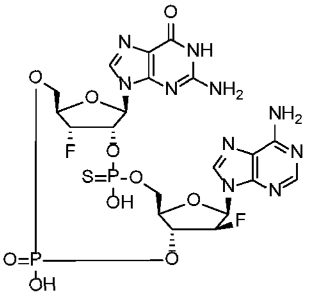
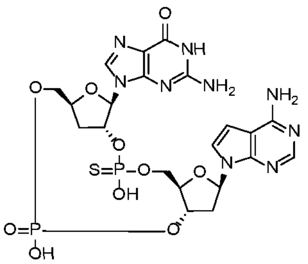
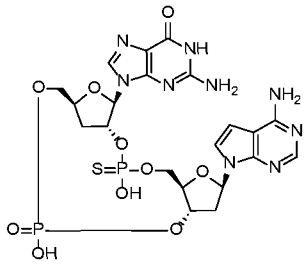
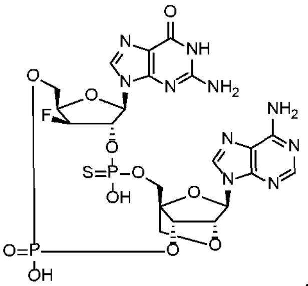
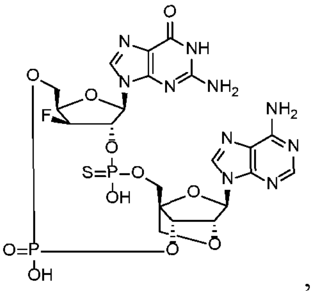
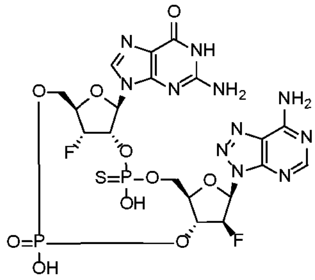
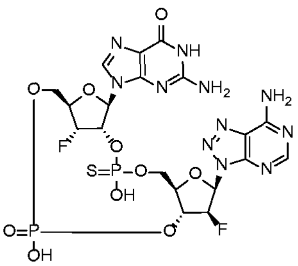
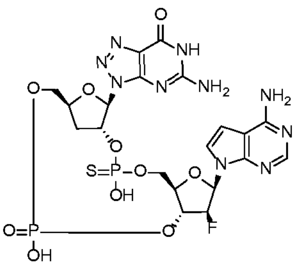
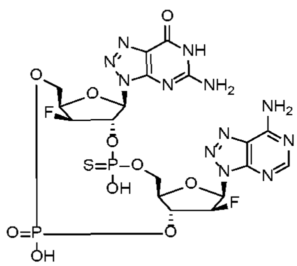
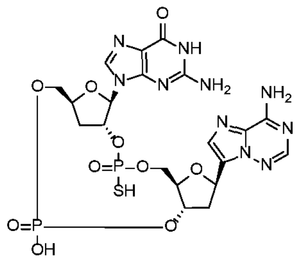
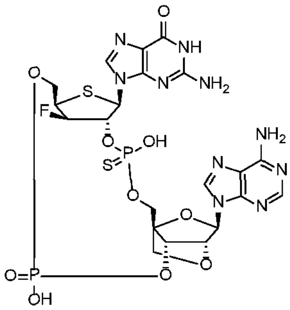
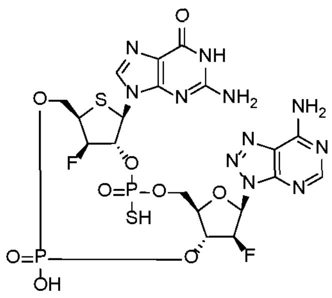
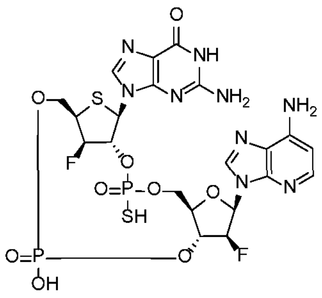
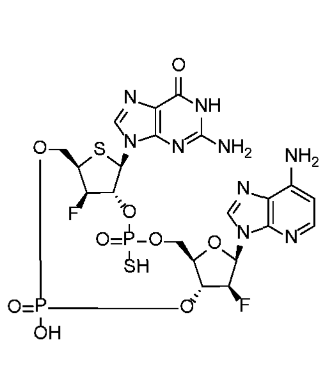
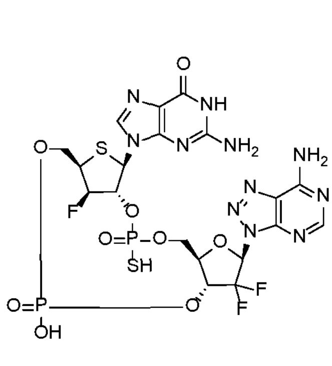
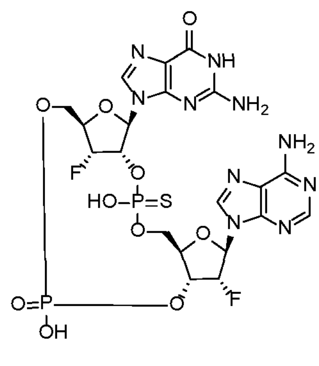
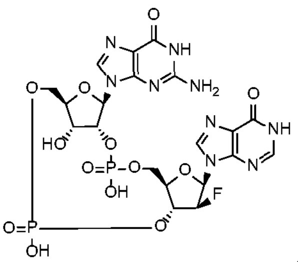
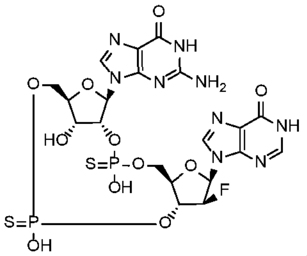
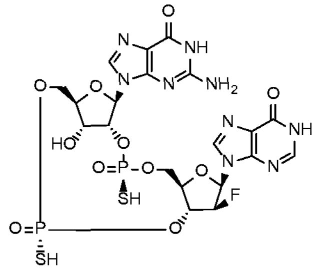
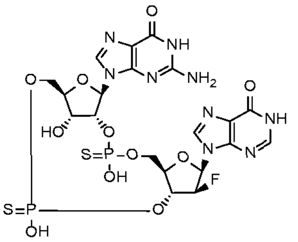
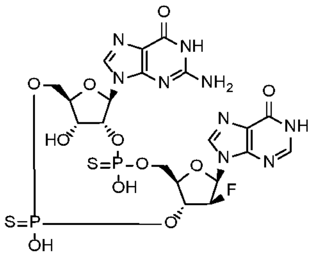
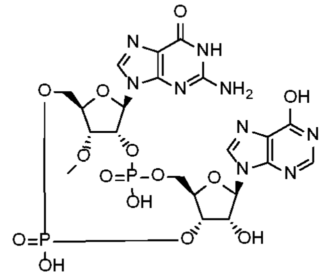
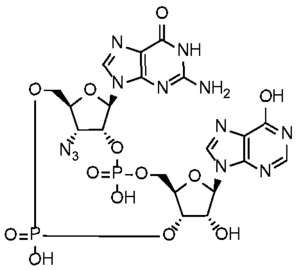
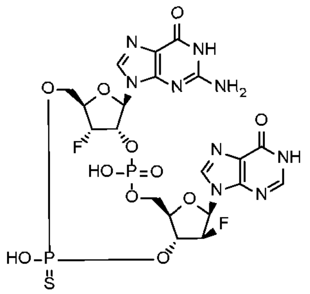
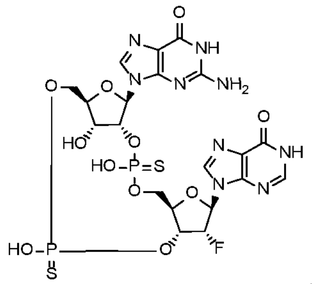
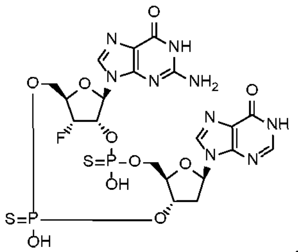
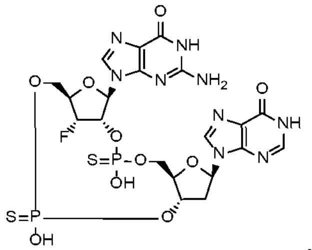
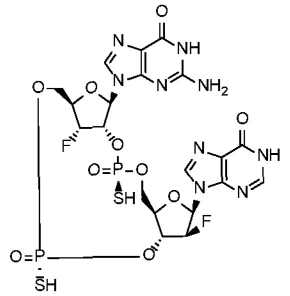
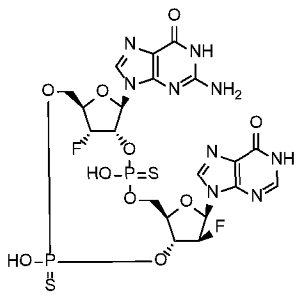


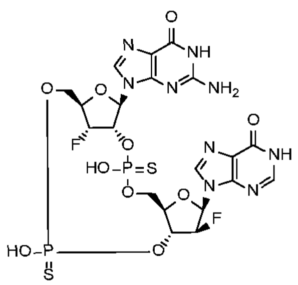
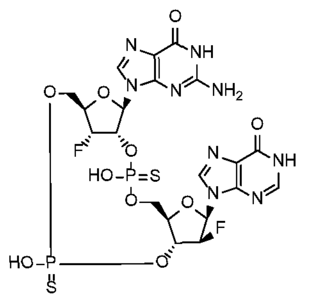
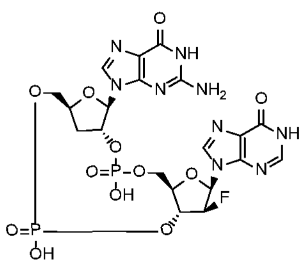
 ,
 ,
 ,
 ,
 ,
 ,
 ,
 ,
 ,
 ,
 ,
 ,
 ,
 ,
 ,
 ,

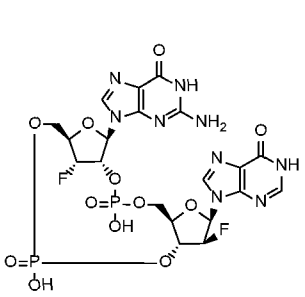
 ,  ,  ,

 ,  ,  ,

 ,  ,  ,

 ,  ,  ,  ,

 ,  ,  ,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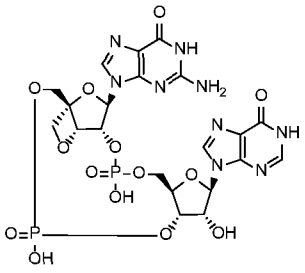
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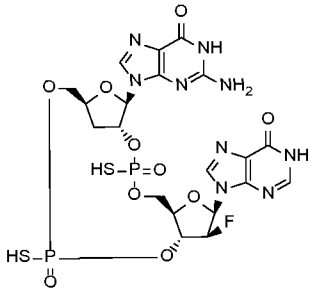
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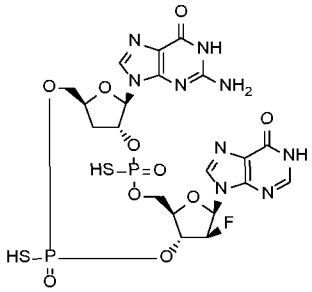
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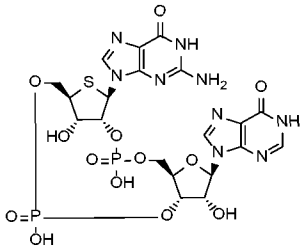
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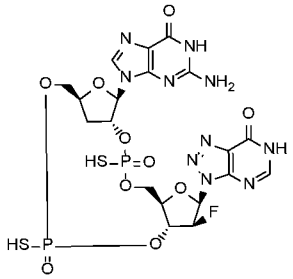
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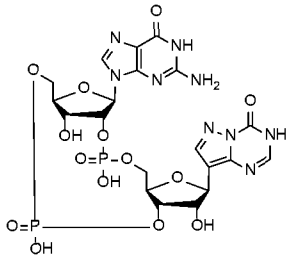
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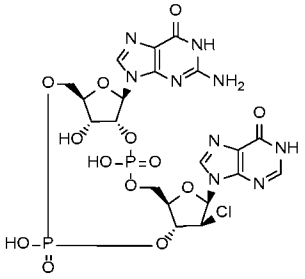
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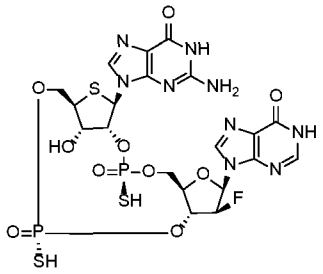
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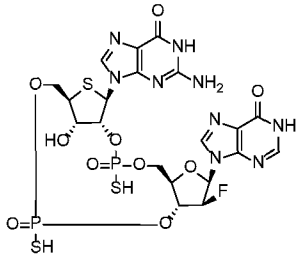
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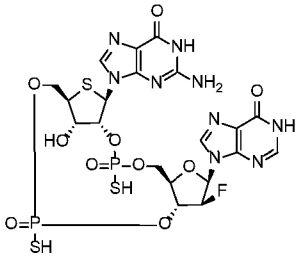
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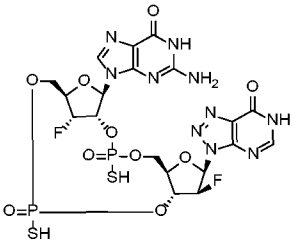
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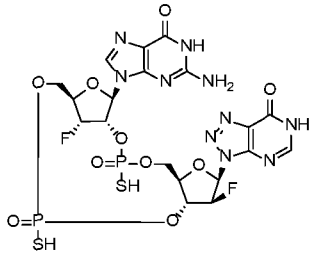
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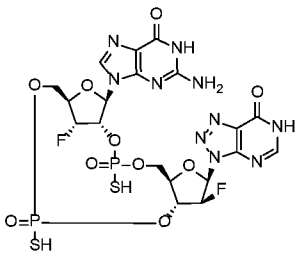
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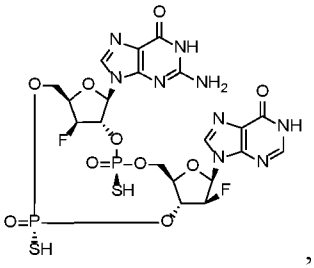
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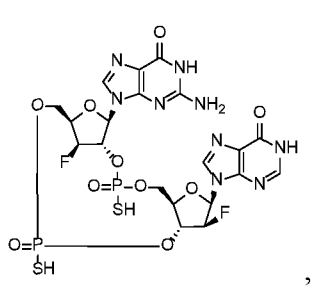
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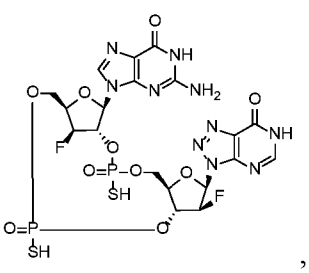
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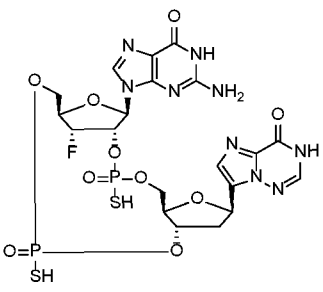
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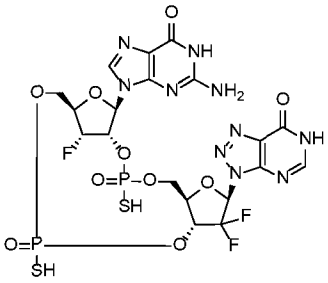
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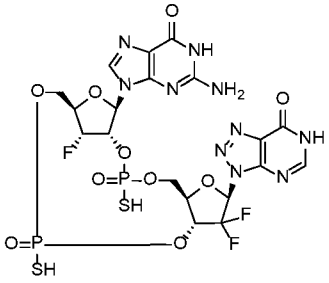
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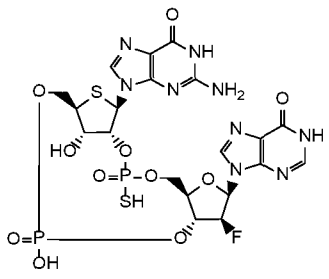
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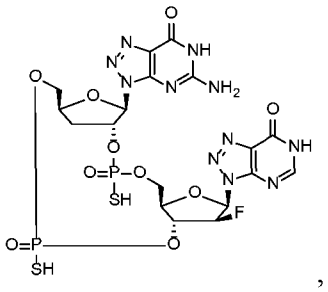
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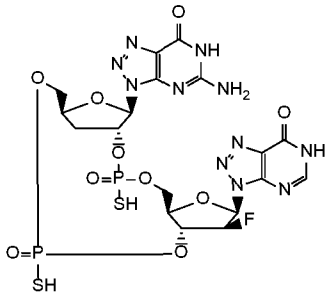
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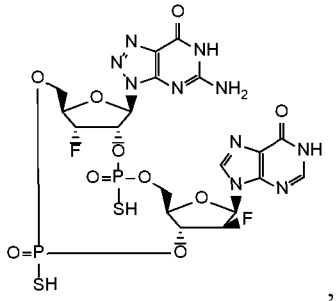
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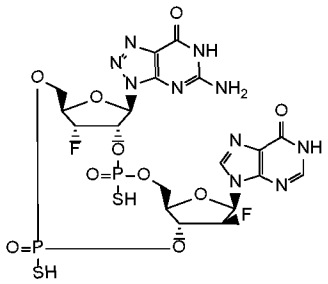
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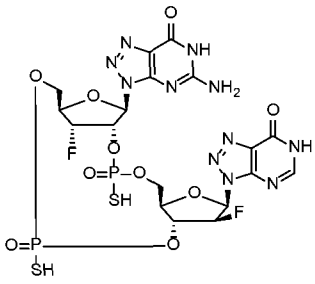
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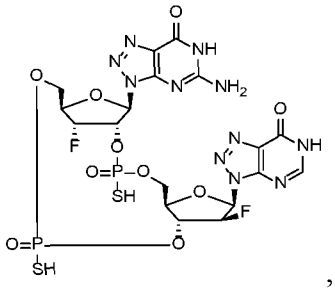
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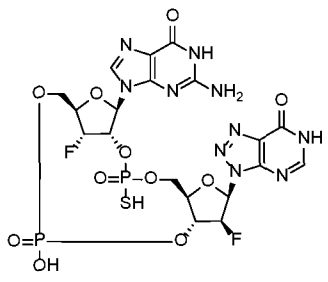
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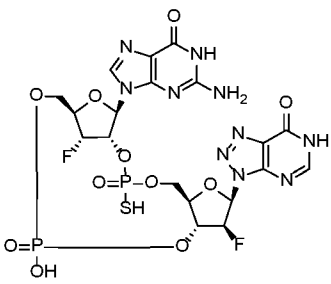
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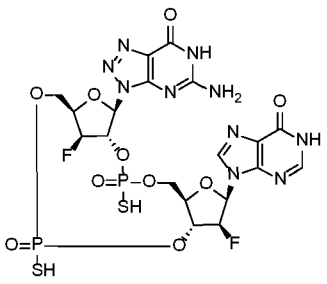
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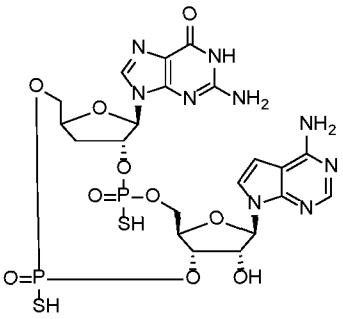
pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof. In particular aspects, the compound is selected from the group consisting of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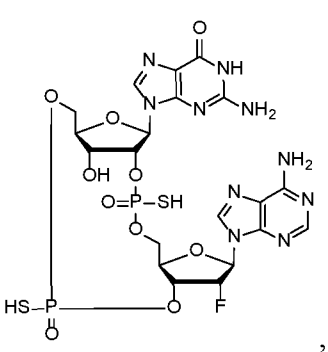
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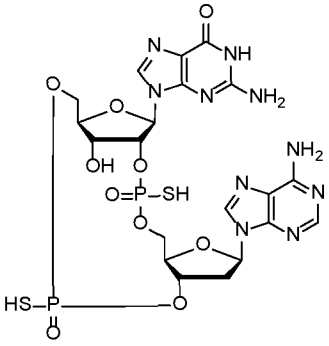
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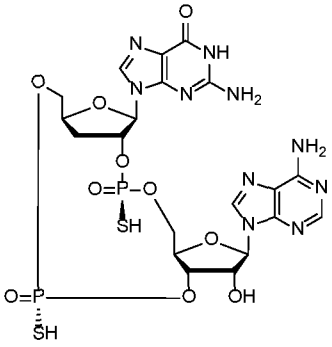
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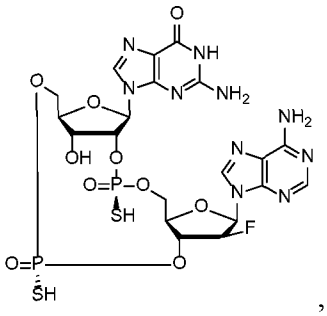
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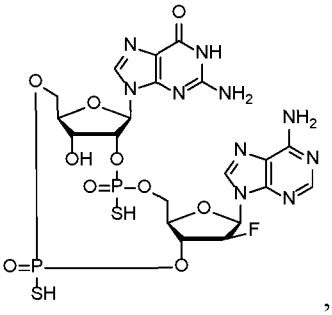
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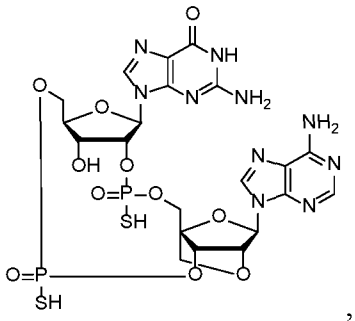
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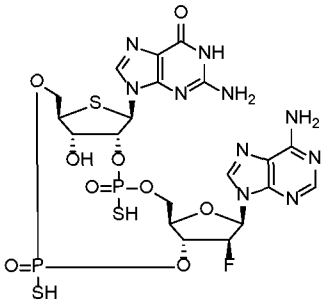
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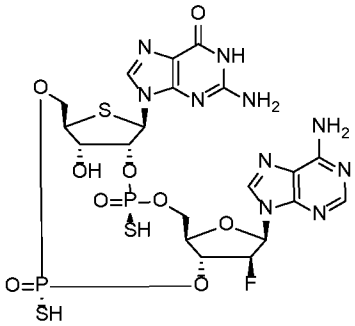
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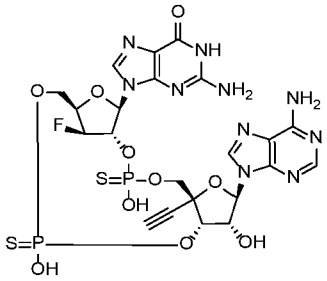
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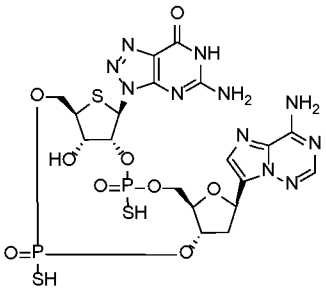
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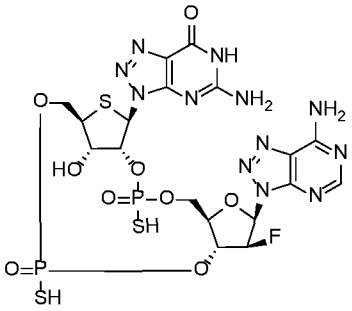
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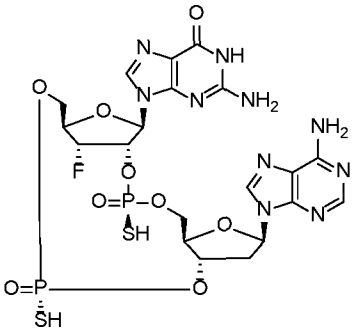
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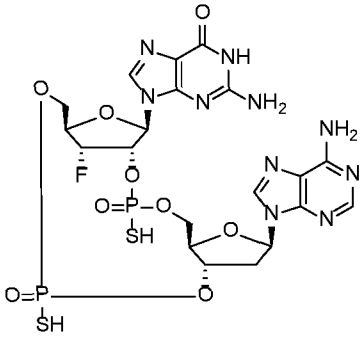
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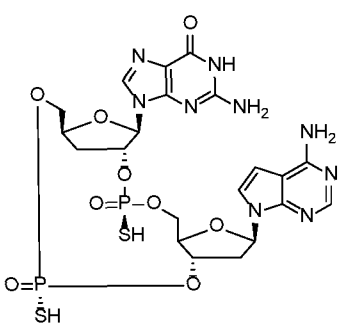
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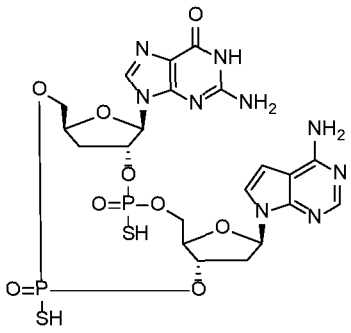
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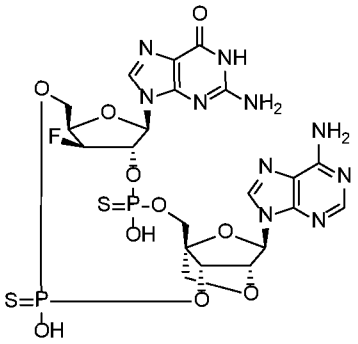
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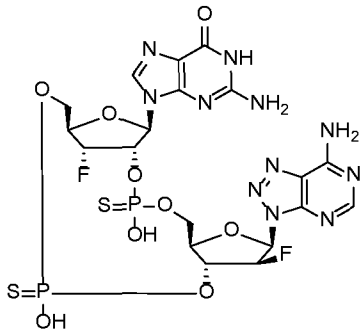
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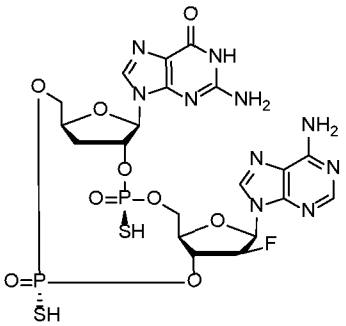
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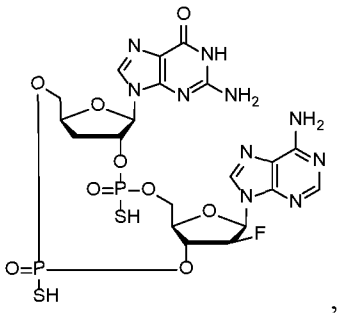
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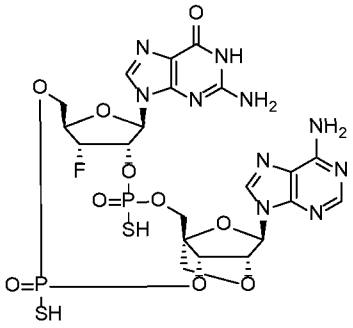
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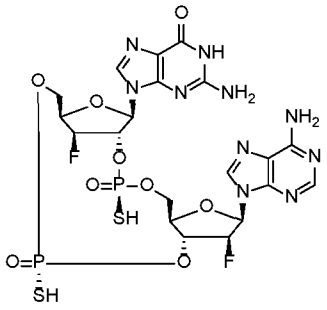
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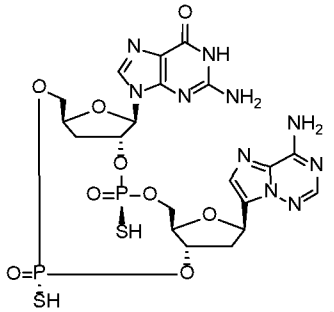
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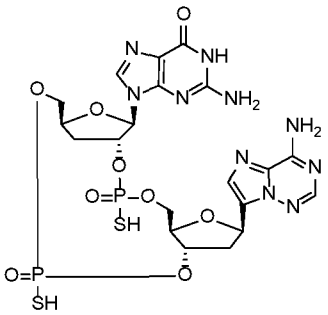
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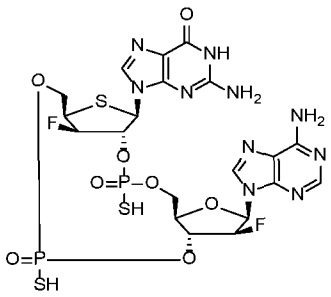
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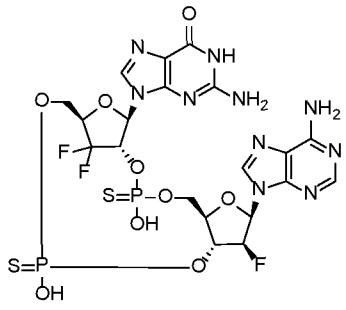
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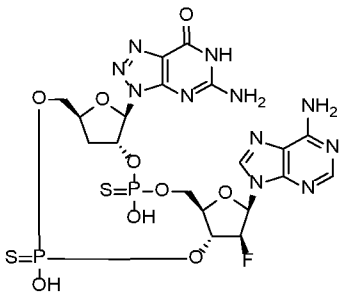
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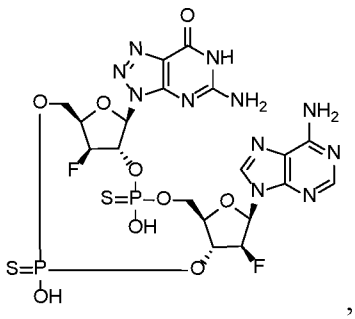
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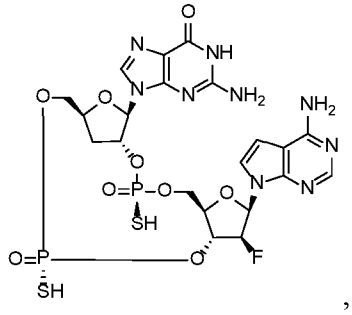
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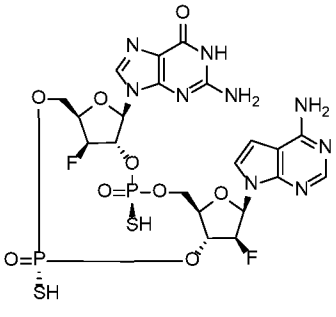
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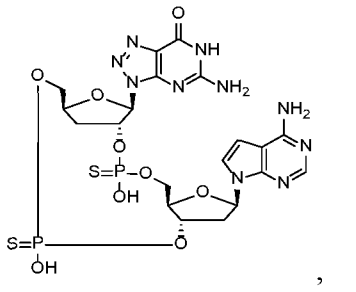
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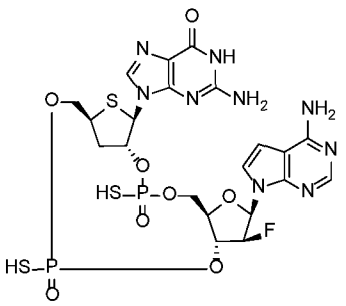
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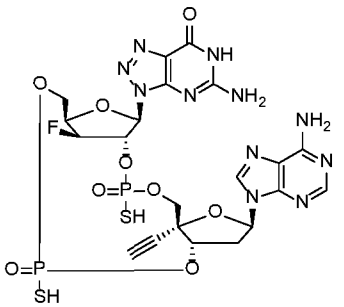
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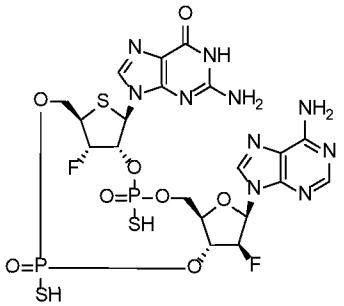
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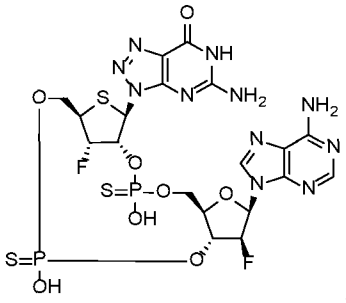
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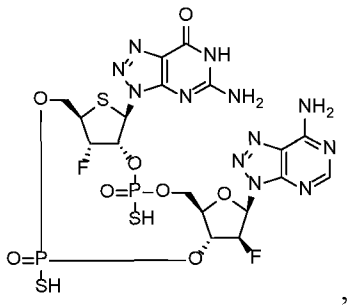
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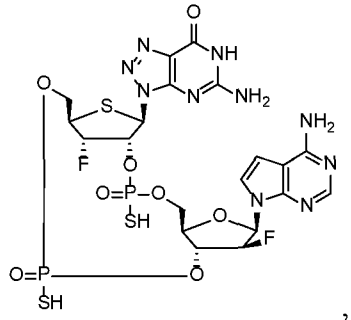
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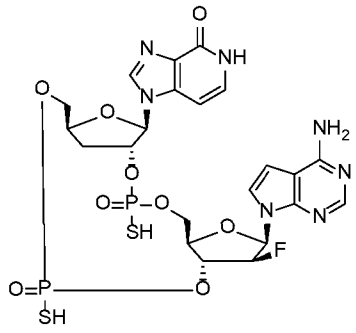
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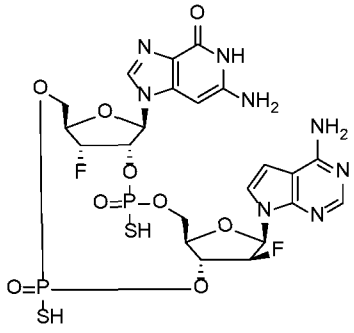
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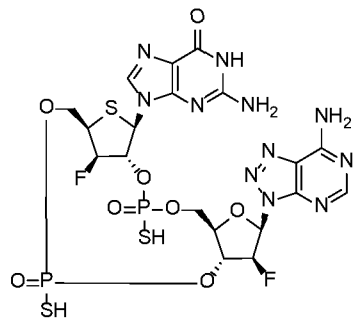
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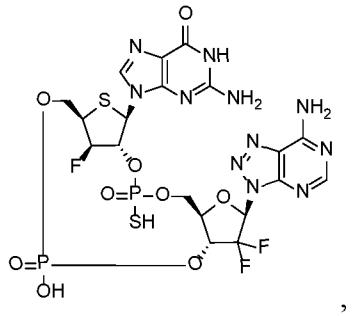
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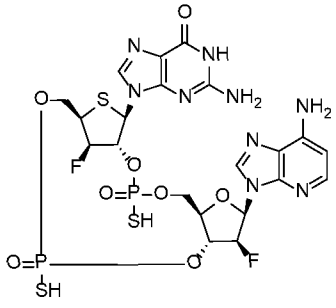
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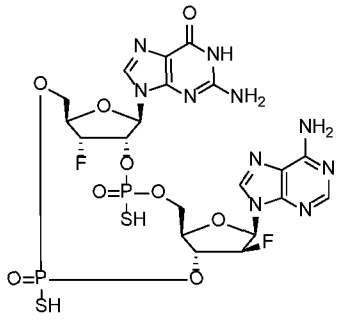
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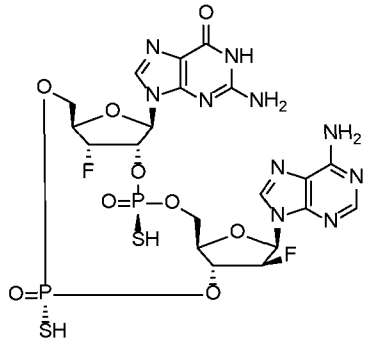
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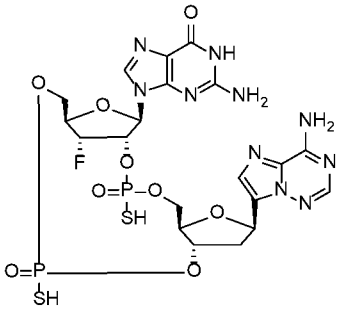
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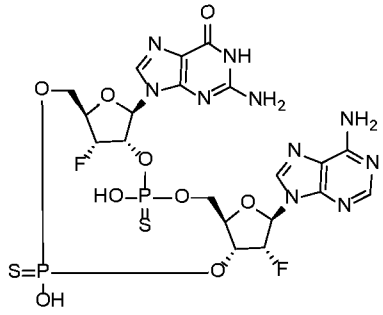
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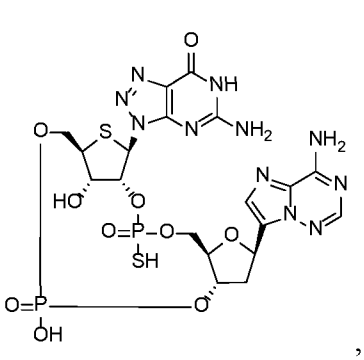
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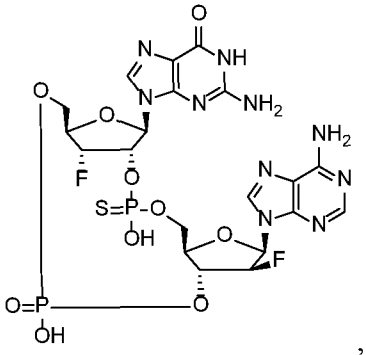
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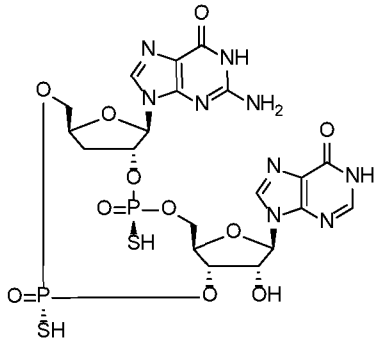
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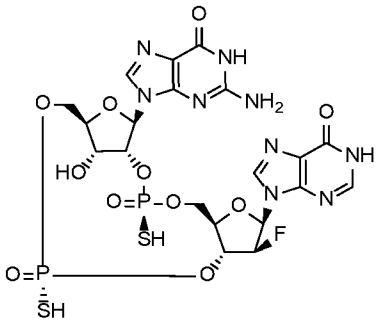
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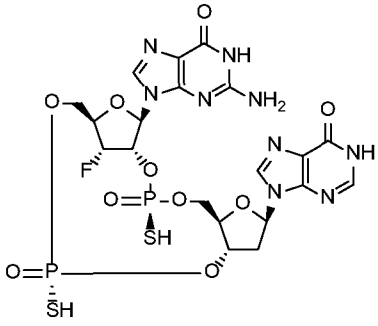
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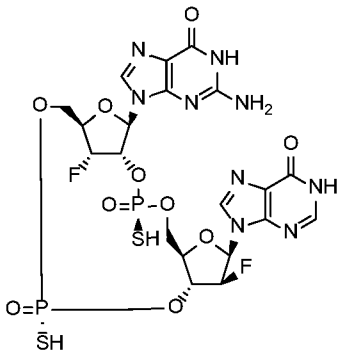
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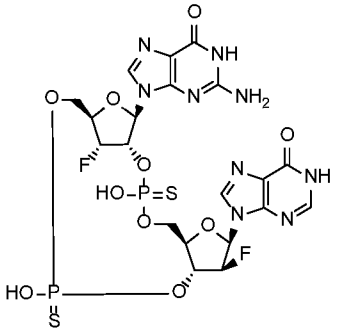
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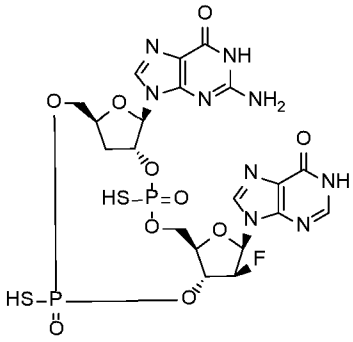
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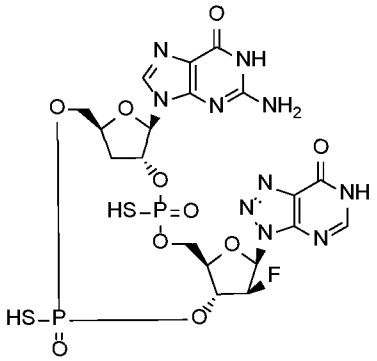
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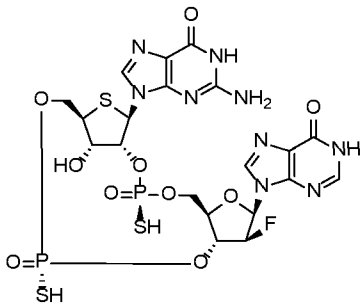
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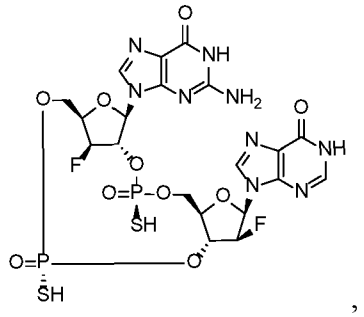
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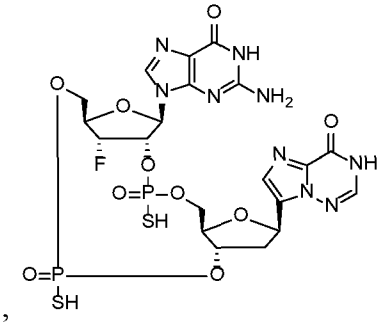
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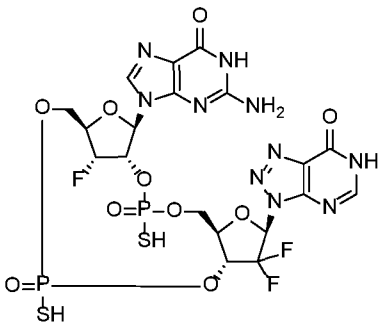
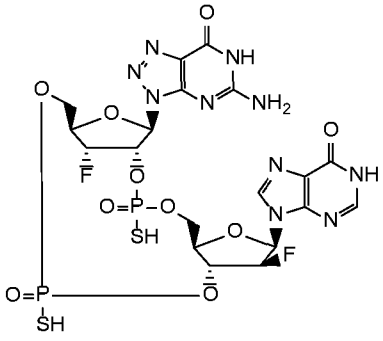
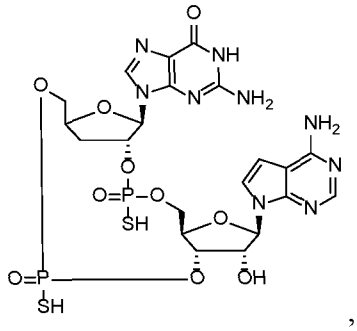
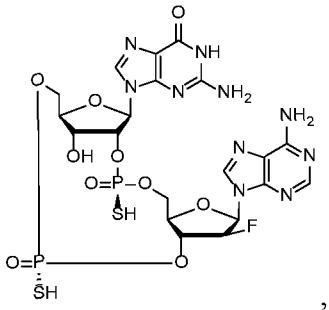
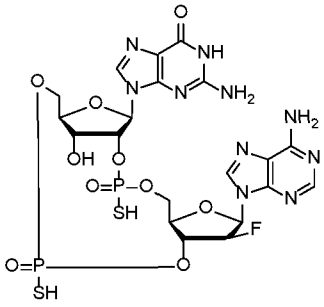
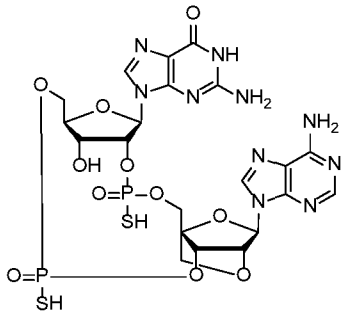
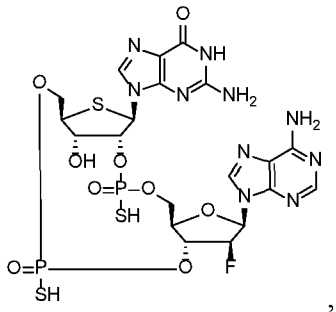
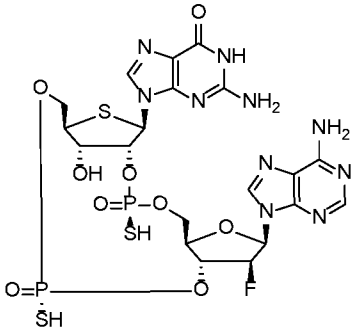
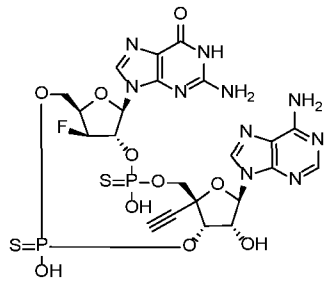
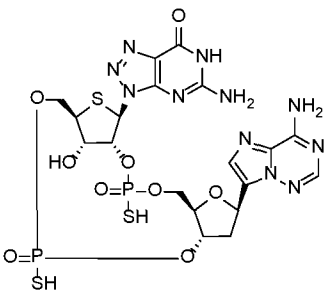
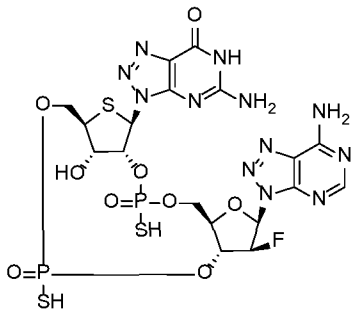
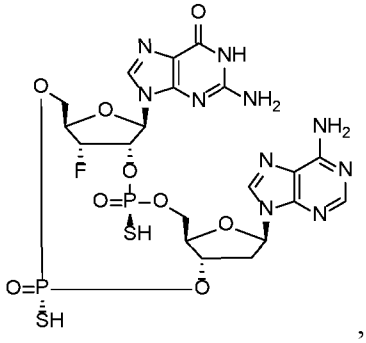
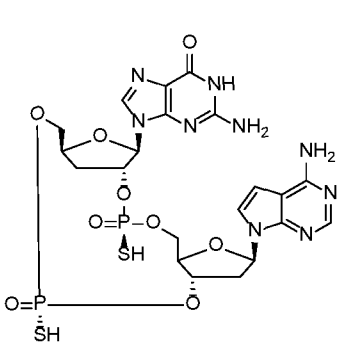


, and  , and pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof. In more particular aspects of this embodiment, the compound is selected from the group consisting of  ,

 ,  ,  ,

 ,  ,  ,

 ,  ,  ,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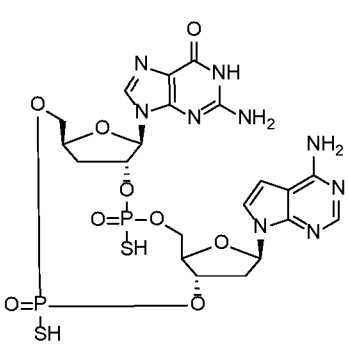
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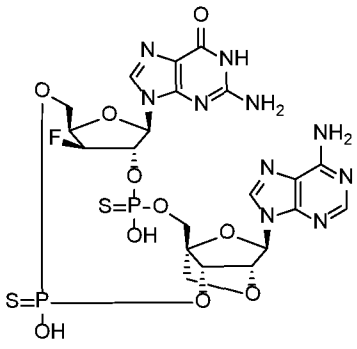
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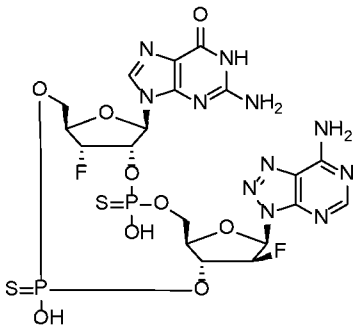
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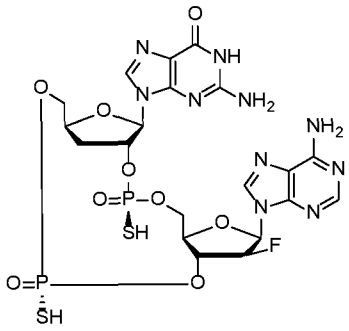
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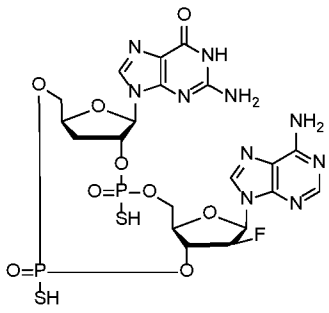
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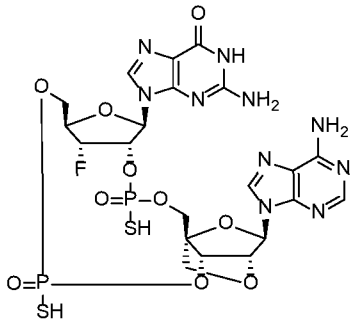
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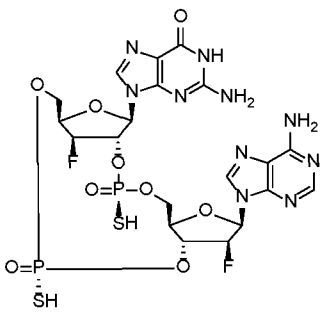
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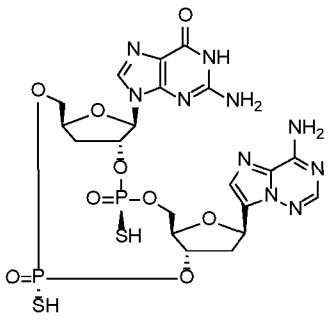
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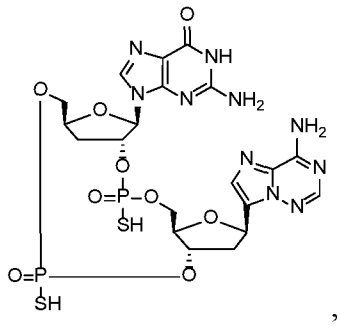
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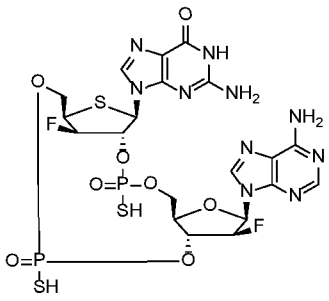
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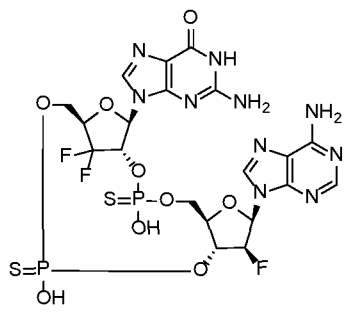
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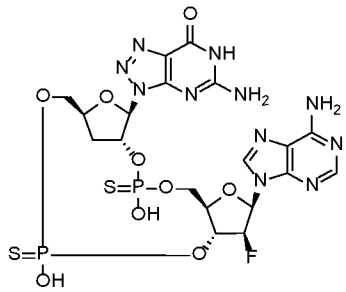
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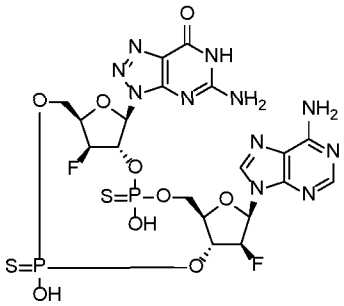
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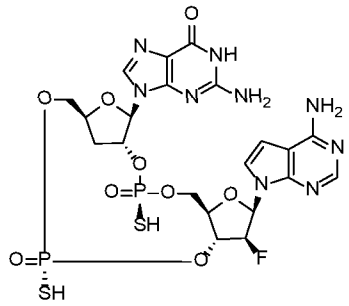
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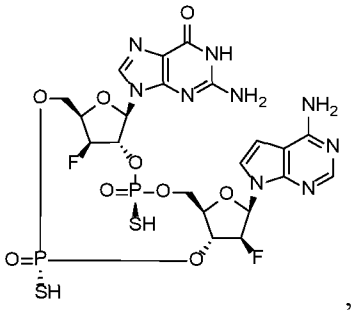
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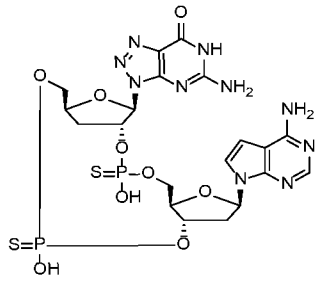
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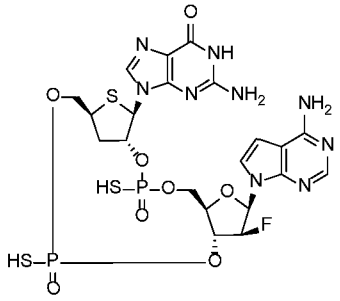
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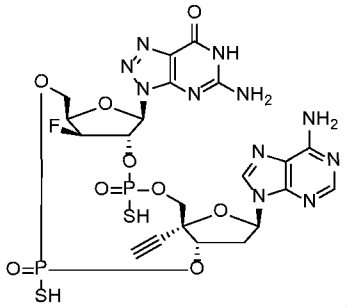
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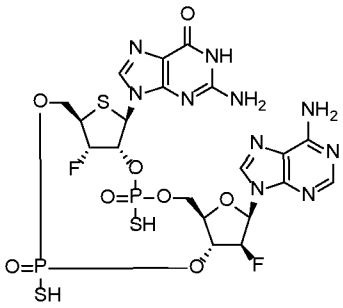
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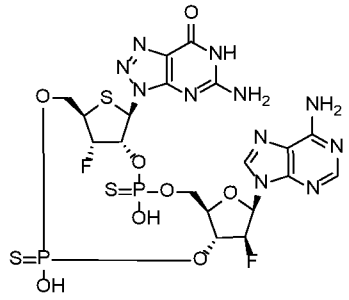
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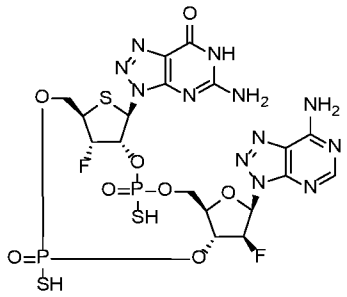
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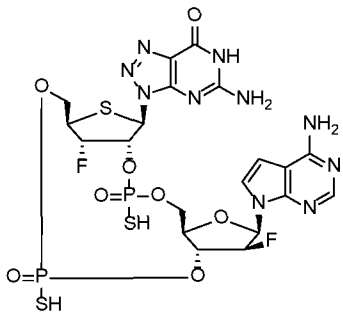
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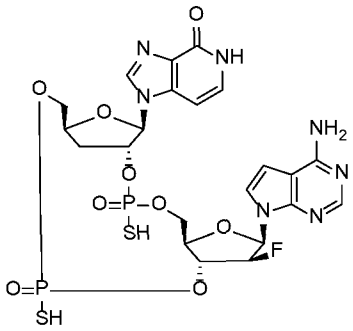
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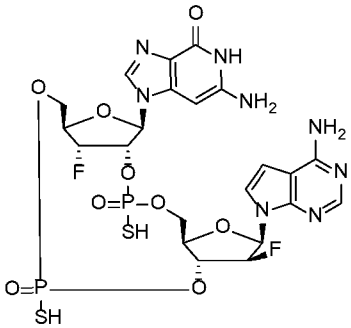
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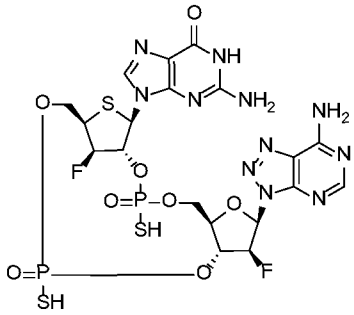
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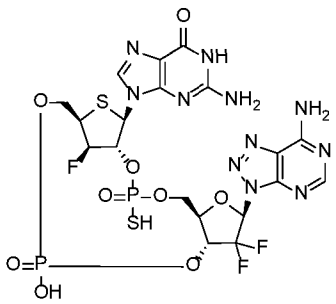
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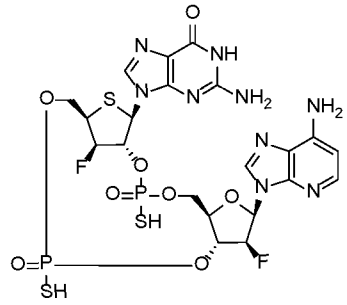
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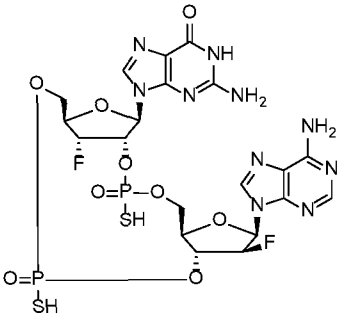
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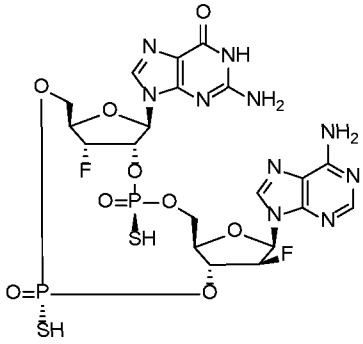
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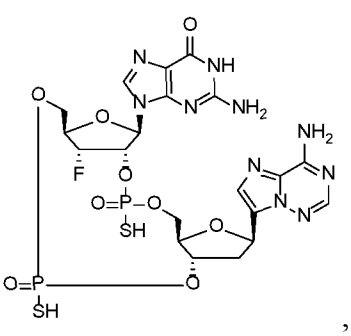
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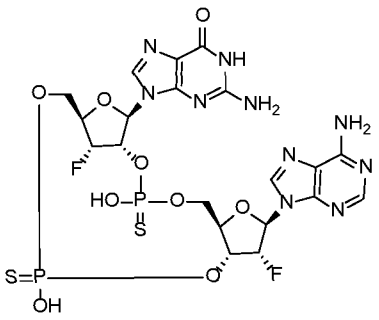
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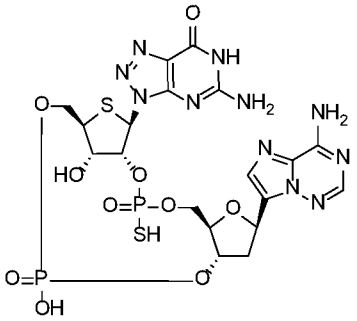
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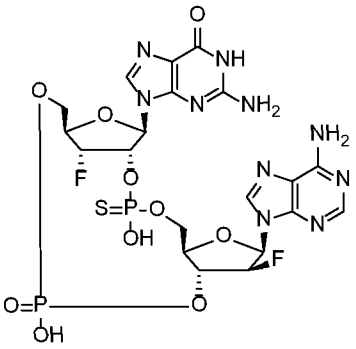
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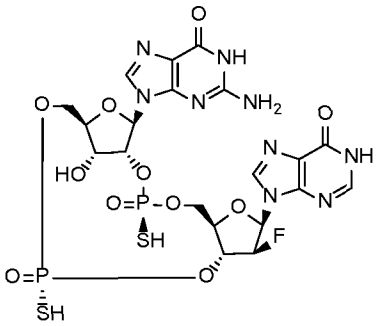
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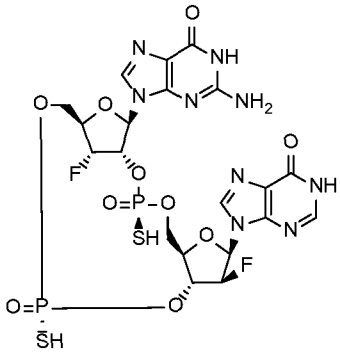
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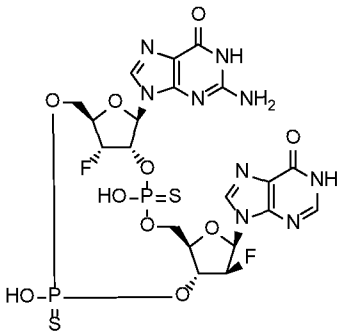
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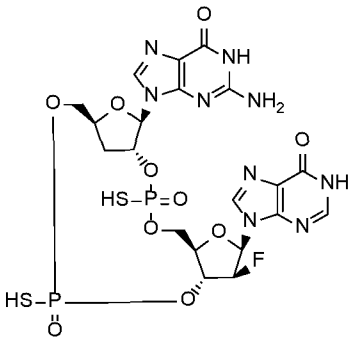
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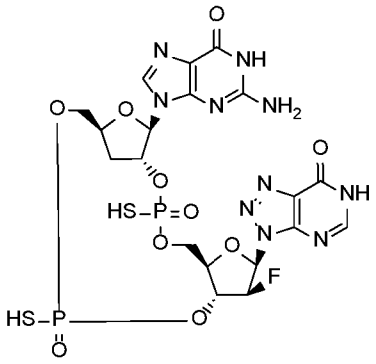
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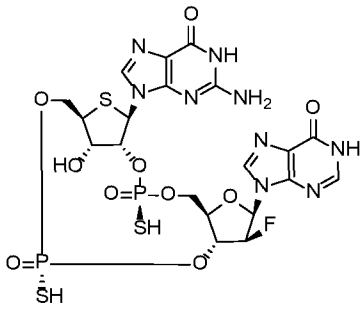
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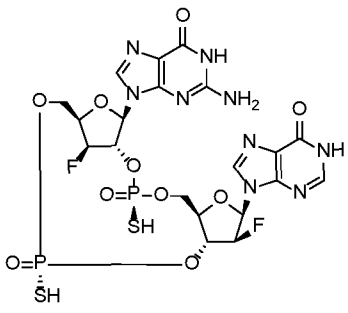
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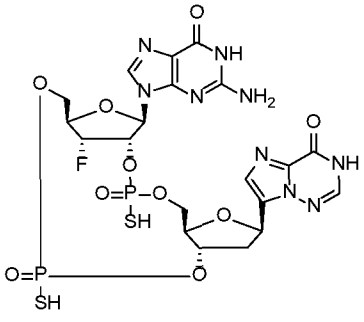
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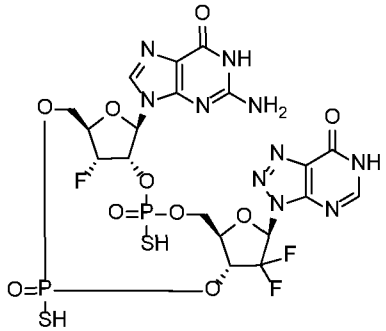
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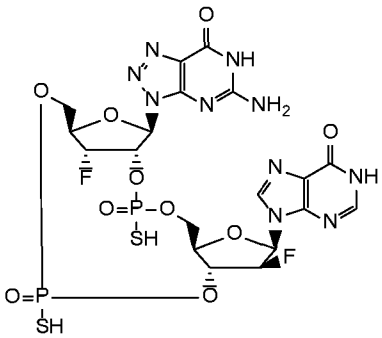
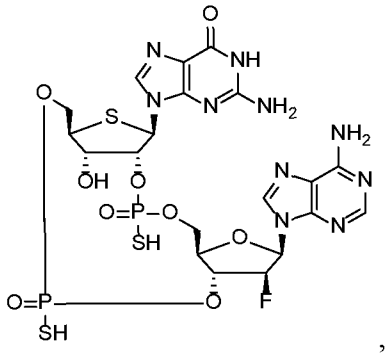
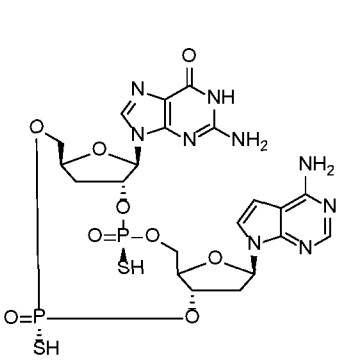
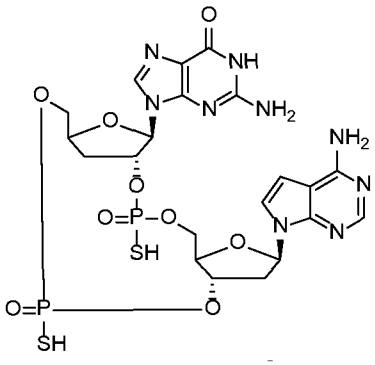
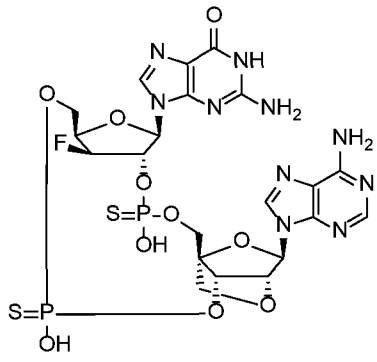
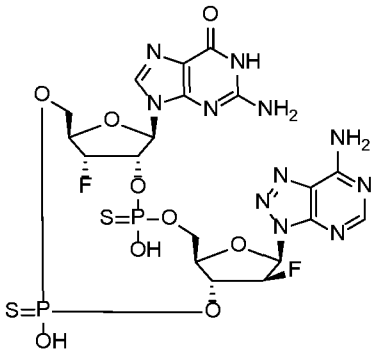
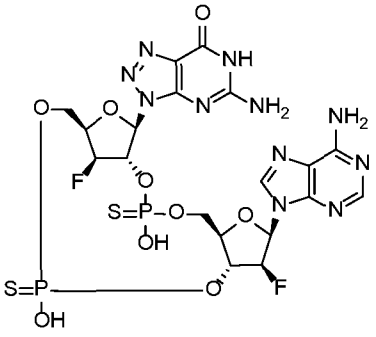
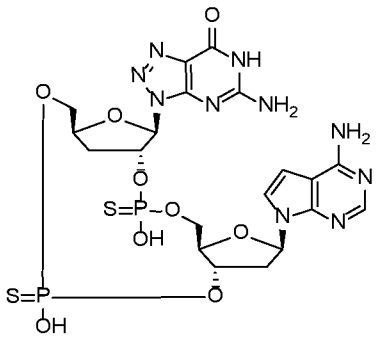
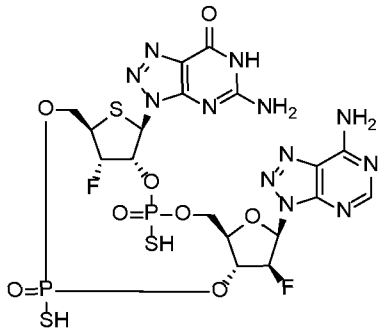
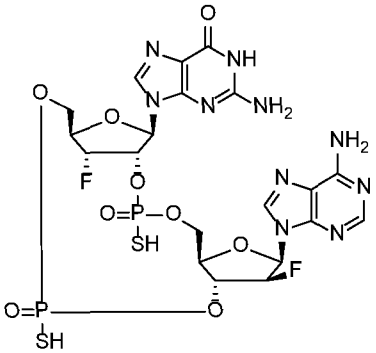
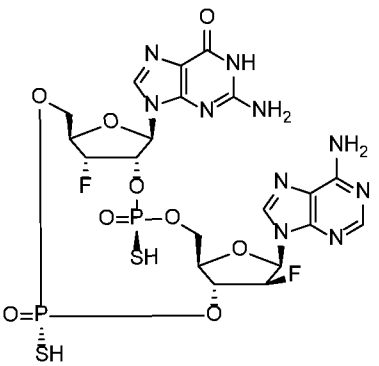

, and pharmaceutically

acceptable salts, hydrates, solvates, or prodrugs thereof. In still more particular aspects, the compound is selected from the group consisting of  ,

 ,  ,  ,

 ,  ,  ,

 ,  ,  ,

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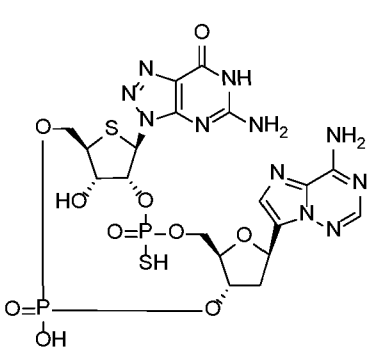
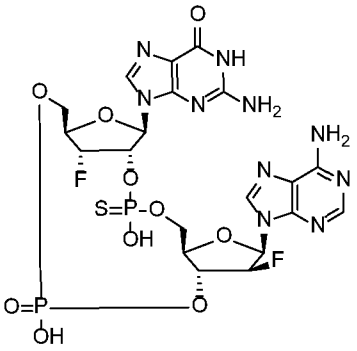
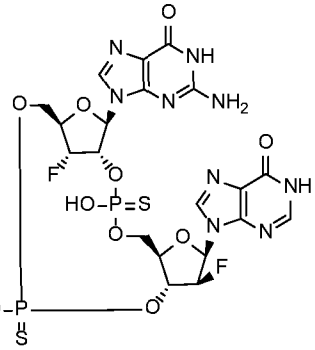
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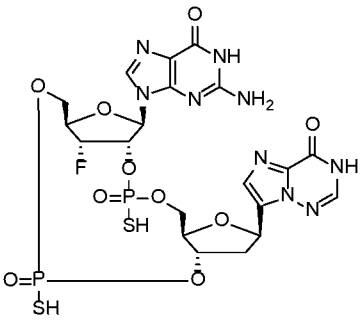

, and pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof.

In another embodiment, for the compounds of general formula (I), compounds of general formula (I') and compounds of general formula (I''), variables $Base^1$, $Base^2$, Y, Y^a, X^a, X^{a1}, X^b, X^{b1}, X^c, X^{c1}, X^d, X^{d1}, R^1, R^{1a}, R^2, R^{2a}, R^3, R^4, R^{4a}, R^5, R^6, R^{6a}, R^7, R^{7a}, R^8, R^{8a}, R^9, and R^{10} are each selected independently from each other.

In another embodiment of the disclosure, the compound of the disclosure is selected from the exemplary species depicted in Examples 1 through 348 shown below.

Other embodiments of the present disclosure include the following:

(a) A pharmaceutical composition comprising an effective amount of a compound of general formula (I) or a compound of general formula (I'), or a compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, and a pharmaceutically acceptable carrier.

(b) The pharmaceutical composition of (a), further comprising a second therapeutic agent selected from the group consisting of STING agonist compounds, anti-viral compounds, antigens, adjuvants, CTLA-4 and PD-1 pathway antagonists and other immunomodulatory agents, lipids, liposomes, peptides, anti-cancer and chemotherapeutic agents.

(c) A pharmaceutical combination that is (i) a compound of general formula (I) or a compound of general formula (I'), or a compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, and (ii) a second therapeutic agent selected from the group consisting of STING agonist compounds, anti-viral compounds, antigens,

adjuvants, CTLA-4 and PD-1 pathway antagonists and other immunomodulatory agents, lipids, liposomes, peptides, anti-cancer and chemotherapeutic agents; wherein the a compound of general formula (I) or compound of general formula (I'), or compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, and the second therapeutic agent are each employed in an amount that renders the combination effective for inducing an immune response in a patient.

(e) A method of inducing an immune response in a patient, which comprises administering to the subject an effective amount of a compound of general formula (I) or a compound of general formula (I'), or a compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof.

(f) A method of inducing an immune response in a patient, which comprises administering to the subject an effective amount of a composition of (a), a composition of (b) or a combination of (c).

(g) A method of inducing STING-dependent type I interferon production in a patient, which comprises administering to the subject an effective amount of a a compound of general formula (I) or a compound of general formula (I'), or a compound of general formula (I'').

(h) A method of inducing STING-dependent type I interferon production in a patient, which comprises administering to the subject an effective amount of a composition of (a), a composition of (b) or a combination of (c).

(i) A method of inducing STING-dependent cytokine production in a patient, which comprises administering to the subject an effective amount of a compound of general formula (I) or a compound of general formula (I'), or a compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof.

(j) A method of inducing STING-dependent cytokine production in a patient, which comprises administering to the subject an effective amount of a composition of (a), a composition of (b) or a combination of (c).

(k) A method of treating a cell proliferation disorder in a subject, said method comprising administering a therapeutically effective amount of a compound of general formula (I) or a compound of general formula (I'), or a compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof to the subject;

(l) The method of (k), wherein the cell proliferation disorder is cancer.

(m). A method of treating a cell proliferation disorder in a subject, said method comprising administering a therapeutically effective amount of a pharmaceutical composition of (a), a composition of (b) or a combination of (c) to the subject.

(n) The method of (m), wherein the cell proliferation disorder is cancer.

The present disclosure also includes a compound of the present disclosure for use (i) in, (ii) as a medicament for, or (iii) in the preparation of a medicament for: (a) inducing an immune response in a patient, or (b) inducing a STING-dependent cytokine production in a patient. In these uses, the compounds of the present disclosure can optionally be employed in combination with one or more second therapeutic agents selected from STING agonist compounds, anti-viral compounds, antigens, adjuvants, CTLA-4 and PD-1 pathway antagonists and other immunomodulatory agents, lipids, liposomes, peptides, anti-cancer and chemotherapeutic agents.

Additional embodiments of the disclosure include the pharmaceutical compositions, combinations and methods set forth in (a)-(n) above and the uses set forth in the preceding paragraph, wherein the compound of the present disclosure employed therein is a compound of one of the embodiments, aspects, classes, sub-classes, or features of the compounds described above. In all of these embodiments, the compound may optionally be used in the form of a pharmaceutically acceptable salt, hydrate, solvate or prodrug as appropriate.

In the embodiments of the compound provided above, it is to be understood that each embodiment may be combined with one or more other embodiments, to the extent that such a combination provides a stable compound and is consistent with the description of the embodiments. It is further to be understood that the embodiments of compositions and methods provided as (a) through (n) above are understood to include all embodiments of the compounds, including such embodiments as result from combinations of embodiments.

The term "subject" (alternatively referred to herein as "patient") as used herein refers to an animal, preferably a mammal, such as a human being, male or female, that has been the object of treatment, observation, or experiment. A subject also refers to one or more of cows, sheep, goats, horses, dogs, cats, rabbits, rats, mice, fish, and birds. In embodiments, the subject is human.

As used herein, the term "immune response" relates to any one or more of the following: specific immune response, non-specific immune response, both specific and non-specific response, innate response, primary immune response, adaptive immunity, secondary immune response, memory immune response, immune cell activation, immune cell proliferation, immune cell differentiation, and cytokine expression. In certain embodiments, the compound of general

formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, is administered in conjunction with one or more additional therapeutic agents including vaccines intended to stimulate an immune response to one or more predetermined anti-viral compounds, antigens, adjuvants, CTLA-4 and PD-1 pathway antagonists and other immunomodulatory agents, lipids, liposomes, peptides, anti-cancer and chemotherapeutic agents, etc. In certain embodiments, the compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, is administered in conjunction with one or more additional compositions including vaccines intended to stimulate an immune response to one or more predetermined anti-viral compounds, antigens, adjuvants, CTLA-4 and PD-1 pathway antagonists and other immunomodulatory agents, lipids, liposomes, peptides, anti-cancer and chemotherapeutic agents, etc.

Compounds

The term "alkyl" refers to a monovalent straight or branched chain, saturated aliphatic hydrocarbon radical having a number of carbon atoms in the specified range. Thus, for example, "C_{1-6} alkyl" (or "C_1-C_6 alkyl") refers to any of the hexyl alkyl and pentyl alkyl isomers as well as n-, iso-, sec- and *tert*-butyl, n- and iso-propyl, ethyl, and methyl. As another example, "C_{1-4} alkyl" refers to n-, iso-, sec- and *tert*-butyl, n- and isopropyl, ethyl, and methyl.

As used herein, the term "alkylene" refers to a bivalent straight chain, saturated aliphatic hydrocarbon radical having a number of carbon atoms in the specified range.

As used herein, the term "alkenyl" refers to a monovalent straight or branched chain, unsaturated aliphatic hydrocarbon radical having a number of carbon atoms in the specified range and including one or more double bond.

As used herein, the term "alkenylene" refers to a bivalent straight chain, unsaturated aliphatic hydrocarbon radical having a number of carbon atoms in the specified range and including one or more double bond.

As used herein, the term "alkynyl" refers to a monovalent straight or branched chain, unsaturated aliphatic hydrocarbon radical having a number of carbon atoms in the specified range and including one or more triple bond.

As used herein, the term "alkynylene" refers to a bivalent straight chain, unsaturated aliphatic hydrocarbon radical having a number of carbon atoms in the specified range and including one or more triple bond.

The term "halogen" (or "halo") refers to fluorine, chlorine, bromine, and iodine (alternatively referred to as fluoro, chloro, bromo, and iodo or F, Cl, Br, and I).

The term "haloalkyl" refers to an alkyl group as defined above in which one or more of the hydrogen atoms have been replaced with a halogen. Thus, for example, "$C_{1\text{-}6}$ haloalkyl" (or "C_1-C_6 haloalkyl") refers to a C_1 to C_6 linear or branched alkyl group as defined above with one or more halogen substituents. The term "fluoroalkyl" has an analogous meaning except the halogen substituents are restricted to fluoro. Suitable fluoroalkyls include the series $(CH_2)_{0\text{-}4}CF_3$ (*i.e.*, trifluoromethyl, 2,2,2-trifluoroethyl, 3,3,3-trifluoro-n-propyl, etc.).

As used herein, the term "haloalkenyl" refers to an alkenyl group as defined above in which one or more of the hydrogen atoms have been replaced with a halogen.

As used herein, the term "haloalkynyl" refers to an alkynyl group as defined above in which one or more of the hydrogen atoms have been replaced with a halogen.

As used herein, the term "spirocycle" or "spirocyclic ring" refers to a pendant cyclic group formed by substituents on a single atom. For example, in general formula (I), a spirocycle may be formed by R^{2a} and R^3.

Unless expressly stated to the contrary, all ranges cited herein are inclusive; *i.e.*, the range includes the values for the upper and lower limits of the range as well as all values in between. As an example, temperature ranges, percentages, ranges of equivalents, and the like described herein include the upper and lower limits of the range and any value in the continuum there between. Numerical values provided herein, and the use of the term "about", may include variations of ± 1%, ± 2%, ±3%, ± 4%, ± 5%, ± 10%, ± 15%, and ± 20% and their numerical equivalents.

As used herein, the term "one or more" item includes a single item selected from the list as well as mixtures of two or more items selected from the list.

In the compounds of general formula (I), compounds of general formula (I'), and compounds of general formula (I''), the atoms may exhibit their natural isotopic abundances, or one or more of the atoms may be artificially enriched in a particular isotope having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number predominantly found in nature. The present disclosure is meant to include all suitable isotopic variations of the compounds of general formula (I), compounds of general formula (I'), and compounds of general formula (I''). For example, different isotopic forms of hydrogen (H) include protium (^1H) and deuterium (^2H). Protium is the predominant hydrogen isotope found in nature. Enriching for deuterium may afford certain therapeutic advantages, such as increasing *in*

vivo half-life or reducing dosage requirements, or may provide a compound useful as a standard for characterization of biological samples. Isotopically-enriched compounds within general formula (I) can be prepared without undue experimentation by conventional techniques well known to those skilled in the art or by processes analogous to those described in the Schemes and Examples herein using appropriate isotopically-enriched reagents and/or intermediates.

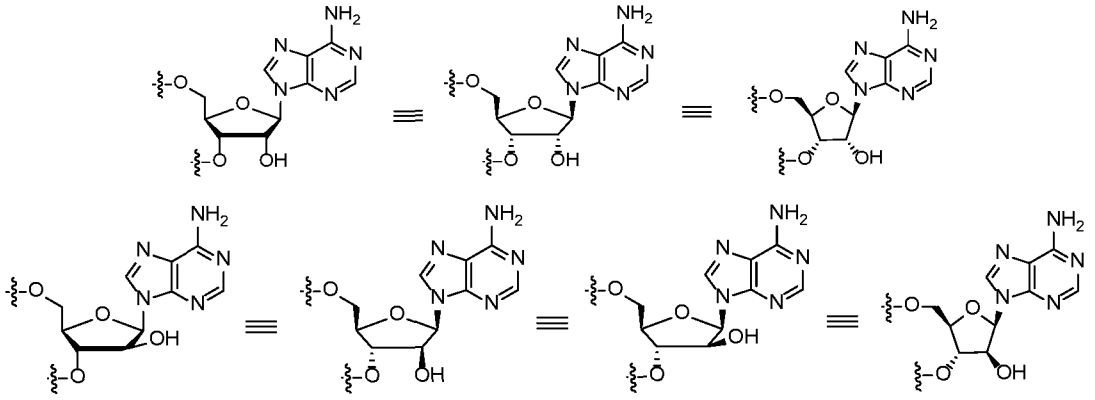
In particular embodiments of the compounds of general formula (I), compounds of general formula (I'), and compounds of general formula (I''), the compounds are isotopically enriched with deuterium. In aspects of these embodiments, one or more of R^1, R^{1a}, R^2, R^{2a}, R^3, R^4, R^{4a}, R^5, R^6, R^{6a}, R^7, R^{7a}, R^8, R^{8a}, R^9, and R^{10} may be deuterium.

As shown in the general structural formulas and the structures of specific compounds as provided herein, a straight line at a chiral center includes both (R) and (S) stereoisomers and mixtures thereof. Also, unless otherwise specified (*e.g.*, 100% purified compound), reference to a particular stereochemistry at a position provides a compound having the indicated stereochemistry, but does not exclude the presence of stereoisomers having different stereochemistry at the indicated position.

Recitation or depiction of a specific compound in the claims (*i.e.*, a species) without a specific stereoconfiguration designation, or with such a designation for less than all chiral centers, is intended to encompass the racemate, racemic mixtures, each individual enantiomer, a diastereoisomeric mixture and each individual diastereomer of the compound where such forms are possible due to the presence of one or more asymmetric centers. The separation of a mixture of stereoisomers can be carried out at an intermediate step during the synthesis of a compound of general formula (I), a compound of general formula (I'), and/or a compound of general formula (I''), or it can be done on a final racemic product. Absolute stereochemistry may be determined by X-ray crystallography of crystalline products or crystalline intermediates, which are derivatized, if necessary, with a reagent containing a stereogenic center of known configuration. Alternatively, absolute stereochemistry may be determined by Vibrational Circular Dichroism (VCD) spectroscopy analysis. The present invention includes all such isomers, as well as salts, solvates (which includes hydrates) and solvated salts of such racemates, enantiomers, diastereomers and tautomers and mixtures thereof.

Those skilled in the art will recognize that chiral compounds, and in particular sugars, can be drawn in a number of different ways that are equivalent. Those skilled in the art will further recognize that the identity and regiochemical position of the substituents on ribose can

vary widely and that the same prinicples of steroechemical equivalence apply regardless of substituent. Non-limiting examples of such equivalence include those exemplified below.



Salts

Compounds described herein having appropriate functional groups can be provided as salts. Examples of such compounds are described herein by reference to possible salts. Such reference is for illustration only. Additional embodiments include salts of any compounds described herein having suitable groups.

Pharmaceutically acceptable salts can be used with compounds for treating patients. Non-pharmaceutical salts may, however, be useful in the preparation of intermediate compounds.

Pharmaceutically acceptable salts are suitable for administration to a patient, preferably, a human. Suitable salts include acid addition salts that may, for example, be formed by mixing a solution of a compound with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, acetic acid, trifluoroacetic acid, or benzoic acid. Compounds carrying an acidic moiety can be mixed with suitable pharmaceutically acceptable salts to provide, for example, alkali metal salts (*e.g.*, sodium or potassium salts), alkaline earth metal salts (*e.g.*, calcium or magnesium salts), and salts formed with suitable organic ligands such as quaternary ammonium salts. Also, in the case of an acid (-COOH) or alcohol group being present, pharmaceutically acceptable esters can be employed to modify the solubility or hydrolysis characteristics of the compound.

Methods of Preparing Compounds

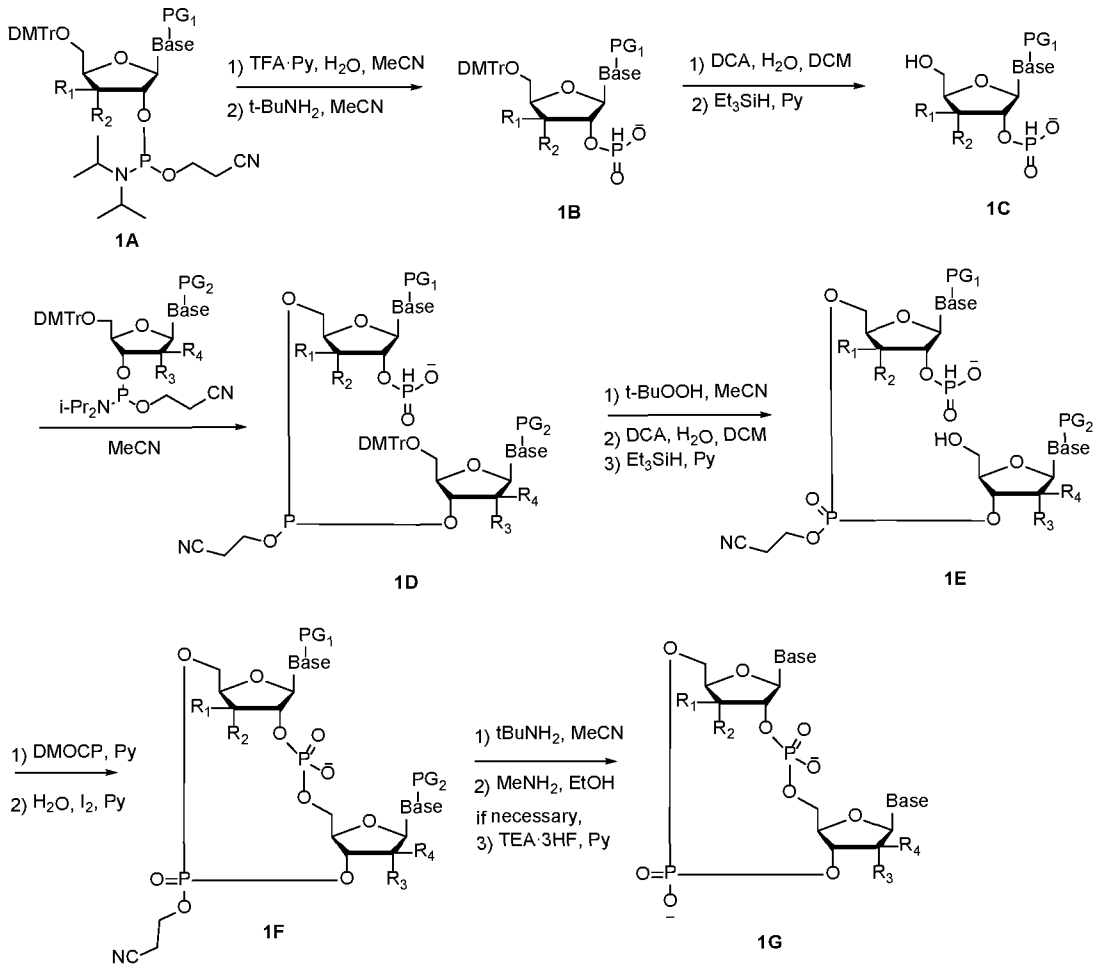
Several methods for preparing the compounds of general formula (I), compounds of general formula (I'), and compounds of general formula (I''), or pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof, are described in the following Schemes and

Examples. Starting materials and intermediates are purchased from commercial sources, made from known procedures, or are otherwise illustrated. In some cases the order of carrying out the steps of the reaction schemes may be varied to facilitate the reaction or to avoid unwanted reaction products.

Method 1

One method for the preparation of examples of the disclosure is detailed in Scheme 1. This procedure was adequately modified from the previously reported procedure for cyclic dinucleotide synthesis (Barbara L. Gaffney *et al.*, *One-Flask Syntheses of c-di-GMP and the [Rp,Rp] and [Rp,Sp] Thiophosphate Analogues*, 12 ORG. LETT. 3269-3271 (2010)). The sequence starts with modified ribo-nucleoside with a nucleobase of which amino group was appropriately protected with an alkyl or phenyl carbonyl group, a phosphoramidite functionality at 2'-O position, and DMTr ether at 5'-O position. It was treated with aqueous TFA/pyridine condition and subsequently t-butylamine to convert the 2'-phosphoramidite moiety to an H-phosphonate. Then, DMTr ether was removed under acidic condition. The resulting 5'-hydroxyl group was reacted with 3'-phosphoramidites of fully protected second modified ribo-nucleoside to give a cyclized compound. It was immediately oxidized with t-butyl hydroperoxide. Then, the 5'-hydroxyl group of the second ribo-nucleoside was deprotected with dichloroacetic acid. Using 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide as a coupling reagent, the H-phosphonate at 2'-O of the first ribo-nucleoside was reacted with 5'-OH of the second ribo-nucleoside to give a cyclic product. It was immediately oxidized with aqueous iodine. Treatment with t-butylamine and methylamine plus fluoride anion in case silyl protection was used provided the desired cyclic dinucleotide 1G.

SCHEME 1

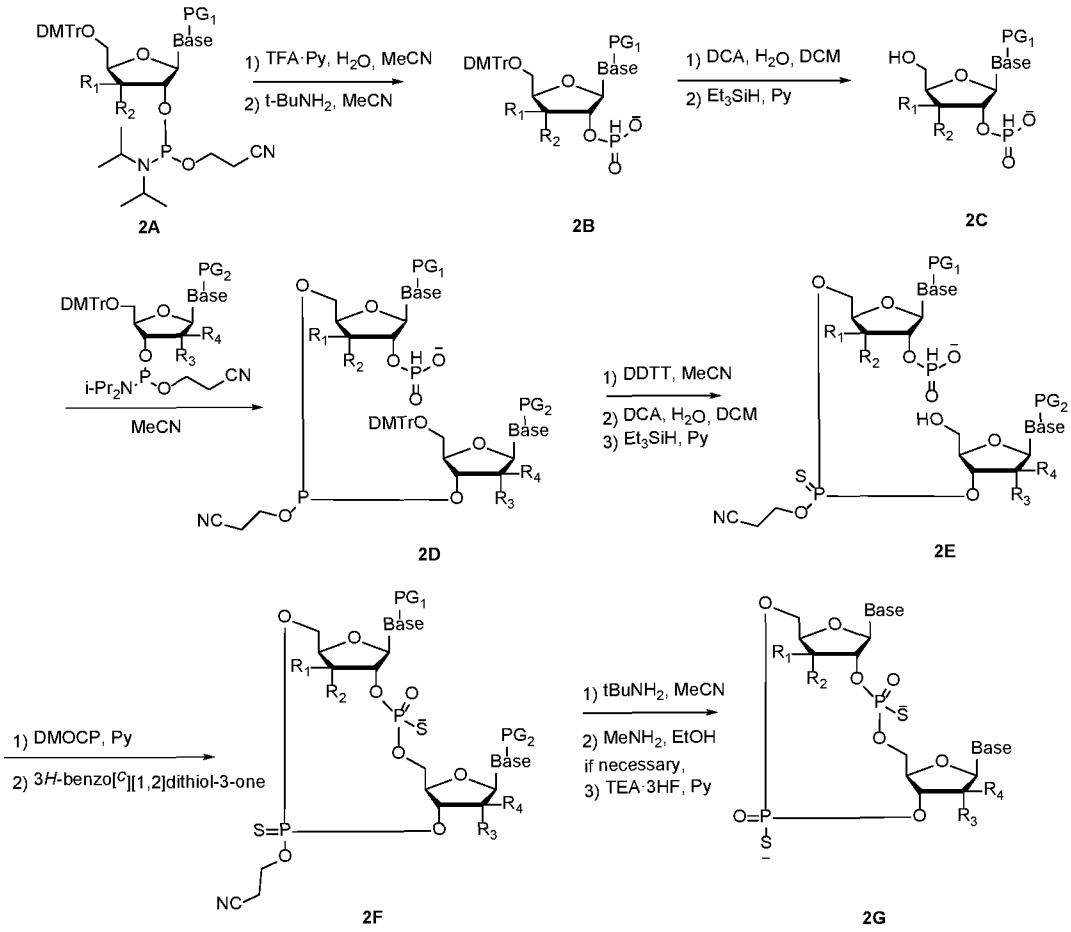


Method 2

Another method for the preparation of examples of the disclosure is detailed in Scheme 2. This procedure was modified from Scheme 1. The sequence starts with modified ribo-nucleoside with a nucleobase of which amino group was appropriately protected with an alkyl or phenyl carbonyl group, a phosphoramidite functionality at 2'-O position, and DMTr ether at 5'-O position. It was treated with aqueous TFA/pyridine condition and subsequently t-butylamine to convert the 2'-phosphoramidite moiety to an H-phosphonate. Then, DMTr ether was removed under acidic condition. The resulting 5'-hydroxyl group was reacted with 3'-phosphoramidites of fully protected second modified ribo-nucleoside to give a cyclized compound. It was immediately thioated with (E)-N,N-dimethyl-N'-(3-thioxo-3H-1,2,4-dithiazol-5-yl)formimidamide. Then, the 5'-hydroxyl group of the second ribo-nucleoside was deprotected with dichloroacetic acid. Using 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide as a coupling reagent, the H-phosphonate at 2'-O of the first ribo-nucleoside was reacted

with 5'-OH of the second ribo-nucleoside to give a cyclic product. It was immediately thioated with 3H-benzo[c][1,2]dithiol-3-one. Treatment with t-butylamine and methylamine plus fluoride anion in case silyl protection was used provided the desired cyclic dinucleotide diphosphorothioate 2G.

SCHEME 2



Methods of Use

Compounds described herein having therapeutic applications, such as the compounds of general formula (I), compounds of general formula (I'), and compounds of general formula (I''), and the compounds of the Examples 1 through 348, can be administered to a patient for the purpose of inducing an immune response, inducing a STING-dependent cytokine production and/or inducing anti-tumor activity. The term "administration" and variants thereof (*e.g.*, "administering" a compound) means providing the compound to the individual in need of treatment. When a compound is provided in combination with one or more other active agents (*e.g.*, antiviral agents useful for treating HCV infection or anti-tumor agents for treating cancers),

"administration" and its variants are each understood to include concurrent and sequential provision of the compound or salt and other agents.

The compounds disclosed herein are STING agonists and inhibitors of viral replication. These compounds are potentially useful in treating diseases or disorders including, but not limited to, cell proliferation disorders, such as cancer.

Cell-proliferation disorders include, but are not limited to, cancer. Examples of such cancers include, but are not limited to, Acute Lymphoblastic Leukemia; Acute Myeloid Leukemia; Adrenocortical Carcinoma; AIDS-Related Lymphoma; AIDS-Related Malignancies; Anal Cancer; Astrocytoma; Bile Duct Cancer; Bladder Cancer; Bone Cancer, Osteosarcoma/Malignant Fibrous Histiocytoma; Brain Stem Glioma; Brain Tumor, Cerebellar Astrocytoma; Brain Tumor, Cerebral Astrocytoma/Malignant Glioma; Brain Tumor, Ependymoma; Brain Tumor, Medulloblastoma; Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors; Brain Tumor, Visual Pathway and Hypothalamic Glioma; Breast Cancer; Bronchial Adenomas/Carcinoids; Carcinoid Tumor; Carcinoid Tumor, Gastrointestinal; Carcinoma, Adrenocortical; Carcinoma, Islet Cell; Central Nervous System Lymphoma, Primary; Cerebral Astrocytoma/Malignant Glioma; Cervical Cancer; Chronic Lymphocytic Leukemia; Chronic Myelogenous Leukemia; Chronic Myeloproliferative Disorders; Clear Cell Sarcoma of Tendon Sheaths; Colon Cancer; Colorectal Cancer; Cutaneous T-Cell Lymphoma; Endometrial Cancer; Ependymoma; Epithelial Cancer, Ovarian; Esophageal Cancer; Esophageal Cancer; Ewing's Family of Tumors; Extracranial Germ Cell Tumor; Extrahepatic Bile Duct Cancer; Eye Cancer, Intraocular Melanoma; Eye Cancer, Retinoblastoma; Gallbladder Cancer; Gastric (Stomach) Cancer; Gastrointestinal Carcinoid Tumor; Germ Cell Tumor, Extracranial, Childhood; Germ Cell Tumor, Extragonadal; Germ Cell Tumor, Ovarian; Gestational Trophoblastic Tumor; Glioma, Childhood Brain Stem; Glioma, Childhood Visual Pathway and Hypothalamic; Hairy Cell Leukemia; Head and Neck Cancer; Hepatocellular (Liver) Cancer; Hodgkin's Lymphoma; Hypopharyngeal Cancer; Hypothalamic and Visual Pathway Glioma; Intraocular Melanoma; Islet Cell Carcinoma (Endocrine Pancreas); Kaposi's Sarcoma; Kidney Cancer; Laryngeal Cancer; Leukemia, Acute Lymphoblastic; Leukemia, Acute Myeloid; Leukemia, Chronic Lymphocytic; Leukemia, Chronic Myelogenous; Leukemia, Hairy Cell; Lip and Oral Cavity Cancer; Liver Cancer; Lung Cancer, Non-Small Cell; Lung Cancer, Small Cell; Lymphoblastic Leukemia; Lymphoma, AIDS- Related; Lymphoma, Central Nervous System (Primary); Lymphoma, Cutaneous T-Cell; Lymphoma, Hodgkin's; Lymphoma, Hodgkin's During Pregnancy; Lymphoma, Non-Hodgkin's; Lymphoma, Primary Central Nervous System;

Macroglobulinemia, Waldenstrom's; Male Breast Cancer; Malignant Mesothelioma; Malignant Thymoma; Medulloblastoma, Childhood; Melanoma; Melanoma, Intraocular; Merkel Cell Carcinoma; Mesothelioma, Malignant; Metastatic Squamous Neck Cancer with Occult Primary; Multiple Endocrine Neoplasia Syndrome, Childhood; Multiple Myeloma/Plasma Cell Neoplasm; Mycosis Fungoides; Myelodysplastic Syndromes; Myelogenous Leukemia, Chronic; Myeloid Leukemia; Myeloma, Multiple; Myeloproliferative Disorders, Chronic; Nasal Cavity and Paranasal Sinus Cancer; Nasopharyngeal Cancer; Neuroblastoma; Non-Hodgkin's Lymphoma; Non-Small Cell Lung Cancer; Oral Cancer; Oral Cavity and Lip Cancer; Oropharyngeal Cancer; steosarcoma/Malignant Fibrous Histiocytoma of Bone; Ovarian Epithelial Cancer; Ovarian Germ Cell Tumor; Ovarian Low Malignant Potential Tumor; Pancreatic Cancer; Paranasal Sinus and Nasal Cavity Cancer; Parathyroid Cancer; Penile Cancer; Pheochromocytoma; Pineal and Supratentorial Primitive Neuroectodermal Tumors; Pituitary Tumor; Plasma Cell Neoplasm/Multiple Myeloma; Pleuropulmonary Blastoma; Pregnancy and Breast Cancer; Pregnancy and Hodgkin's Lymphoma; Pregnancy and Non-Hodgkin's Lymphoma; Primary Central Nervous System Lymphoma; Primary Liver Cancer; Prostate Cancer; Rectal Cancer; Renal Cell (Kidney) Cancer; Renal Pelvis and Ureter, Transitional Cell Cancer; Retinoblastoma; Rhabdomyosarcoma; Salivary Gland Cancer; Sarcoma, Ewing's Family of Tumors; Sarcoma, Kaposi's; Sarcoma (Osteosarcoma)/Malignant Fibrous Histiocytoma of Bone; Sarcoma, Soft Tissue; Sezary Syndrome; Skin Cancer; Skin Cancer (Melanoma); Skin Carcinoma, Merkel Cell; Small Cell Lung Cancer; Small Intestine Cancer; Soft Tissue Sarcoma; Squamous Neck Cancer with Occult Primary, Metastatic; Stomach (Gastric) Cancer; Supratentorial Primitive Neuroectodermal Tumors; T- Cell Lymphoma, Cutaneous; Testicular Cancer; Thymoma, Malignant; Thyroid Cancer; Transitional Cell Cancer of the Renal Pelvis and Ureter; Trophoblastic Tumor, Gestational; Ureter and Renal Pelvis, Transitional Cell Cancer; Urethral Cancer; Uterine Sarcoma; Vaginal Cancer; Visual Pathway and Hypothalamic Glioma; Vulvar Cancer; Waldenstrom's Macro globulinemia; and Wilms' Tumor.

In one embodiment, the cancer is brain cancer, such as an astrocytic tumor (*e.g.*, pilocytic astrocytoma, subependymal giant-cell astrocytoma, diffuse astrocytoma, pleomorphic xanthoastrocytoma, anaplastic astrocytoma, astrocytoma, giant cell glioblastoma, glioblastoma, secondary glioblastoma, primary adult glioblastoma, and primary pediatric glioblastoma); oligodendroglial tumor (*e.g.*, oligodendroglioma, and anaplastic oligodendroglioma); oligoastrocytic tumor (*e.g.*, oligoastrocytoma, and anaplastic oligoastrocytoma); ependymoma (*e.g.*, myxopapillary ependymoma, and anaplastic ependymoma); medulloblastoma; primitive

neuroectodermal tumor, schwannoma, meningioma, atypical meningioma, anaplastic meningioma; and pituitary adenoma. In another embodiment, the brain cancer is glioma, glioblastoma multiforme, paraganglioma, or suprantentorial primordial neuroectodermal tumors (sPNET).

In another embodiment, the cancer is leukemia, such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic myelogenous leukemia (CML), myeloproliferative neoplasm (MPN), post-MPN AML, post-MDS AML, del(5q)-associated high risk MDS or AML, blast-phase chronic myelogenous leukemia, angioimmunoblastic lymphoma, and acute lymphoblastic leukemia.

In one embodiment, the cancer is skin cancer, including melanoma. In another embodiment, the cancer is prostate cancer, breast cancer, thyroid cancer, colon cancer, or lung cancer. In another embodiment, the cancer is sarcoma, including central chondrosarcoma, central and periosteal chondroma, and fibrosarcoma. In another embodiment, the cancer is cholangiocarcinoma.

As used herein, the terms "treatment" and "treating" refer to all processes wherein there may be a slowing, interrupting, arresting, controlling, or stopping of the progression of a disease or disorder described herein. The terms do not necessarily indicate a total elimination of all disease or disorder symptoms.

The terms "administration of" and or "administering" a compound should be understood to include providing a compound described herein, or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof, and compositions of the foregoing to a subject.

The amount of a compound administered to a subject is an amount sufficient to induce an immune response and/or to induce STING-dependent type I interferon production in the subject. In an embodiment, the amount of a compound can be an "effective amount" or "therapeutically effective amount," wherein the subject compound is administered in an amount that will elicit, respectively, a biological or medical (*i.e.*, intended to treat) response of a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician. An effective amount does not necessarily include considerations of toxicity and safety related to the administration of a compound.

An effective amount of a compound will vary with the particular compound chosen (*e.g.*, considering the potency, efficacy, and/or half-life of the compound); the route of administration chosen; the condition being treated; the severity of the condition being treated; the age, size, weight, and physical condition of the subject being treated; the medical history of the subject

being treated; the duration of the treatment; the nature of a concurrent therapy; the desired therapeutic effect; and like factors and can be routinely determined by the skilled artisan.

The compounds disclosed herein may be administered by any suitable route including oral and parenteral administration. Parenteral administration is typically by injection or infusion and includes intravenous, intramuscular, and subcutaneous injection or infusion.

The compounds disclosed herein may be administered once or according to a dosing regimen wherein a number of doses are administered at varying intervals of time for a given period of time. For example, doses may be administered one, two, three, or four times per day. Doses may be administered until the desired therapeutic effect is achieved or indefinitely to maintain the desired therapeutic effect. Suitable dosing regimens for a compound disclosed herein depend on the pharmacokinetic properties of that compound, such as absorption, distribution and half-life which can be determined by a skilled artisan. In addition, suitable dosing regimens, including the duration such regimens are administered, for a compound disclosed herein depend on the disease or condition being treated, the severity of the disease or condition, the age and physical condition of the subject being treated, the medical history of the subject being treated, the nature of concurrent therapy, the desired therapeutic effect, and like factors within the knowledge and expertise of the skilled artisan. It will be further understood by such skilled artisans that suitable dosing regimens may require adjustment given an individual subject's response to the dosing regimen or over time as the individual subject needs change. Typical daily dosages may vary depending upon the particular route of administration chosen.

One embodiment of the present disclosure provides for a method of treating a cell proliferation disorder comprising administration of a therapeutically effective amount of a compound of general formula (I), a compound of general formula (I'), or a compound of general formula (I'') to a subject in need of treatment thereof. In one embodiment, the cell proliferation disorder is cancer.

In one embodiment, the cancer is brain cancer, leukemia, skin cancer, prostate cancer, thyroid cancer, colon cancer, lung cancer or sarcoma. In another embodiment the cancer is selected from the group consisting of glioma, glioblastoma multiforme, paraganglioma, suprantentorial primordial neuroectodermal tumors, acute myeloid leukemia, myelodysplastic syndrome, chronic myelogenous leukemia, melanoma, breast, prostate, thyroid, colon, lung, central chondrosarcoma, central and periosteal chondroma tumors, fibrosarcoma, and cholangiocarcinoma.

In one embodiment, disclosed herein is the use of a compound of general formula (I), compound of general formula (I'), and/or compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof, in a therapy. The compound may be useful in a method of inducing an immune response and/or inducing STING-dependent type I interferon production in a subject, such as a mammal in need of such inhibition, comprising administering an effective amount of the compound to the subject.

In one embodiment, disclosed herein is a pharmaceutical composition comprising at least one compound of general formula (I), compound of general formula (I'), and/or compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof, for use in potential treatment to induce an immune response and/or to induce STING-dependent type I interferon production.

In one embodiment, disclosed herein is the use of a compound of general formula (I), compound of general formula (I'), and/or compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof, in the manufacture of a medicament for the treatment to induce an immune response and/or to induce STING-dependent type I interferon production. In one embodiment, the disease or disorder to be treated is a cell proliferation disorder. In another embodiment, the cell proliferation disorder is cancer. In another embodiment, the cancer is brain cancer, leukemia, skin cancer, breast, prostate cancer, thyroid cancer, colon cancer, lung cancer, or sarcoma. In another embodiment, the cancer is glioma, glioblastoma multiforme, paraganglioma, suprantentorial primordial neuroectodermal tumors, acute myeloid leukemia, myelodysplastic syndrome, chronic myelogenous leukemia, melanoma, breast, prostate, thyroid, colon, lung, central chondrosarcoma, central and periosteal chondroma tumors, fibrosarcoma, and/or cholangiocarcinoma.

Compositions

The term "composition" as used herein is intended to encompass a dosage form comprising a specified compound in a specified amount, as well as any dosage form which results, directly or indirectly, from combination of a specified compound in a specified amount. Such term is intended to encompass a dosage form comprising a compound of general formula (I), a compound of general formula (I'), and/or a compound of general formula (I''), or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, and one or more pharmaceutically acceptable carriers or excipients. Accordingly, the compositions of the present disclosure encompass any composition made by admixing a compound of the present disclosure

and one or more pharmaceutically acceptable carrier or excipients. By "pharmaceutically acceptable", it is meant the carriers or excipients are compatible with the compound disclosed herein and with other ingredients of the composition.

For the purpose of inducing an immune response and/or inducing a STING-dependent type I interferon production, the compounds, optionally in the form of a salt, hydrate, solvate or prodrug, can be administered by means that produces contact of the active agent with the agent's site of action. They can be administered by conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or in a combination of therapeutic agents. They can be administered alone, but typically are administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

In one embodiment, disclosed herein is a composition comprising a compound of general formula (I), a compound of general formula (I'), and/or a compound of general formula (I''), or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable carriers or excipients. The composition may be prepared and packaged in bulk form wherein an effective amount of a compound of the disclosure can be extracted and then given to a subject, such as with powders or syrups. Alternatively, the composition may be prepared and packaged in unit dosage form wherein each physically discrete unit contains an effective amount of a compound of general formula (I), a compound of general formula (I'), and/or a compound of general formula (I'').

The compounds disclosed herein and a pharmaceutically acceptable carrier or excipient(s) will typically be formulated into a dosage form adapted for administration to a subject by a desired route of administration. For example, dosage forms include those adapted for (1) oral administration, such as tablets, capsules, caplets, pills, troches, powders, syrups, elixirs, suspensions, solutions, emulsions, sachets, and cachets; and (2) parenteral administration, such as sterile solutions, suspensions, and powders for reconstitution. Suitable pharmaceutically acceptable carriers or excipients will vary depending upon the particular dosage form chosen. In addition, suitable pharmaceutically acceptable carriers or excipients may be chosen for a particular function that they may serve in the composition. For example, certain pharmaceutically acceptable carriers or excipients may be chosen for their ability to facilitate the production of uniform dosage forms. Certain pharmaceutically acceptable carriers or excipients may be chosen for their ability to facilitate the production of stable dosage forms. Certain pharmaceutically acceptable carriers or excipients may be chosen for their ability to facilitate the

carrying or transporting of a compound disclosed herein, once administered to the subject, from one organ or portion of the body to another organ or another portion of the body. Certain pharmaceutically acceptable carriers or excipients may be chosen for their ability to enhance patient compliance.

Suitable pharmaceutically acceptable excipients include the following types of excipients: diluents, lubricants, binders, disintegrants, fillers, glidants, granulating agents, coating agents, wetting agents, solvents, co-solvents, suspending agents, emulsifiers, sweeteners, flavoring agents, flavor masking agents, coloring agents, anti-caking agents, hemectants, chelating agents, plasticizers, viscosity increasing agents, antioxidants, preservatives, stabilizers, surfactants, and buffering agents.

A skilled artisan possesses the knowledge and skill in the art to select suitable pharmaceutically acceptable carriers and excipients in appropriate amounts for the use in the disclosure. In addition, there are a number of resources available to the skilled artisan, which describe pharmaceutically acceptable carriers and excipients and may be useful in selecting suitable pharmaceutically acceptable carriers and excipients. Examples include Remington's Pharmaceutical Sciences (Mack Publishing Company), The Handbook of Pharmaceutical Additives (Gower Publishing Limited), and The Handbook of Pharmaceutical Excipients (the American Pharmaceutical Association and the Pharmaceutical Press).

The compositions of the disclosure are prepared using techniques and methods known to those skilled in the art. Some methods commonly used in the art are described in Remington's Pharmaceutical Sciences (Mack Publishing Company).

In one embodiment, the disclosure is directed to a solid oral dosage form such as a tablet or capsule comprising an effective amount of a compound of the disclosure and a diluent or filler. Suitable diluents and fillers include lactose, sucrose, dextrose, mannitol, sorbitol, starch (*e.g.*, corn starch, potato starch, and pre-gelatinized starch), cellulose and its derivatives, (*e.g.*, microcrystalline cellulose), calcium sulfate, and dibasic calcium phosphate. The oral solid dosage form may further comprise a binder. Suitable binders include starch (*e.g.*, corn starch, potato starch, and pre-gelatinized starch) gelatin, acacia, sodium alginate, alginic acid, tragacanth, guar gum, povidone, and cellulose and its derivatives (*e.g.*, microcrystalline cellulose). The oral solid dosage form may further comprise a disintegrant. Suitable disintegrants include crospovidone, sodium starch glycolate, croscarmelose, alginic acid, and sodium carboxymethyl cellulose. The oral solid dosage form may further comprise a lubricant. Suitable lubricants include stearic acid, magnesium stearate, calcium stearate, and talc.

Where appropriate, dosage unit formulations for oral administration can be microencapsulated. The composition can also be prepared to prolong or sustain the release as, for example, by coating or embedding particulate material in polymers, wax, or the like.

The compounds disclosed herein may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyrancopolymer, polyhydroxypropylmethacrylamidephenol, polyhydroxyethylaspartamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds of the disclosure may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example polylactic acid, polepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanacrylates and cross-linked or amphipathic block copolymers of hydrogels.

In one embodiment, the disclosure is directed to a liquid oral dosage form. Oral liquids such as solution, syrups and elixirs can be prepared in dosage unit form so that a given quantity contains a predetermined amount of a compound disclosed herein. Syrups can be prepared by dissolving the compound of the disclosure in a suitably flavored aqueous solution; while elixirs are prepared through the use of a non-toxic alcoholic vehicle. Suspensions can be formulated by dispersing a compound disclosed herein in a non-toxic vehicle. Solubilizers and emulsifiers such as ethoxylated isostearyl alcohols and polyoxy ethylene sorbitol ethers, preservatives, flavor additives such as peppermint oil or other natural sweeteners or saccharin or other artificial sweeteners and the like can also be added.

In one embodiment, the disclosure is directed to compositions for parenteral administration. Compositions adapted for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions that may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

Combinations

The compounds of general formula (I), compounds of general formula (I'), and/or compounds of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, may be administered in combination with one or more additional therapeutic agents. In embodiments, one or more a compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, and the one or more additional therapeutic agents may be co-adminstered. The additional therapeutic agent(s) may be administered in a single dosage form with the compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, or the additional therapeutic agent(s) may be administered in separate dosage form(s) from the dosage form containing the compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof. The additional therapeutic agent(s) may be one or more agents selected from the group consisting of STING agonist compounds, anti-viral compounds, antigens, adjuvants, anti-cancer agents, CTLA-4, LAG-3 and PD-1 pathway antagonists, lipids, peptides, cytotoxic agents, chemotherapeutic agents, immunomodulatory cell lines, checkpoint inhibitors, vascular endothelial growth factor (VEGF) receptor inhibitors, topoisomerase II inhibitors, smoothen inhibitors, alkylating agents, anti-tumor antibiotics, anti-metabolites, retinoids, and immunomodulatory agents including but not limited to anti-cancer vaccines. It will be understood the descriptions of the above additional therapeutic agents may be overlapping. It will also be understood that the treatment combinations are subject to optimization, and it is understood that the best combination to use of the compounds of general formula (I), compounds of general formula (I'), or compounds of general formula (I'') and one or more additional therapeutic agents will be determined based on the individual patient needs.

A compound disclosed herein may be used in combination with one or more other active agents, including but not limited to, other anti-cancer agents that are used in the prevention, treatment, control, amelioration, or reduction of risk of a particular disease or condition (*e.g.*, cell proliferation disorders). In one embodiment, a compound disclosed herein is combined with one or more other anti-cancer agents for use in the prevention, treatment, control amelioration, or reduction of risk of a particular disease or condition for which the compounds disclosed herein are useful. Such other active agents may be administered, by a route and in an amount

commonly used therefor, contemporaneously or sequentially with a compound of the present disclosure.

When a compound disclosed herein is used contemporaneously with one or more other active agents, a composition containing such other active agents in addition to the compound disclosed herein is contemplated. Accordingly, the compositions of the present disclosure include those that also contain one or more other active ingredients, in addition to a compound disclosed herein. A compound disclosed herein may be administered either simultaneously with, or before or after, one or more other therapeutic agent(s). A compound disclosed herein may be administered separately, by the same or different route of administration, or together in the same pharmaceutical composition as the other agent(s).

Products provided as combinations may include a composition comprising a compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, and one or more other active agent(s) together in the same pharmaceutical composition, or may include a composition comprising a compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, and a composition comprising one or more other therapeutic agent(s) in separate form, *e.g.* in the form of a kit or in any form designed to enable separate administration either concurrently or on separate dosing schedules.

The weight ratio of a compound disclosed herein to a second active agent may be varied and will depend upon the effective dose of each agent. Generally, an effective dose of each will be used. Combinations of a compound disclosed herein and other active agents will generally also be within the aforementioned range, but in each case, an effective dose of each active agent should be used. In such combinations, the compound disclosed herein and other active agents may be administered separately or in conjunction. In addition, the administration of one element may be prior to, concurrent to, or subsequent to the administration of other agent(s).

In one embodiment, this disclosure provides a composition comprising a compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, and at least one other therapeutic agent as a combined preparation for simultaneous, separate or sequential use in therapy. In one embodiment, the therapy is the treatment of a cell proliferation disorder, such as cancer.

In one embodiment, the disclosure provides a kit comprising two or more separate pharmaceutical compositions, at least one of which contains a compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof. In one embodiment, the kit comprises means for separately retaining said compositions, such as a container, divided bottle, or divided foil packet. An example of such a kit is a blister pack, as typically used for the packaging of tablets, capsules, and the like.

A kit of this disclosure may be used for administration of different dosage forms, for example, oral and parenteral, for administration of the separate compositions at different dosage intervals, or for titration of the separate compositions against one another. To assist with compliance, a kit of the disclosure typically comprises directions for administration.

Disclosed herein is a use of a compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, for treating a cell proliferation disorder, wherein the medicament is prepared for administration with another active agent. The disclosure also provides the use of another active agent for treating a cell proliferation disorder, wherein the medicament is administered with a compound of general formula (I).

The disclosure also provides the use of a compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, for treating a cell proliferation disorder, wherein the patient has previously (*e.g.*, within 24 hours) been treated with another active agent. The disclosure also provides the use of another therapeutic agent for treating a cell proliferation disorder, wherein the patient has previously (*e.g.*, within 24 hours) been treated with a compound of general formula (I), compound of general formula (I'), or compound of general formula (I''), or pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof. The second agent may be applied a week, several weeks, a month, or several months after the administration of a compound disclosed herein.

STING agonist compounds that may be used in combination with the compounds of general formula (I), compounds of general formula (I'), and compounds of general formula (I'') disclosed herein include but are not limited to cyclic di-nucleotide compounds.

Anti-viral compounds that may be used in combination with the compounds of general formula (I), compounds of general formula (I'), and compounds of general formula (I'') disclosed herein include hepatitis B virus (HBV) inhibitors, hepatitis C virus (HCV) protease inhibitors,

HCV polymerase inhibitors, HCV NS4A inhibitors, HCV NS5A inhibitors, HCV NS5b inhibitors, and human immunodeficiency virus (HIV) inhibitors.

Antigens and adjuvants that may be used in combination with the compounds of general formula (I), compounds of general formula (I'), and compounds of general formula (I'') disclosed herein include B7 costimulatory molecule, interleukin-2, interferon-y, GM-CSF, CTLA-4 antagonists, OX-40/0X-40 ligand, CD40/CD40 ligand, sargramostim, levamisol, vaccinia virus, Bacille Calmette-Guerin (BCG), liposomes, alum, Freund's complete or incomplete adjuvant, detoxified endotoxins, mineral oils, surface active substances such as lipolecithin, pluronic polyols, polyanions, peptides, and oil or hydrocarbon emulsions. Adjuvants, such as aluminum hydroxide or aluminum phosphate, can be added to increase the ability of the vaccine to trigger, enhance, or prolong an immune response. Additional materials, such as cytokines, chemokines, and bacterial nucleic acid sequences, like CpG, a toll-like receptor (TLR) 9 agonist as well as additional agonists for TLR 2, TLR 4, TLR 5, TLR 7, TLR 8, TLR9, including lipoprotein, LPS, monophosphoryllipid A, lipoteichoic acid, imiquimod, resiquimod, and in addition retinoic acid-inducible gene I (RIG-I) agonists such as poly I:C, used separately or in combination with the described compositions are also potential adjuvants.

CLTA-4 and PD-1 pathways are important negative regulators of immune response. Activated T-cells upregulate CTLA-4, which binds on antigen-presenting cells and inhibits T-cell stimulation, IL-2 gene expression, and T-cell proliferation; these anti-tumor effects have been observed in mouse models of colon carcinoma, metastatic prostate cancer, and metastatic melanoma. PD-1 binds to active T-cells and suppresses T-cell activation; PD-1 antagonists have demonstrated anti-tumor effects as well. CTLA-4 and PD-1 pathway antagonists that may be used in combination with the compounds of general formula (I) disclosed herein include ipilimumab, tremelimumab, nivolumab, pembrolizumab, CT-011, AMP-224, and MDX-1106.

"PD-1 antagonist" or "PD-1 pathway antagonist" means any chemical compound or biological molecule that blocks binding of PD-L1 expressed on a cancer cell to PD-1 expressed on an immune cell (T-cell, B-cell, or NKT-cell) and preferably also blocks binding of PD-L2 expressed on a cancer cell to the immune-cell expressed PD-1. Alternative names or synonyms for PD-1 and its ligands include: PDCD1, PD1, CD279, and SLEB2 for PD-1; PDCD1L1, PDL1, B7H1, B7-4, CD274, and B7-H for PD-L1; and PDCD1L2, PDL2, B7-DC, Btdc, and CD273 for PD-L2. In any of the treatment method, medicaments and uses of the present disclosure in which a human individual is being treated, the PD-1 antagonist blocks binding of human PD-L1 to human PD-1, and preferably blocks binding of both human PD-L1 and PD-L2 to human PD-1.

Human PD-1 amino acid sequences can be found in NCBI Locus No.: NP_005009. Human PD-L1 and PD-L2 amino acid sequences can be found in NCBI Locus No.: NP_054862 and NP_079515, respectively.

PD-1 antagonists useful in any of the treatment method, medicaments and uses of the present disclosure include a monoclonal antibody (mAb), or antigen binding fragment thereof, which specifically binds to PD-1 or PD-L1, and preferably specifically binds to human PD-1 or human PD-L1. The mAb may be a human antibody, a humanized antibody, or a chimeric antibody and may include a human constant region. In some embodiments, the human constant region is selected from the group consisting of IgG1, IgG2, IgG3, and IgG4 constant regions, and in preferred embodiments, the human constant region is an IgG1 or IgG4 constant region. In some embodiments, the antigen binding fragment is selected from the group consisting of Fab, Fab'-SH, $F(ab')_2$, scFv, and Fv fragments.

Examples of mAbs that bind to human PD-1, and useful in the treatment method, medicaments and uses of the present disclosure, are described in U.S. Patent Nos. US7488802, US7521051, US8008449, US8354509, and US8168757, PCT International Patent Application Publication Nos. WO2004/004771, WO2004/072286, and WO2004/056875, and U.S. Patent Application Publication No. US2011/0271358.

Examples of mAbs that bind to human PD-L1, and useful in the treatment method, medicaments and uses of the present disclosure, are described in PCT International Patent Application Nos. WO2013/019906 and WO2010/077634 A1 and in U.S. Patent No. US8383796. Specific anti-human PD-L1 mAbs useful as the PD-1 antagonist in the treatment method, medicaments and uses of the present disclosure include MPDL3280A, BMS-936559, MEDI4736, MSB0010718C, and an antibody that comprises the heavy chain and light chain variable regions of SEQ ID NO:24 and SEQ ID NO:21, respectively, of WO2013/019906.

Other PD-1 antagonists useful in any of the treatment method, medicaments, and uses of the present disclosure include an immune-adhesion that specifically binds to PD-1 or PD-L1, and preferably specifically binds to human PD-1 or human PD-L1, *e.g.*, a fusion protein containing the extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region such as an Fc region of an immunoglobulin molecule. Examples of immune-adhesion molecules that specifically bind to PD-1 are described in PCT International Patent Application Publication Nos. WO2010/027827 and WO2011/066342. Specific fusion proteins useful as the PD-1 antagonist in the treatment method, medicaments, and uses of the present disclosure include AMP-224 (also known as B7-DCIg), which is a PD-L2-FC fusion protein and binds to human PD-1.

Examples of cytotoxic agents that may be used in combination with the compounds of general formula (I), compounds of general formula (I'), and compounds of general formula (I''), or pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof, disclosed herein include, but are not limited to, arsenic trioxide (sold under the tradename TRISENOX®), asparaginase (also known as L-asparaginase, and Erwinia L-asparaginase, sold under the tradenames ELSPAR® and KIDROLASE®).

Chemotherapeutic agents that may be used in combination with the compounds of general formula (I), compounds of general formula (I'), and compounds of general formula (I''), or pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof, disclosed herein include abiraterone acetate, altretamine, anhydrovinblastine, auristatin, bexarotene, bicalutamide, BMS 184476, 2,3,4,5,6-pentafluoro-N-(3-fluoro-4-methoxyphenyl)benzene sulfonamide, bleomycin, N,N-dimethyl-L-valyl-L-valyl-N-methyl-L-valyl-L-proly- 1-Lproline-tbutylamide, cachectin, cemadotin, chlorambucil, cyclophosphamide, 3',4'-didehydro-4'deoxy-8'-norvin-caleukoblastine, docetaxol, doxetaxel, cyclophosphamide, carboplatin, carmustine, cisplatin, cryptophycin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, daunorubicin, decitabine dolastatin, doxorubicin (adriamycin), etoposide, 5-fluorouracil, finasteride, flutamide, hydroxyurea and hydroxyureataxanes, ifosfamide, liarozole, lonidamine, lomustine (CCNU), MDV3100, mechlorethamine (nitrogen mustard), melphalan, mivobulin isethionate, rhizoxin, sertenef, streptozocin, mitomycin, methotrexate, taxanes, nilutamide, nivolumab, onapristone, paclitaxel, pembrolizumab, prednimustine, procarbazine, RPR109881, stramustine phosphate, tamoxifen, tasonermin, taxol, tretinoin, vinblastine, vincristine, vindesine sulfate, and vinflunine.

Examples of vascular endothelial growth factor (VEGF) receptor inhibitors include, but are not limited to, bevacizumab (sold under the trademark AVASTIN by Genentech/Roche), axitinib (described in PCT International Patent Publication No. WO01/002369), Brivanib Alaninate ((S)-((R)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate, also known as BMS-582664), motesanib (N-(2,3-dihydro-3,3-dimethyl-1H-indol-6-yl)-2-[(4-pyridinylmethyl)amino]-3-pyridinecarboxamide. and described in PCT International Patent Application Publication No. WO02/068470), pasireotide (also known as SO 230, and described in PCT International Patent Publication No. WO02/010192), and sorafenib (sold under the tradename NEXAVAR).

Examples of topoisomerase II inhibitors, include but are not limited to, etoposide (also known as VP-16 and Etoposide phosphate, sold under the tradenames TOPOSAR, VEPESID, and ETOPOPHOS), and teniposide (also known as VM-26, sold under the tradename VUMON).

Examples of alkylating agents, include but are not limited to, 5-azacytidine (sold under the trade name VIDAZA), decitabine (sold under the trade name of DECOGEN), temozolomide (sold under the trade names TEMODAR and TEMODAL by Schering-Plough/Merck), dactinomycin (also known as actinomycin-D and sold under the tradename COSMEGEN), melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, sold under the tradename ALKERAN), altretamine (also known as hexamethylmelamine (HMM), sold under the tradename HEXALEN), carmustine (sold under the tradename BCNU), bendamustine (sold under the tradename TREANDA), busulfan (sold under the tradenames BUSULFEX® and MYLERAN®), carboplatin (sold under the tradename PARAPLATIN®), lomustine (also known as CCNU, sold under the tradename CEENU®), cisplatin (also known as CDDP, sold under the tradenames PLATINOL® and PLATINOL®-AQ), chlorambucil (sold under the tradename LEUKERAN®), cyclophosphamide (sold under the tradenames CYTOXAN® and NEOSAR®), dacarbazine (also known as DTIC, DIC and imidazole carboxamide, sold under the tradename DTIC-DOME®), altretamine (also known as hexamethylmelamine (HMM) sold under the tradename HEXALEN®), ifosfamide (sold under the tradename IFEX®), procarbazine (sold under the tradename MATULANE®), mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, sold under the tradename MUSTARGEN®), streptozocin (sold under the tradename ZANOSAR®), thiotepa (also known as thiophosphoamide, TESPA and TSPA, and sold under the tradename THIOPLEX®.

Examples of anti-tumor antibiotics include, but are not limited to, doxorubicin (sold under the tradenames ADRIAMYCIN® and RUBEX®), bleomycin (sold under the tradename LENOXANE®), daunorubicin (also known as dauorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, sold under the tradename CERUBIDINE®), daunorubicin liposomal (daunorubicin citrate liposome, sold under the tradename DAUNOXOME®), mitoxantrone (also known as DHAD, sold under the tradename NOVANTRONE®), epirubicin (sold under the tradename ELLENCE™), idarubicin (sold under the tradenames IDAMYCIN®, IDAMYCIN PFS®), and mitomycin C (sold under the tradename MUTAMYCIN®).

Examples of anti-metabolites include, but are not limited to, claribine (2-chlorodeoxyadenosine, sold under the tradename LEUSTATIN®), 5-fluorouracil (sold under the tradename ADRUCIL®), 6-thioguanine (sold under the tradename PURINETHOL®), pemetrexed (sold under the tradename ALIMTA®), cytarabine (also known as arabinosylcytosine (Ara-C), sold under the tradename CYTOSAR-U®), cytarabine liposomal (also known as Liposomal Ara-C, sold under the tradename DEPOCYT™), decitabine (sold under the tradename DACOGEN®),

hydroxyurea (sold under the tradenames HYDREA®, DROXIA™ and MYLOCEL™), fludarabine (sold under the tradename FLUDARA®), floxuridine (sold under the tradename FUDR®), cladribine (also known as 2-chlorodeoxyadenosine (2-CdA) sold under the tradename LEUSTATIN™), methotrexate (also known as amethopterin, methotrexate sodium (MTX), sold under the tradenames RHEUMATREX® and TREXALL™), and pentostatin (sold under the tradename NIPENT®).

Examples of retinoids include, but are not limited to, alitretinoin (sold under the tradename PANRETIN®), tretinoin (all-trans retinoic acid, also known as ATRA, sold under the tradename VESANOID®), Isotretinoin (13-c/s-retinoic acid, sold under the tradenames ACCUTANE®, AMNESTEEM®, CLARAVIS®, CLARUS®, DECUTAN®, ISOTANE®, IZOTECH®, ORATANE®, ISOTRET®, and SOTRET®), and bexarotene (sold under the tradename TARGRETIN®).

Activity: STING Biochemical [3H]cGAMP Competition Assay

The individual compounds described in the Examples herein are defined as STING agonists by demonstrating binding to the STING protein with an EC_{50} of 20uM or less in the STING Biochemical [3H]cGAMP Competition Assay and demonstrating interferon production with a 20% or greater luminescence induction at 30uM in the IFN-β secretion in the THP1 cell assay.

The ability of compounds to bind STING is quantified by the ability to compete with tritiated cGAMP ligand for human STING receptor membrane using a radioactive filter-binding assay. The binding assay employs STING receptor obtained from Hi-Five cell membranes overexpressing full-length HAQ STING prepared in-house and tritiated cGAMP ligand also purified in-house.

ABBREVIATIONS

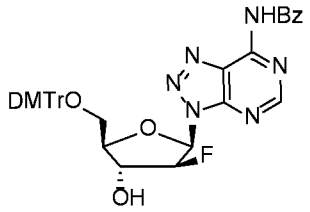
^{1}H -NMR	Proton nuclear magnetic resonance spectroscopy
^{19}F-NMR	^{19}F nuclear magnetic resonance spectroscopy
^{31}P-NMR	^{31}P nuclear magnetic resonance spectroscopy
Å	Angstrom
A^{Bz}	6-*N*-benzoyladenine
aq	Aqueous
Ar	Argon
ATP	Adenosine 5'-triphosphate

Bz	Benzoyl
CD_3OD	Deuterium-enriched methyl alcohol, deuterium-enriched methanol
$CHCl_3$	Trichloromethane
Ci	Curie, a non-standard unit of radioactivity; 1Ci = 3.7×10^{10}Bq, where Bq is Becquerel, the SI unit of radioactivity, equivalent to 1 disintegration per second (dps)
CO_2	Carbon dioxide
d	Doublet
d	Day(s)
D_2O	Deuterium-enriched water
DCA	Dichloroacetic acid
DCM, CH_2Cl_2	Dichloromethane
ddd	Doublet of doublet of doublet
ddt	Doublet of doublet of triplet
DDTT	(E)-N,N-dimethyl-N'-(3-thioxo-3H-1,2,4-dithiazol-5-yl)formimidamide
DMF	N,N-dimethylformamide
DMOCP	2-chloro-5,5-dimethyl-1,3,2-dioxaphosphineane 2-oxide
DMSO	Dimethyl sulfoxide
DMTr	4,4'-dimethoxytrityl
DMTrCl	4,4'-dimethoxytrityl chloride
dq	Doublet of quartet
EC_{50}	half maximal effective concentration, concentration of a drug, antibody or toxicant that induces a response halfway between the baseline and maximum after a specified exposure time
eq	Equivalents
ES	Electron spray
Et	Ethyl
Et_2O	Diethyl ether
Et_3SiH	Triethylsilane
EtOAc	Ethyl acetate
EtOH	Ethyl alcohol, ethanol
g	Gram
GTP	Guanosine 5'-triphosphate

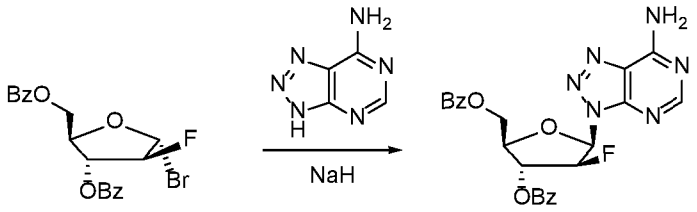
h	Hour
H_2O	Water
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid, a zwitterionic organic chemical buffering agent
hept	Heptet
Hex	Hexanes
HF-Pyr	Hydrogen fluoride – pyridine complex
HPLC	High performance liquid chromatography
Hz	Hertz
ITP	Inosine 5'-triphosphate
J	NMR Coupling constant
LCMS	Liquid chromatography – mass spectroscopy
m	Multiplet
M	Molar, moles per liter
mCi	Millicurie
Me	Methyl
MeCN	Acetonitrile
$MeNH_2$	Methylamine
mg	Milligram
$MgCl_2$	Magnesium chloride
MHz	Megahertz
min	Minute(s)
mL,ml	Milliliter
mM	Millimole per liter
mmol	Millimole
MOI	Multiplicity of infection
MPLC	Medium pressure liquid chromatography
MTBE	Methyl t-butyl ether, methyl tertiary butyl ether
Na_2SO_4	Sodium sulfate
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
$NaHSO_3$	Sodium bisulfite
NaOH	Sodium hydroxide

ng	Nanogram(s)
NH_4HCO_3	Ammonium bicarbonate
NH_4OH	Ammonium hydroxide
nL	Nanoliter
nm	Nanometer
nM	Nanomolar
P_2O_5	Phosphorus pentoxide
Py	Pyridine
q	Quartet
RPM, rpm	Revolutions per minute
RT, rt	Room temperature, approximately 25°C
s	Singlet
sat	Saturated
t	Triplet
TBS	t-Butyldimethylsilyl
TMA	Trimethylamine
TEA, Et_3N	Triethylamine
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography
TMSCl	Trimethylsilyl chloride
T_R	Retention time
TrisCl	Tris(hydroxymethyl)aminomethane hydrochloride
v/v	Volume/volume
λ_{em}	Emission wavelength
λ_{ex}	Excitation wavelength
µg	Microgram
µL, uL	Microliter
µM, uM	Micromolar

Preparation 1: N-(3-((2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)-methyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)benzamide

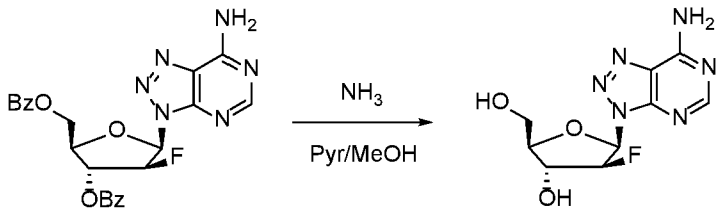


Step 1: (2R,3R,4S,5R)-5-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((benzoyloxy)methyl)-4-fluorotetrahydrofuran-3-yl benzoate



To a mixture of 3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-amine (2.36g, 17.4mmol) in NMP (50ml) was added NaH (60%, 0.744g, 18.6mmol). The mixture was vigorously stirred and after 1h, generation of bubbles had completely ceased. The mixture was added to ((2R,3R,4S,5R)-3-(benzoyloxy)-5-bromo-4-fluorotetrahydrofuran-2-yl)methyl benzoate (neat, 5.25g, 12.4mmol) in one portion. The reaction was stirred for 18h. LCMS showed several peaks with the desired mass (m/e = 479). EtOAc (70mL) and water (70mL) were added to the reaction. Layers were separated, and the organic layer was washed with half saturated brine (3x10mL) and brine (1x10mL), dried ($MgSO_4$), and concentrated. The crude was purified via silica column eluting with 0 to 50% EtOAc in Hex to give the product. LCMS (ES, m/z): 479.3 $[M + H]^+$. ^{1}H-NMR (500MHz, DMSO-d_6): δ 8.62 (s, 1H), 8.34 (s, 1H), 8.28 (s, 1H), 8.10-8.04 (m, 2H), 7.97-7.90 (m, 2H), 7.77-7.69 (m, 1H), 7.67-7.55 (m, 3H), 7.49-7.42 (m, 2H), 6.97 (dd, J = 6.5, 3.1Hz, 1H), 6.49 (dt, J = 17.6, 6.9Hz, 1H), 6.16 (dt, J = 56, 6.6Hz, 1H), 4.76-4.62 (m, 3H).

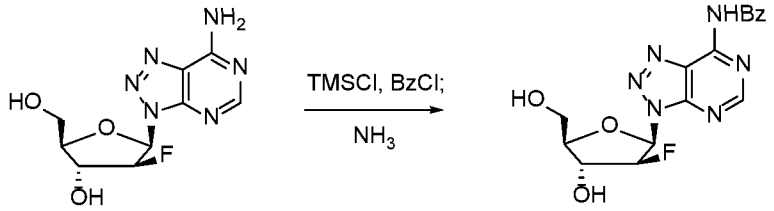
Step 2: (2R,3R,4S,5R)-5-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol



To a solution of (2R,3R,4S,5R)-5-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((benzoyloxy)methyl)-4-fluorotetrahydrofuran-3-yl benzoate (2.00g, 4.18mmol) in pyridine (10mL) at was added NH_3 in MeOH (7N, 20mL, 140mmol). It was stirred for 48h. LCMS showed completion of the reaction (m/e = 271). It was concentrated and purified by silica column chromatography eluting with 10% MeOH in CH_2Cl_2 to give the desired product. LCMS

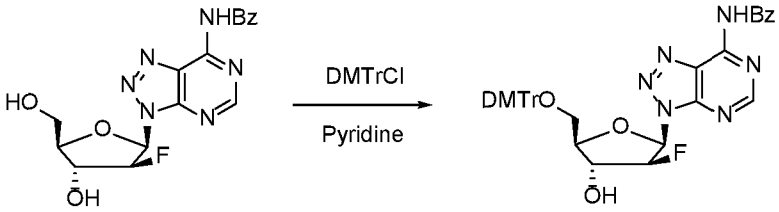
(ES, m/z): 271.1 $[M + H]^+$. ^{1}H-NMR (500MHz, DMSO-d6): δ 8.54 (s, 1H), 8.33 (s, 1H), 8.22 (s, 1H), 6.73 (dd, *J* = 6.5, 2.6Hz, 1H), 6.00 (d, *J* = 5.4Hz, 1H), 5.51 (ddd, *J* = 53, 7.2, 6.5Hz, 1H), 4.93 (t, *J* = 5.8Hz, 1H), 4.86-4.74 (m, 1H), 3.91-3.83 (m, 1H), 3.77-3.61 (m, 2H).

Step 3: N-(3-((2R,3S,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)benzamide



To a solution of (2R,3R,4S,5R)-5-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (1.34g, 4.96mmol) in pyridine (30mL) at 0°C was added TMSCl (1.46mL, 11.4mmol). It was warmed to rt and stirred for 1h. Then, it was recooled to 0°C and BzCl (0.921mL, 7.93mmol) was added dropwise. The reaction was slowly warmed to rt over 2h. LCMS showed completion of reaction (m/e = 375, 479). Water (3mL) was added. It was cooled to 0°C and NH_3 in MeOH (7N, 2.8mL, 20mmol) was added. After 1h, the reaction mixture was concentrated. It was purified by silica column chromatography eluting with 0 to 10% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 375.2 $[M + H]^+$. ^{1}H-NMR (500MHz, DMSO-d_6): δ 11.95 (s, 1H), 8.98 (s, 1H), 8.10 (d, *J* = 7.6Hz, 2H), 7.73-7.66 (m, 1H), 7.59 (t, *J* = 7.7Hz, 2H), 6.91 (d, *J* = 6.2Hz, 1H), 6.06 (d, *J* = 5.6Hz, 1H), 5.59 (t, *J* = 53, 6.8Hz, 1H), 4.90 (t, *J* = 5.8Hz, 1H), 4.82 (dq, *J* = 19.8, 7.0Hz, 1H), 3.92 (td, *J* = 7.6, 2.9Hz, 1H), 3.75 (ddd, *J* = 12.1, 5.6, 3.0Hz, 1H), 3.66 (dt, *J* = 12.0, 6.6Hz, 1H).

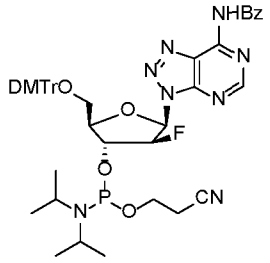
Step 4: N-(3-((2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)benzamide



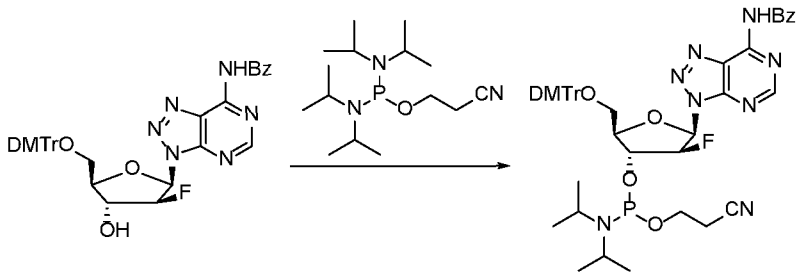
To a solution of N-(3-((2R,3S,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)benzamide (1.25g, 3.34mmol) in pyridine (15mL) at 0°C was added DMTrCl (1.58g, 4.68mmol). It was stirred at rt for 1h. LCMS showed a peak with the desired mass (m/e = 677). It was partly concentrated (to 5mL), and EtOAc (20mL) and water (10mL) were added. Layers were separated, and the aq layer was extracted with EtOAc (2x10mL). The combined organics were washed with brine (5mL), dried

($MgSO_4$), concentrated and purified by silica column chromatography eluting with 0 to 60% EtOAc in Hex to give the product. LCMS (ES, m/z): 675.5 $[M - H]^-$. ^{1}H-NMR (500MHz, DMSO-d_6): δ 8.13 - 8.07 (m, 2H), 7.69 (t, J = 7.4Hz, 1H), 7.59 (t, J = 7.6Hz, 2H), 7.35-7.29 (m, 2H), 7.23-7.10 (m, 6H), 6.97 (d, J = 6.5Hz, 1H), 6.81-6.74 (m, 2H), 6.74-6.67 (m, 2H), 6.07 (d, J = 5.7Hz, 1H), 5.62 (dt, J = 53, 7.0Hz, 1H), 4.91-4.79 (m, 1H), 4.15-4.07 (m, 1H), 3.69 (s, 3H), 3.67 (s, 3H), 3.44 (dd, J = 10.4, 8.0Hz, 1H), 3.21 (dd, J = 10.3, 2.4Hz, 1H).

Preparation 2: (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite



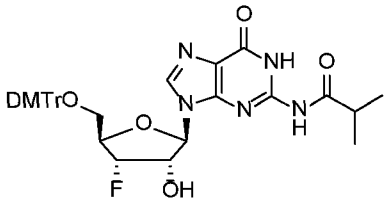
Step 1: (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite



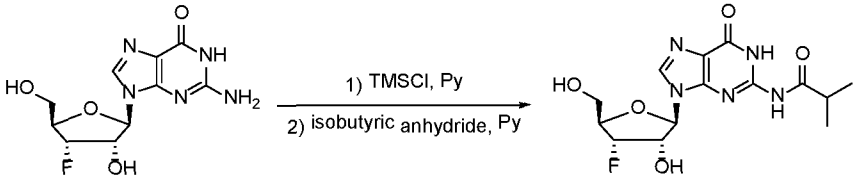
To a solution of 3-((bis(diisopropylamino)phosphino)oxy)propanenitrile (8.02g, 26.6mmol) in ACN (90mL) at rt was added pyridin-1-ium 2,2,2-trifluoroacetate (3.85g, 19.95mmol) and a solution of N-(3-((2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy) methyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)benzamide (9g, 13.30mmol) in ACN (90mL). The resulting mixture was stirred for 1h. Then, it was concentrated and the residue was dissolved in CH_2Cl_2 (1000mL). It was washed with aq $NaHCO_3$ (1%, 2x300mL), water (300mL) and brine (300mL), dried (Na_2SO_4), concentrated, and purified by reverse phase (C18) chromatography eluting with 0 to 95% ACN in water to give the product. LCMS (ES, m/z): 877.5 $[M + H]^+$. ^{1}H-NMR: (400MHz, DMSO-d_6): δ 12.01 (s, 1H), 8.92 (s, 1H), 8.11 (d, J = 7.6Hz, 2H), 7.66 (dt, J = 42.3, 7.5Hz, 3H), 7.32 (td J = 7.2, 6.6, 2.9Hz,

2H), 7.22-7.00 (m, 9H), 6.83-6.63 (m, 4H), 5.86 (ddt, J = 52.8, 17.6, 6.9Hz, 1H), 5.16 (td, J = 17.7, 17.2, 8.8Hz, 1H), 3.78-3.63 (m, 7H), 3.59-3.35 (m, 5H), 2.74 (t, J = 5.9Hz, 1H), 2.63 (t, J = 5.9Hz, 1H), 1.23-0.99 (m, 10H), 0.91 (d, J = 6.7Hz, 2H). ^{31}P-NMR: (162MHz, DMSO-d_6): δ 150.26, 149.60 (2 s, 1P).

Preparation 3. N-(9-((2R,3S,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide

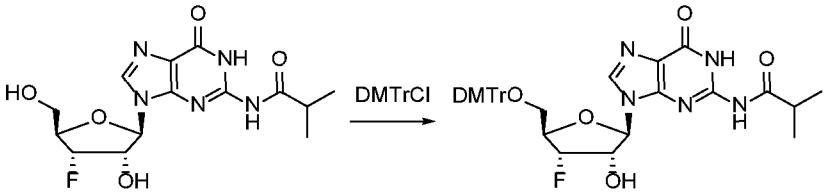


Step 1: N-(9-((2R,3S,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



To a suspension of 2-amino-9-((2R,3S,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one (Carbosynth catalog # ND10826, 1.50g, 5.26mmol) in pyridine (30mL) at 0-5°C was added TMSCl (2.86g, 26.3mmol), and the mixture was stirred at rt for 30min. Then, isobutyric anhydride (2.50g, 15.8mmol) was added dropwise, and it was stirred for an additional for 2h. Then, MeOH (5.3mL) was added. After 5min, NH_4OH (10.5mL) was added dropwise and stirring was continued for 30min. The reaction mixture was concentrated under reduced pressure, and MeOH (2mL) in CH_2Cl_2 (18mL) was added to the residue. Insolubles were filtered off, and the filtrate was concentrated and purified by flash column chromatography with 2-10% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 356.1 $[M + H]^+$. ^{1}H-NMR: (400MHz, DMSO-d_6): δ 12.11 (s, 1H), 11.68 (s, 1H), 8.28 (s, 1H), 5.98 (d, J = 6.1Hz, 1H), 5.85 (d, J = 8.0Hz, 1H), 5.24 (t, J = 5.4Hz, 1H), 5.14 (d, J = 4.1Hz, 0.5H), 5.01 (d, J = 4.2Hz, 0.5H), 4.87-4.69 (m, 1H), 4.26 (t, J = 4.4Hz, 0.5H), 4.19 (t, J = 4.4Hz, 0.5H), 3.61 (t, J = 4.9Hz, 2H), 2.77 (hept, J = 6.8Hz, 1H), 1.13 (d, J = 6.7Hz, 6H). ^{19}F-NMR: (376MHz, DMSO-d_6): δ -197.5 (s).

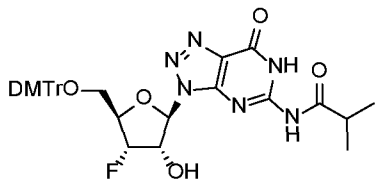
Step 2: N-(9-((2R,3S,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



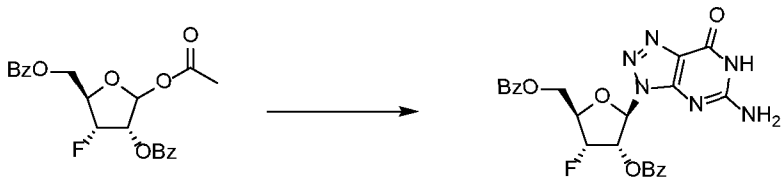
N-(9-((2R,3S,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (1.30g, 3.66mmol) was co-evaporated with pyridine (3x10mL) and re-dissolved in pyridine (26mL). To the solution at 0-5°C was added DMTrCl (1.36g, 4.02mmol). It was stirred at rt for 3h and then, concentrated. CH_2Cl_2 (40mL, with 1% Et_3N) was added, and it was washed with sat aq $NaHCO_3$ (15mL), water (10mL) and brine (10mL). The organic solution was dried (Na_2SO_4), concentrated and purified by silica gel column chromatography using 0-10% MeOH in CH_2Cl_2 (1% Et_3N) to give the product. LCMS (ES, m/z): 656.2 $[M - H]^-$. ^{1}H-NMR: (400MHz, DMSO-d_6): δ 12.10 (s, 1H), 11.61 (s, 1H), 8.14 (s, 1H), 7.40-7.31 (m, 2H), 7.31-7.19 (m, 7H), 6.89-6.78 (m, 4H), 6.08 (d, J = 6.1Hz, 1H), 5.87 (d, J = 7.3Hz, 1H), 5.23 (dd, J = 4.1, 1.8Hz, 0H), 5.10 (d, J = 4.4Hz, 0H), 4.96 (dq, J = 22.4, 5.9Hz, 1H), 4.30 (dt, J = 26.1, 4.6Hz, 1H), 3.74 (d, J = 1.1Hz, 6H), 3.39 (dd, J = 10.6, 5.7Hz, 1H), 3.22 (dd, J = 10.6, 3.8Hz, 1H), 2.76 (p, J = 6.8Hz, 1H), 1.13 (d, J = 6.8Hz, 6H). ^{19}F-NMR: (376MHz, DMSO-d_6): δ -198.1 (s, 1F).

The product of Preparation 3 may optionally be treated according to the procedures of Preparation 22, Steps 4 and 5 (below), to afford (2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate. LCMS (ES, m/z): 720 $[M-H]^-$.

Preparation 4: N-(3-((2R,3S,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide

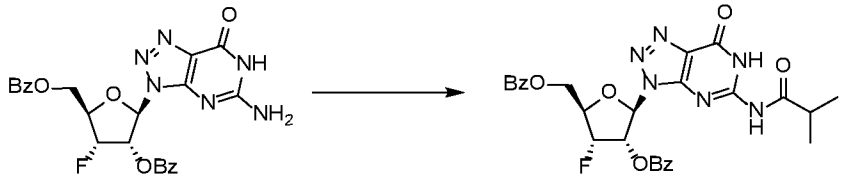


Step 1: ((2R,3R,4S,5R)-5-(5-amino-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-4-(benzoyloxy)-3-fluorotetrahydrofuran-2-yl)methyl benzoate



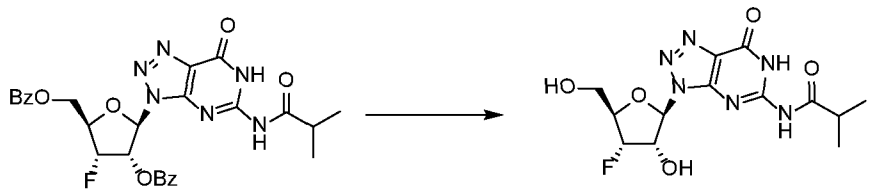
To a suspension of 8-azaguanine (5.14g, 33.8mmol) in anhydrous CH_3CN (100mL) at rt was added dropwise (E)-trimethylsilyl N-(trimethylsilyl)acetimidate (16.53mL, 67.6mmol), then the mixture was stirred at 70°C for 2h. The reaction was cooled to rt, and a solution of ((2R,3R,4S)-5-acetoxy-4-(benzoyloxy)-3-fluorotetrahydrofuran-2-yl)methyl benzoate (6.8g, 16.90mmol) in anhydrous CH_3CN (20mL) was added followed by dropwise addition of tin(IV) chloride (67.6mL, 67.6mmol). The homogeneous solution was stirred at 70°C for 2h. The reaction was cooled to rt and concentrated. The residue was dissolved in EtOAc (1000mL) and neutralized by pouring into sat aq $NaHCO_3$ (500mL). The organic layer was separated, and the aq layer was extracted with EtOAc (4x500mL). The organic layers were combined and washed with water (3x700mL), brine, dried over anhydrous sodium sulfate, filtered, and concentrated to afford title compound without further purification. LCMS (ES, m/z): 495.3 $[M + H]^+$.

Step 2: ((2R,3R,4S,5R)-4-(benzoyloxy)-3-fluoro-5-(5-isobutyramido-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)tetrahydrofuran-2-yl)methyl benzoate



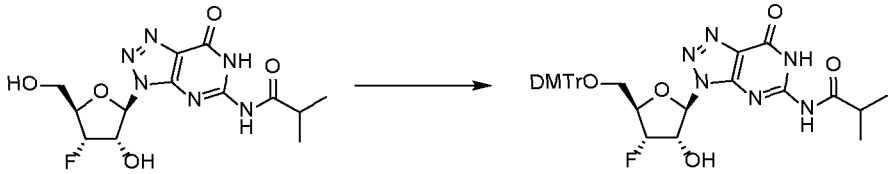
To a solution of ((2R,3R,4S,5R)-5-(5-amino-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-4-(benzoyloxy)-3-fluorotetrahydrofuran-2-yl)methyl benzoate (8g, 16.18mmol) from Step 1 in anhydrous DMA (40mL) at rt was added dropwise isobutyric anhydride (4.02mL, 24.27mmol). The mixture was stirred at 140°C for 4h. The reaction was cooled and diluted with EtOAc (600mL), washed with sat aq NH_4Cl (4x500mL), brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by MPLC (220 g silica gel, eluting with a gradient of 100% hexanes to 100% ethyl acetate) to afford ((2R,3R,4S,5R)-4-(benzoyloxy)-3-fluoro-5-(5-isobutyramido-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)tetrahydrofuran-2-yl)methyl benzoate. LCMS (ES, m/z): 565.3 $[M + H]^+$.

Step 3: N-(3-((2R,3S,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide



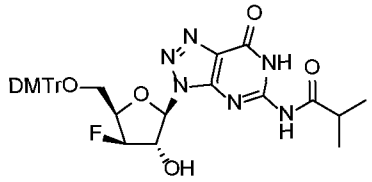
To a solution of ((2R,3R,4S,5R)-4-(benzoyloxy)-3-fluoro-5-(5-isobutyramido-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)tetrahydrofuran-2-yl)methyl benzoate (6g, 10.63mmol) in THF (20mL), CH_3OH (16mL), and water (4mL) at 0°C was added 5N aqueous NaOH (4.89mL, 24.45mmol) and stirred for 1h. The reaction was neutralized with formic acid (1.223mL, 31.9mmol). The solvent was removed, and the residue was purified by MPLC (120g, silica gel, eluting with a gradient of 100% CH_2Cl_2 to 20% CH_3OH/CH_2Cl_2) to afford N-(3-((2R,3S,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide. LCMS (ES, m/z): 357.2 $[M + H]^+$.

Step 4: N-(3-((2R,3S,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide



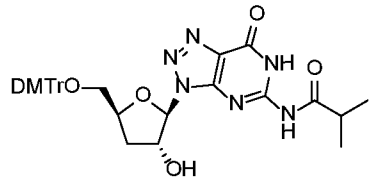
To a solution of N-(3-((2R,3S,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide (3g, 8.42mmol) in anhydrous pyridine (40mL) at 0°C was added 4,4'-dimethoxytrityl chloride (3.42g, 10.10mmol). The ice bath was removed, and the reaction mixture was allowed to reach RT and was stirred for 2h. The mixture was diluted with EtOAc (400mL), washed with sat aq $NaHCO_3$ (100mL), water (3x100mL), brine, and dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by MPLC (120 g silica gel, eluting with a gradient of 100% CH_2Cl_2 to 15% CH_3OH/CH_2CH_2 to afford N-(3-((2R,3S,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide. LCMS (ES, m/z): 659.3 $[M + H]^+$.

Preparation 5: N-(3-((2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d] pyrimidin-5-yl)isobutyramide



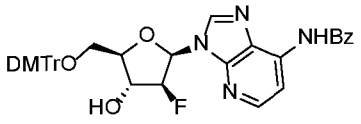
N-(3-((2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide was prepared according to procedures analogous to those described for Preparation **4** using the appropriately substituted ribose in Step 1. LCMS (ES, m/z): 659.4 [M + H]$^+$.

Preparation 6: N-(3-((2R,3R,5S)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-hydroxytetrahydrofuran-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl) isobutyramide

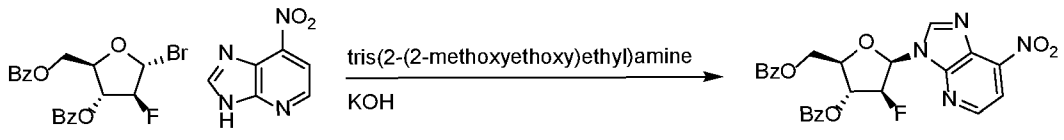


N-(3-((2R,3R,5S)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-hydroxytetrahydrofuran-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide was prepared according to procedures analogous to those described for Preparation **4** using the appropriately substituted ribose in Step 1. LCMS (ES, m/z): 641.2 [M + H]$^+$.

Preparation 7: 3-{5-*O*-[bis(4-methoxyphenyl)(phenyl)methyl]-2-deoxy-2-fluoro-β-D-arabinofuranosyl}-*N*-(phenylcarbonyl)-3*H*-imidazo[4,5-*b*]pyridin-7-amine

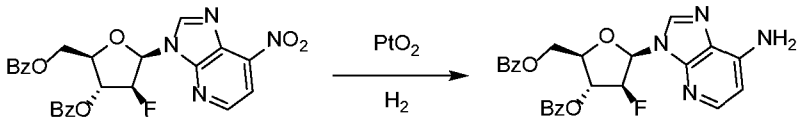


Step 1: 3-[2-deoxy-2-fluoro-3,5-bis-O-(phenylcarbonyl)-β-D-arabinofuranosyl]-7-nitro-3H-imidazo[4,5-b]pyridine



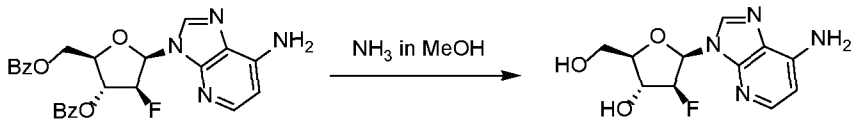
To a stirred mixture of freshly ground KOH (308mg, 5.48mmol) in acetonitrile (50mL) was added tris(2-(2-methoxyethoxy)ethyl)amine (0.070mL, 0.219mmol). The reaction mixture was aged for 15min at ambient temperature followed by addition of 7-nitro-3H-imidazo[4,5-b]pyridine (600mg, 3.66mmol) in a single portion. The resulting mixture was stirred at ambient temperature for 15min. A solution of 2-deoxy-2-fluoro-3,5-bis-*O*-(phenylcarbonyl)-α-D-arabinofuranosyl bromide (1700mg, 4.02mmol) in acetonitrile (10mL) was added dropwise and the resulting mixture was vigorously stirred at RT for 17h. The reaction mixture was diluted with sat aq ammonium chloride (80mL) and extracted with DCM (3x150mL). The organic extracts were combined, dried over sodium sulphate and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel; 120 g prepacked, (0-70% ethyl acetate/hexanes) to afford 3-[2-deoxy-2-fluoro-3,5-bis-*O*-(phenylcarbonyl)-β-D-arabinofuranosyl]-7-nitro-3*H*-imidazo[4,5-*b*]pyridine. MS: 507 $(M+H)^+$.

Step 2: 3-[2-deoxy-2-fluoro-3,5-bis-O-(phenylcarbonyl)-β-D-arabinofuranosyl]-3H-imidazo[4,5-b]pyridin-7-amine



To a stirred solution of 3-[2-deoxy-2-fluoro-3,5-bis-*O*-(phenylcarbonyl)-β-D-arabinofuranosyl]-7-nitro-3*H*-imidazo[4,5-*b*]pyridine (1380mg, 2.72mmol) in methanol (55mL) at RT was added platinum(IV) oxide (61.9mg, 0.272mmol). The reaction mixture was placed under an atmosphere of hydrogen and stirred at RT for 72h. The catalyst was removed by filtration through a plug of CELITE. The filtrate was concentrated under reduced pressure, and the resulting residue was purified by column chromatography on silica gel; 80g prepacked, ((0-40% (3:1, ethyl acetate:ethanol)/hexanes) to afford 3-[2-deoxy-2-fluoro-3,5-bis-*O*-(phenylcarbonyl)-β-D-arabinofuranosyl]-3*H*-imidazo[4,5-*b*]pyridin-7-amine. MS: 477 $(M+H)^+$.

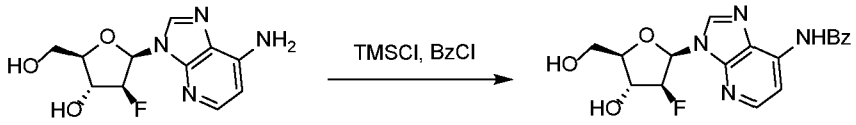
Step 3: 3-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-3H-imidazo[4,5-b]pyridin-7-amine



To a stirred solution of 3-[2-deoxy-2-fluoro-3,5-bis-*O*-(phenylcarbonyl)-β-D-arabinofuranosyl]-3*H*-imidazo[4,5-*b*]pyridin-7-amine (995mg, 2.09mmol) in methanol (36mL) at ambient temperature was added ammonia (7N in methanol, 12mL, 84.0mmol). The resulting solution was brought to 80°C and stirred for 5h. The reaction mixrure was cooled to RT and

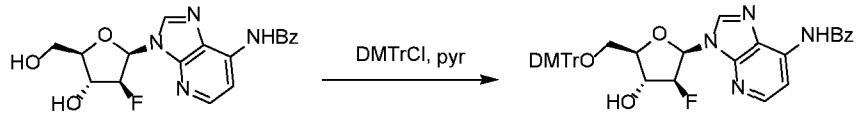
concentrated under reduced pressure. The resulting residue was suspended in methanol/dichloromethane and sonicated until a solid precipitated out of solution. Solid was collected by filtering through a glass frit affording 3-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-3*H*-imidazo[4,5-*b*]pyridin-7-amine. MS: 269 $(M+H)^+$.

Step 4: 3-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N-(phenylcarbonyl)-3H-imidazo[4,5-b]pyridin-7-amine



To a stirred solution of 3-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-3*H*-imidazo[4,5-*b*]pyridin-7-amine (550mg, 2.05mmol) in pyridine (6.5mL) at RT was added TMSCl (2.62mL, 20.5mmol). The resulting solution was stirred for 1.5h followed by addition of benzyol chloride (0.357mL, 3.08mmol). After stirring for an additional hour, water (2.15mL) was added to the reaction mixture, which was then stirred for 45min. The reaction mixture was cooled to 0°C and aqueous ammonia (28% w/w) (0.370mL, 4.79mmol) was added. The reaction mixture was returned to RT and stirred for 45min and then concentrated under reduced pressure. The resulting residue was taken up in water (20mL) and extracted with ethyl acetate (3x40mL). The organic extracts were combined washed with brine, dried over sodium sulphate and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel; 120 g prepacked, (0-7% methanol/dichloromethane) to afford 3-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-*N*-(phenylcarbonyl)-3*H*-imidazo[4,5-*b*]pyridin-7-amine. MS: 373 $(M+H)^+$.

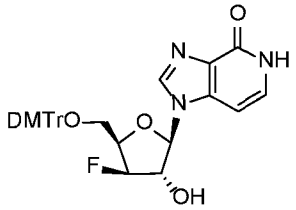
Step 5: 3-{5-O-[bis(4-methoxyphenyl)(phenyl)methyl]-2-deoxy-2-fluoro-β-D-arabinofuranosyl}-N-(phenylcarbonyl)-3H-imidazo[4,5-b]pyridin-7-amine



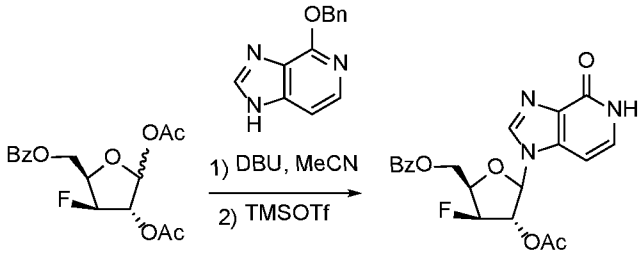
To a stirred mixture of 3-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-*N*-(phenylcarbonyl)-3*H*-imidazo[4,5-*b*]pyridin-7-amine (185mg, 0.497mmol) and 4Å molecular sieves in pyridine (3mL) at 0°C was added 4,4'-dimethoxytrityl chloride (253mg, 0.745mmol) in a single portion. The reaction mixture was allowed to warm to RT and was stirred for 18h. Sieves were removed by filtration and the filtrate was concentrated under reduced pressure. The resulting residue was taken up in a mixture of methanol/ether and added to water. The phases were separated, and the aqueous layer was extracted with ether (3 times). The organic extracts were combined, washed with brine, dried over sodium sulphate and concentrated under reduced pressure. The resulting

residue was purified by column chromatography on silica gel; 40g prepacked, ((0-40% (3:1, ethyl acetate:ethanol)/hexanes) to afford 3-{5-*O*-[bis(4-methoxyphenyl)(phenyl)methyl]-2-deoxy-2-fluoro-β-D-arabinofuranosyl}-*N*-(phenylcarbonyl)-3*H*-imidazo[4,5-*b*]pyridin-7-amine. MS: 675 $(M+H)^+$.

Preparation 8: 1-((2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-1,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one

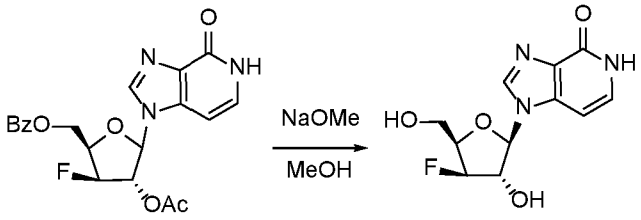


Step 1: ((2R,3S,4S)-4-acetoxy-3-fluoro-5-(4-oxo-4,5-dihydro-1H-imidazo[4,5-c]pyridin-1-yl)tetrahydrofuran-2-yl)methyl benzoate



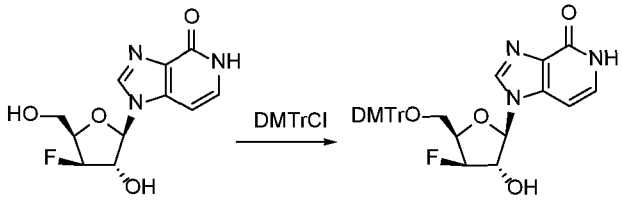
To a suspension of 4-(benzyloxy)-1*H*-imidazo[4,5-*c*]pyridine (0.795g, 3.53mmol) and (3*S*,4*S*,5*R*)-5-((benzoyloxy)methyl)-4-fluorotetrahydrofuran-2,3-diyl diacetate (1g, 2.94mmol) in ACN (20mL) and CH_2Cl_2 (10mL) at 0°C under Ar was added 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (1.34g, 8.815mmol). The resulting mixture was stirred at 0°C for 30min. Then, trimethylsilyl trifluoromethanesulfonate (3.92g, 17.65mmol) was added to the solution, and it was stirred at 0°C for 30min. Then, it was heated to 80°C for 16h. The reaction was cooled to rt and sat aq $NaHCO_3$ (10mL) and water (30mL) were added. It was extracted with EtOAc (3x50mL). The combined organic layers was washed with brine, dried over (Na_2SO_4), concentrated, and the residue was purified by silica gel column chromatography, eluted with 1 to 10% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 416.3 $[M + H]^+$.

Step 2: 1-((2R,3S,4R,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one



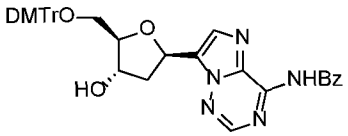
To a solution of ((2*R*,3*S*,4*S*)-4-acetoxy-3-fluoro-5-(4-oxo-4,5-dihydro-1*H*-imidazo[4,5-*c*]pyridin-1-yl)tetrahydrofuran-2-yl)methyl benzoate (2.5g, 5.3mmol) in MeOH (10mL) was added sodium methanolate (3.47g, 21.2mmol). The solution was stirred at rt for 1h. It was neutralized with AcOH, and the solution was concentrated. The residue was purified by reverse phase (AQ C18) chromatography eluted with 0 to 30% ACN in aq NH_4HCO_3 (5mM) to give the product. LCMS (ES, m/z): 270.0 $[M + H]^+$. ^1H NMR (400MHz, DMSO-d_6) δ 11.31 (s, 1H), 8.06 (s, 1H), 7.23 (d, J = 7.1Hz, 1H), 6.62 (d, J = 7.1Hz, 1H), 6.37 (d, J = 2.9Hz, 1H), 5.85 (d, J = 2.8Hz, 1H), 5.22 – 4.98 (m, 2H), 4.54 (d, J = 17.7Hz, 1H), 4.28 (dtd, J = 29.6, 6.0, 3.1Hz, 1H), 3.93 – 3.62 (m, 2H).

Step 3: 1-((2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-1,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one

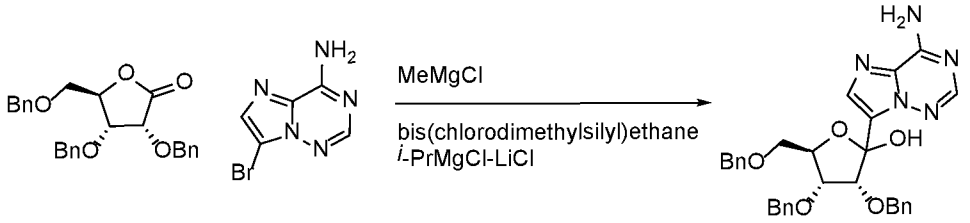


To a stirred solution of 1-((2*R*,3*S*,4*R*,5*R*)-4-fluoro-3-hydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-4(5*H*)-one (340mg, 1.26mmol) in pyridine (3mL) at rt was added 4,4'-(chloro(phenyl)methylene)bis(methoxybenzene) (426mg, 1.1mmol). It was stirred for 4h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography eluted with 1 to 10% MeOH in CH_2Cl_2 (0.5% Et_3N) to give the product. LCMS (ES, m/z): 572.3 $[M + H]^+$. ^1H NMR (400MHz, DMSO-d_6) δ 11.32 (d, J = 5.9Hz, 1H), 7.95 (s, 1H), 7.41 (d, J = 7.8Hz, 2H), 7.37 – 7.15 (m, 8H), 6.86 (dd, J = 10.5, 8.6Hz, 4H), 6.60 (d, J = 7.1Hz, 1H), 6.50 – 6.36 (m, 1H), 5.92 (d, J = 2.6Hz, 1H), 5.77 (s, 1H), 5.27 – 5.06 (m, 1H), 4.65 – 4.42 (m, 2H), 3.73 (d, J = 2.6Hz, 6H), 3.30 – 3.24 (m, 1H).

Preparation 9: *N*-(7-((2*R*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)imidazo[2,1-*f*][1,2,4]triazin-4-yl)benzamide

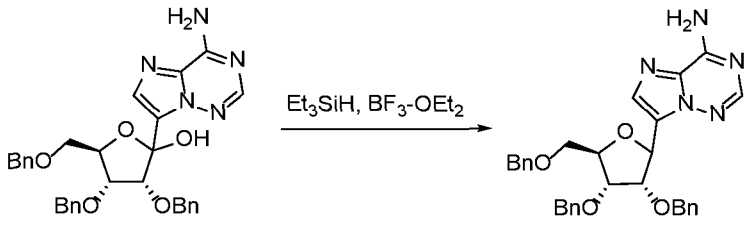


Step 1: (3R,4R,5R)-2-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-ol



To a stirring mixture of 7-bromoimidazo[2,1-*f*][1,2,4]triazin-4-amine (41g, 0.19mol) in THF (0.50L) at 0°C was added MeMgBr (3.0M in THF, 66mL, 0.19mol) dropwise to maintain the internal temperature below 10°C. Bis(chlorodimethylsilyl)ethane (41g, 190mmol) was added in one portion. MeMgBr (3.0M in diethyl ether, 66mL, 0.19 mol) was then added dropwise to maintain the internal temperature below 10°C. *i*-PrMgCl-LiCl (1.3 M in THF, 0.16L, 0.21mol) was added while maintaining the internal temperature below 10°C. A mixture of (3*R*,4*R*,5*R*)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)dihydrofuran-2(3*H*)-one (160g, 0.38 mol) in THF was added dropwise at 0°C, and the mixture was then allowed to warm to rt and was stirred for 12h. The mixture was diluted with saturated aqueous ammonium chloride (100mL) and extracted with ethyl acetate (3x1000mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by column chromatography (column height: 2500 mm, diameter: 1000 mm, 25% to 75% ethyl acetate gradient in hexanes) to afford (3*R*,4*R*,5*R*)-2-(4-aminoimidazo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-tetrahydrofuran-2-ol.

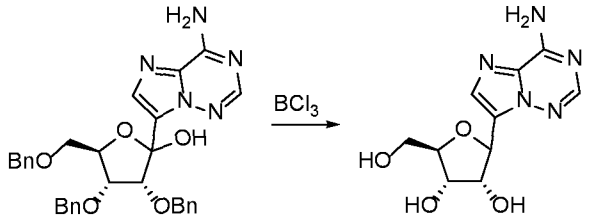
Step 2: 7-((3S,4R,5R)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)imidazo[2,1-f][1,2,4]triazin-4-amine



To a stirring mixture of (3*R*,4*R*,5*R*)-2-(4-aminoimidazo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-ol (64g, 0.12mmol) in DCM (1.3L) at 0°C was added triethylsilane (81g, 0.69mol), and then boron trifluoride diethyl etherate (21g,

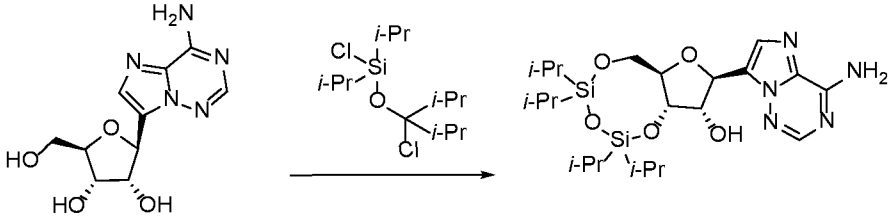
0.15 mol). The mixture was then allowed to warm to 25°C, and the mixture was stirred for 1h. More boron trifluoride diethyl etherate (57g, 0.40mol) was added, and the mixture was then heated to 35°C for 4h. Upon cooling to rt, the mixture was quenched with saturated aqueous sodium bicarbonate (200mL) and then extracted with ethyl acetate (3x300mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by column chromatography (15-75% ethyl acetate gradient in hexanes) to afford 7-((3*S*,4*R*,5*R*)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)imidazo[2,1-*f*][1,2,4]triazin-4-amine. MS (ES, m/z) = 538 [M + H]+.

Step 3: (3R,4S,5R)-2-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol



To a stirring mixture of 7-((3*S*,4*R*,5*R*)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-tetrahydrofuran-2-yl)imidazo[2,1-*f*][1,2,4]triazin-4-amine (12g, 22mmol) in DCM (850mL) at -78°C was added boron trichloride (18g, 0.16 mol) dropwise. Upon completion, the mixture was stirred at -78°C for 3h. After 3h, the mixture was quenched with methanol (50mL) at -78°C, and the mixture was allowed to warm to 25°C. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography (9-25% methanol gradient in dichloromethane) to afford (3*R*,4*S*,5*R*)-2-(4-aminoimidazo[2,1-*f*][1,2,4]triazin-7-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol.

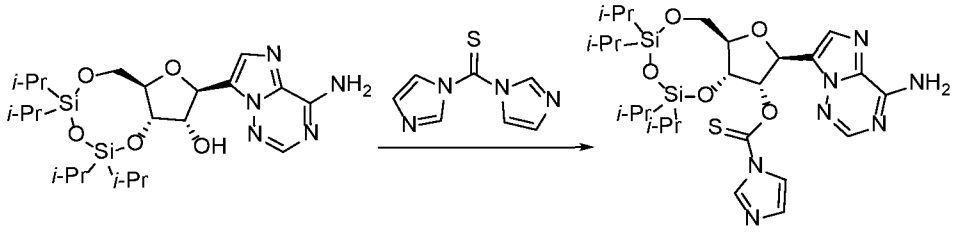
Step 4: (6aR,8S,9S,9aS)-8-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,2,4,4-tetraisopropyltetrahydro-6H-furo[3,2-f][1,3,5,2,4]trioxadisilocin-9-ol



To a stirred mixture of (2*S*,3*R*,4*S*,5*R*)-2-(4-aminoimidazo[2,1-*f*][1,2,4]triazin-7-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (4.0g, 15mmol) in pyridine (0.10L) was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (5.8mL, 18mmol). After 3h, the mixture was diluted with toluene (50mL) and then concentrated. The resulting mixture was taken up in DCM and

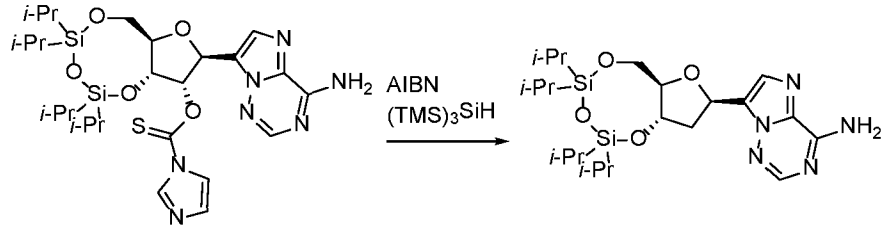
methanol and then silica gel (40g) was added. The mixture was concentrated, placed under vacuum for 1h and then purified by column chromatography (0-80% ethyl acetate gradient in hexanes) to afford (6a*R*,8*S*,9*S*,9a*S*)-8-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-9-ol. MS (ES, m/z) = 510 [M + H]+.

Step 5: O-((6aR,8S,9S,9aR)-8-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,2,4,4-tetraisopropyltetrahydro-6H-furo[3,2-f][1,3,5,2,4]trioxadisilocin-9-yl) 1H-imidazole-1-carbothioate



To a mixture of (6a*R*,8*S*,9*S*,9a*S*)-8-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-9-ol (6.45g, 12.7mmol) in acetonitrile (63.0mL) and pyridine (63.0mL) was added 1,1'-thiocarbonyldiimidazole (2.71g, 15.2mmol). After 90min, more 1,1'-thiocarbonyldiimidazole (2.71g, 15.2mmol) was added, and the mixture was stirred overnight. After stirring overnight, the mixture was concentrated and purified by column chromatography (0-100% ethyl acetate gradient in hexanes) to afford *O*-((6a*R*,8*S*,9*S*,9a*R*)-8-(4-aminoimidazo[2,1-*f*][1,2,4]triazin-7-yl)-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-9-yl) 1H-imidazole-1-carbothioate. MS (ES, m/z) = 620 [M + H]+.

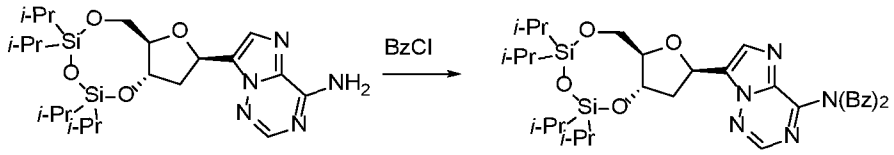
Step 6: 7-((6aR,8R,9aS)-2,2,4,4-tetraisopropyltetrahydro-6H-furo[3,2-f][1,3,5,2,4]trioxadisilocin-8-yl)imidazo[2,1-f][1,2,4]triazin-4-amine



To a mixture of *O*-((6a*R*,8*S*,9*S*,9a*R*)-8-(4-aminoimidazo[2,1-*f*][1,2,4]triazin-7-yl)-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-9-yl) (5.65g, 9.11mmol) in toluene (91.0mL) was added 2,2'-azobis(2-methylpropionitrile (0.300g, 1.82mmol) and tris(trimethylsilyl)silane (4.22mL, 13.7mmol). The mixture was heated to 85°C for 30min. After 30min, the mixture was allowed to cool to rt and placed directly on the column and

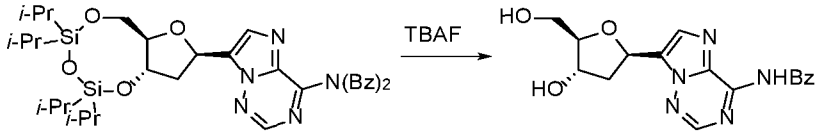
purified (0-80% ethyl acetate gradient in hexanes) to afford 7-((6a*R*,8*R*,9a*S*)-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl)imidazo[2,1-*f*][1,2,4]triazin-4-amine. MS (ES, m/z) = 494 [M + H]+ 494.

Step 7: N-benzoyl-N-(7-((6aR,8R,9aS)-2,2,4,4-tetraisopropyltetrahydro-6H-furo[3,2-f][1,3,5,2,4]trioxadisilocin-8-yl)imidazo[2,1-f][1,2,4]triazin-4-yl)benzamide



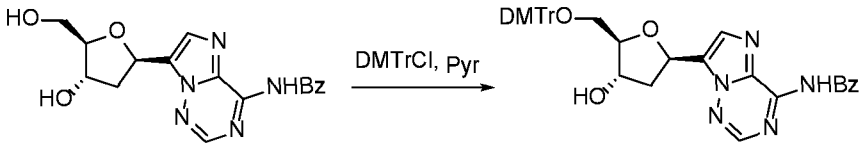
To a mixture of 7-((6a*R*,8*R*,9a*S*)-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl)imidazo[2,1-*f*][1,2,4]triazin-4-amine (15.7g, 31.8mmol) in pyridine (64.0mL) was added benzoyl chloride (11.0mL, 95.0mmol), and the mixture was heated to 50°C for 45min. After 45min, the mixture was allowed to cool to rt. After cooling, a precipitate formed and was filtered off. The filtrate was diluted with DCM (50mL) and toluene (50mL). The mixture was concentrated to about 50mL. The mixture was filtered, and the solids washed with DCM. The filtrate and washes were combined, loaded onto a column, and purified (0-50% ethyl acetate gradient in hexanes) to afford *N*-benzoyl-*N*-(7-((6a*R*,8*R*,9a*S*)-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl)imidazo[2,1-*f*][1,2,4]triazin-4-yl)benzamide. MS (ES, m/z) = 702 [M + H]+.

Step 8: N-(7-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)imidazo[2,1-f][1,2,4]triazin-4-yl)benzamide



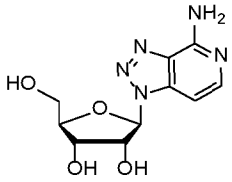
To a mixture of *N*-benzoyl-*N*-(7-((6a*R*,8*R*,9a*S*)-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl)imidazo[2,1-*f*][1,2,4]triazin-4-yl)benzamide (10g, 14mmol) in tetrahydrofuran (0.14L) was added TBAF ((1.0M in THF, 29mL, 29mmol), and the mixture was stirred for 1h. After 1h, the mixture was concentrated and purified by column chromatography to afford *N*-(7-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydro-furan-2-yl)imidazo[2,1-f][1,2,4]triazin-4-yl)benzamide. MS (ES, m/z) = 356 [M + H]+.

Step 9: N-(7-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)imidazo[2,1-f][1,2,4]triazin-4-yl)benzamide

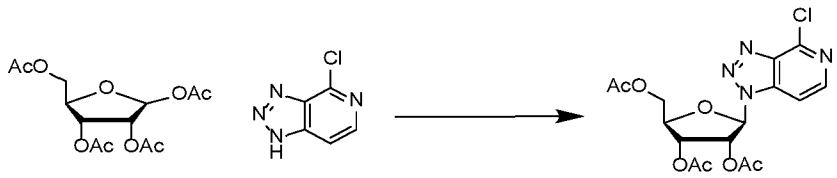


To a mixture of *N*-(7-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)imidazo[2,1-f][1,2,4]triazin-4-yl)benzamide (6.1g, 17mmol) in pyridine (86mL) at 0°C was added 4,4'-(chloro(phenyl)methylene)bis(methoxybenzene) (5.8g, 17mmol), and the mixture was allowed to warm to RT overnight. After stirring overnight, the mixture was diluted with toluene and then concentrated under reduced pressure to afford the crude product. The crude product was purified by silica gel chromatography (0-100% ethyl acetate gradient in hexanes) to afford *N*-(7-((2*R*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)imidazo[2,1-*f*][1,2,4]triazin-4-yl)benzamide. MS (ES, m/z) = 658 [M + H]+.

Preparation 10: (2R,3R,4S,5R)-2-(4-amino-1H-[1,2,3]triazolo[4,5-c]pyridin-1-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol



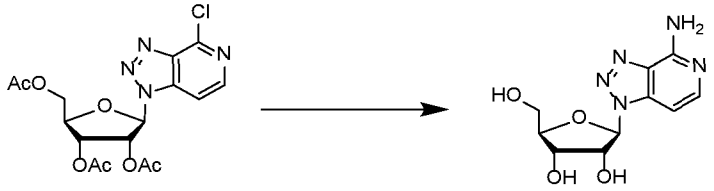
Step 1: (2R,3R,4R,5R)-2-(acetoxymethyl)-5-(4-chloro-1H-[1,2,3]triazolo[4,5-c]pyridin-1-yl)tetrahydrofuran-3,4-diyl diacetate



To a suspension of 4-chloro-1H-[1,2,3]triazolo[4,5-c]pyridine (1.0g, 6.5mmol) and (3R,4R,5R)-5-(acetoxymethyl)tetrahydrofuran-2,3,4-triyl triacetate (3.1g, 9.7mmol) in nitromethane (50mL) was added BF_3•Et_2O (1.23mL, 9.7mmol), and the resulting mixture was heated at 50°C for 2h. The reaction mixture was cooled to rt, diluted with 100mL of DCM and washed with sat aq. $NaHCO_3$ (100mL) and brine (100mL). The separated organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by a silica gel column, eluting with 0-100% EtOAc:EtOH (3:1)/Hexane. LCMS (ES, m/z): 413.07 $[M+H]^+$. ^1H NMR (500MHz, Chloroform-*d*) δ 8.40 (d, *J* = 5.8Hz, 1H), 7.56 (d, *J* = 5.8Hz, 1H), 6.43 (d, *J* = 4.1Hz, 1H), 6.17 (dd, *J* = 5.3, 4.1Hz, 1H), 5.74 (t, *J* = 5.3Hz, 1H), 5.32 (s, 1H), 4.58 (ddd, *J* = 5.3, 3.9,

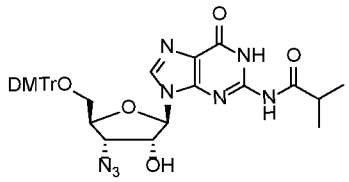
2.9Hz, 1H), 4.42 (dd, *J* = 12.5, 3.0Hz, 1H), 4.25 (dd, *J* = 12.5, 3.9Hz, 1H), 2.17 (d, *J* = 18.8Hz, 6H), 2.03 (s, 3H).

Step 2: (2R,3R,4S,5R)-2-(4-amino-1H-[1,2,3]triazolo[4,5-c]pyridin-1-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol



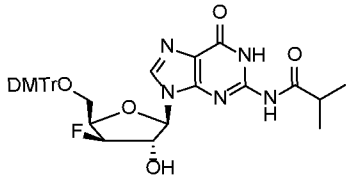
To a solution of (2R,3R,4R,5R)-2-(acetoxymethyl)-5-(4-chloro-1H-[1,2,3]triazolo[4,5-c]pyridin-1-yl)tetrahydrofuran-3,4-diyl diacetate (1.2g, 2.9mmol) in MeOH (8.3mL) was added a 7N solution of ammonia in MeOH (8.3mmol, 58.1mmol). The reaction mixture was stirred at 150°C in a sealed Q-Tube™ Pressure Tube Reactor for 18h. Excess solvent was removed under reduced pressure, and the residue was purified by a reverse phase silica gel column, eluting with 0-10% Acetonitrile/H_2O containing 0.05% TFA. LCMS (ES, m/z): 268.17 $[M+H]^+$. ^1H NMR (500MHz, DMSO-d_6) δ 13.87 (s, 2H), 9.34 (s, 4H), 7.89 (d, *J* = 7.1Hz, 2H), 7.51 (d, *J* = 7.0Hz, 2H), 7.42 (s, 4H), 7.32 (s, 6H), 7.22 (s, 5H), 6.69 (s, 2H), 6.30 (d, *J* = 4.9Hz, 2H), 5.72 (s, 2H), 5.41 (s, 2H), 4.75 – 4.67 (m, 0H), 4.65 (t, *J* = 5.0Hz, 2H), 4.27 (t, *J* = 4.6Hz, 2H), 4.07 (q, *J* = 4.2Hz, 2H), 3.86 – 3.67 (m, 2H), 3.69 – 3.58 (m, 2H), 3.54 (dd, *J* = 12.0, 4.7Hz, 2H), 3.48 – 3.38 (m, 1H), 3.28 (s, 1H), 3.23 (s, 0H), 1.76 (d, *J* = 0.5Hz, 5H), 1.11 (dt, *J* = 26.0, 7.1Hz, 1H).

Preparation 11: 9-{3-azido-5-O-[bis(4-methoxyphenyl)(phenyl)methyl]-3-deoxy-β-D-ribofuranosyl}-2-[(2-methylpropanoyl)amino]-1,9-dihydro-6H-purin-6-one



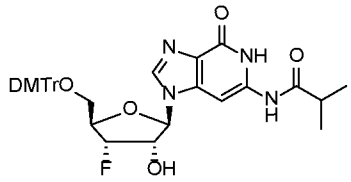
The title compound was prepared according to published procedures (*Nucleosides, Nucleotides & Nucleic Acids* **2005**, *24*(10-12), 1707-1727).

Preparation 12: 9-{5-*O*-[bis(4-methoxyphenyl)(phenyl)methyl]-3-deoxy-3-fluoro-β-D-xylofuranosyl}-2-[(2-methylpropanoyl)amino]-1,9-dihydro-6*H*-purin-6-one



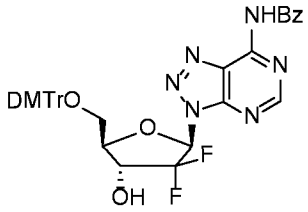
The title compound was prepared according to published procedures (*Tetrahedron Letters*, **1989**, *30*(24), 3171-3174).

Preparation 13: 1-{5-*O*-[bis(4-methoxyphenyl)(phenyl)methyl]-3-deoxy-3-fluoro-β-D-ribofuranosyl}-6-[(2-methylpropanoyl)amino]-1,5-dihydro-4*H*-imidazo[4,5-c]pyridin-4-one

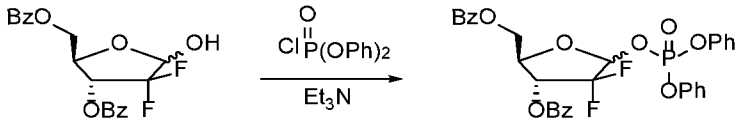


The title compound was prepared according to published procedures (WO2002057425).

Preparation 14: N-(3-((2R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3,3-difluoro-4-hydroxytetrahydrofuran-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)benzamide



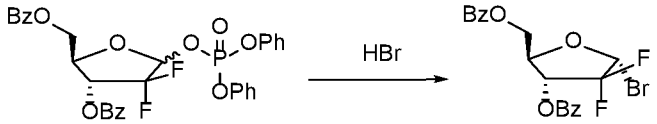
Step 1: (((2R,3R)-3-(benzoyloxy)-5-((diphenoxyphosphoryl)oxy)-4,4-difluorotetrahydrofuran-2-yl)methyl benzoate



To ((2R,3R)-3-(benzoyloxy)-4,4-difluoro-5-hydroxytetrahydrofuran-2-yl)methyl benzoate (20.0g, 52.9mmol) in toluene (150mL) at 0°C were added Et_3N (7.74mL, 55.5mmol) and diphenyl phosphoryl chloride (12.1mL, 58.2mmol) in toluene (20mL) dropwise. The reaction was warmed to rt and stirred for 3h. LCMS showed completion of the reaction (m/e = 611). Water (30mL) and aq HCl (1 M, 5mL) were added. Layers were separated, and the aq layer was extracted with CH_2Cl_2 (2x30mL). The combined organic solution was washed with sat aq $NaHCO_3$ (20mL), and Brine (20mL), dried ($MgSO_4$), and concentrated. It was purified by

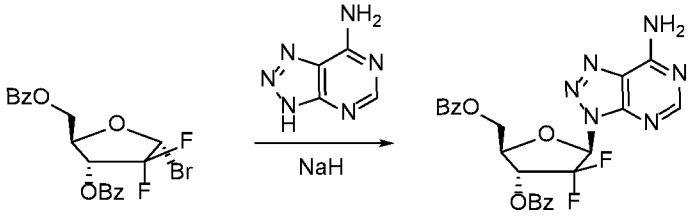
silica column chromatography eluting with 0 to 30% EtOAc in Hex to give the product. LCMS (ES, m/z): 611.3 $[M + H]^+$.

Step 2: ((2R,3R,5R)-3-(benzoyloxy)-5-bromo-4,4-difluorotetrahydrofuran-2-yl)methyl benzoate



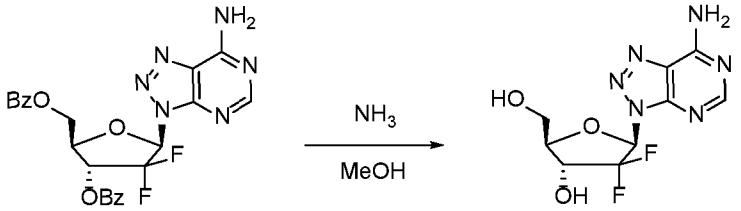
To ((2R,3R)-3-(benzoyloxy)-5-((diphenoxyphosphoryl)oxy)-4,4-difluorotetrahydrofuran-2-yl)methyl benzoate (23.8g, 39.0mmol) at 0°C was added HBr in acetic acid (33%, 51.2mL, 292mmol). It was stirred as it warmed to rt. After 6h, LCMS showed completion of the reaction (m/e = 441 and 443). CH_2Cl_2 (200mL) was added, and the organic layer was washed with water (2x50mL), sat aq $NaHCO_3$ (2x50mL) and brine (50mL). It was dried ($MgSO_4$) and concentrated to give the crude product. It was used in the next step without purification. LCMS (ES, m/z): 441.1, 443.1 $[M + H]^+$.

Step 3: (2R,3R,5R)-5-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((benzoyloxy)methyl)-4,4-difluorotetrahydrofuran-3-yl benzoate



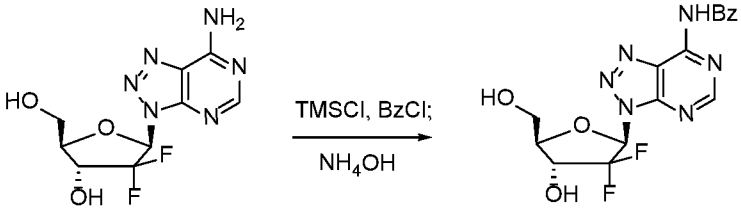
To a mixture of 3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-amine (3.21g, 23.6mmol) in NMP (60mL) was added NaH (60%, 0.979g, 24.5mmol). The mixture was vigorously stirred, and after 1h, generation of bubbles had completely ceased. To the mixture was added to ((2R,3R,5R)-3-(benzoyloxy)-5-bromo-4,4-difluorotetrahydrofuran-2-yl)methyl benzoate (neat, 8.00g, 18.1mmol). The mixture was stirred vigorously for 30min. Then, it was heated to 90°C for 5h. It was cooled to rt, and CH_2Cl_2 (300mL) and water (150mL) were added. The phases were separated, and the organic layer was washed with water (8x150mL) and brine (50mL), dried ($MgSO_4$) and concentrated. The residue was purified by silica column chromatography eluting with 0% to 30% EtOAc to give the desired product. LCMS (ES, m/z): 497.1 $[M + H]^+$. ^1H NMR (500MHz, Chloroform-*d*) δ 8.44 (s, 1H), 8.15-8.08 (m, 2H), 8.08 - 7.99 (m, 2H), 7.63 (ddt, *J* = 8.7, 7.1, 1.3Hz, 1H), 7.56-7.44 (m, 3H), 7.40-7.32 (m, 2H), 6.79-6.68 (m, 2H), 6.04 (bs, 2H), 4.92-4.84 (m, 1H), 4.80-4.72 (m, 2H).

Step 4: (2R,3R,5R)-5-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-4,4-difluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol



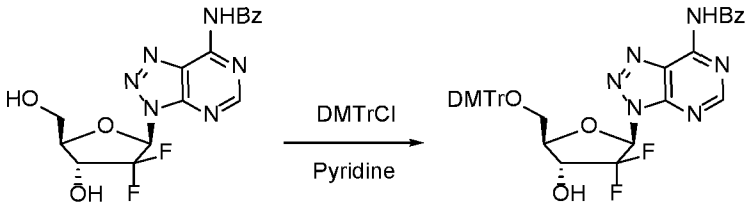
To (2R,3R,5R)-5-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((benzoyloxy) methyl)-4,4-difluorotetrahydrofuran-3-yl benzoate (1.05g, 2.11mmol) was added ammonia in MeOH (7N, 9.0mL, 63mmol), and the mixture was stirred for 24h. LCMS showed completion of reaction (m/e = 289). It was concentrated and purified by silica column chromatography eluting with 0 to 15% MeOH in CH_2Cl_2 to give the desired product. LCMS (ES, m/z): 289.1 [M + H]$^+$. ^{1}H-NMR (500MHz, DMSO-d_6): δ 8.65 (s, 1H), 8.36 (s, 1H), 8.32 (s, 1H), 6.65 (d, J = 12.4Hz, 1H), 6.40 (d, J = 6.7Hz, 1H), 5.03 (dd, J = 6.4, 5.3Hz, 1H), 4.83 (dq, J = 16.5, 8.9Hz, 1H), 4.00 (t, J = 6.7Hz, 1H), 3.77 (ddd, J = 12.3, 5.2, 2.5Hz, 1H), 3.68 (dt, J = 12.5, 6.3Hz, 1H).

Step 5: N-(3-((2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)benzamide



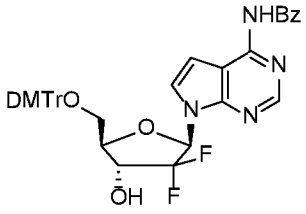
To a solution of (2R,3R,5R)-5-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-4,4-difluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (0.57g, 2.0mmol) in pyridine (15mL) at 0°C was added TMSCl (0.55mL, 4.3mmol). It was warmed to rt and stirred for 1h. Then, it was recooled to 0°C and BzCl (0.34mL, 2.9mmol) was added dropwise. The reaction was slowly warmed rt over 2h. LCMS showed completion of reaction (m/e = 393, 497). It was cooled to 0°C, and ammonium hydroxide (28%, 1.1mL, 7.9mmol) was added. After 30min, it was concentrated and purified by silica column chromatography eluting with 0 to 10% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 393.3 [M + H]$^+$. ^{1}H-NMR (500MHz, DMSO-d_6): δ 12.02 (s, 1H), 9.01 (s, 1H), 8.13-8.07 (m, 2H), 7.73-7.66 (m, 1H), 7.64-7.55 (m, 2H), 6.86 (d, J = 11.6Hz, 1H), 6.46 (d, J = 6.7Hz, 1H), 5.01 (dd, J = 6.3, 5.4Hz, 1H), 4.86 (dt, J = 16.7, 8.3Hz, 1H), 4.04 (ddd, J = 8.8, 6.2, 2.6Hz, 1H), 3.78 (ddd, J = 12.3, 5.4, 2.7Hz, 1H), 3.68 (dt, J = 12.4, 6.2Hz, 1H).

Step 6: N-(3-((2R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3,3-difluoro-4-hydroxytetrahydrofuran-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)benzamide

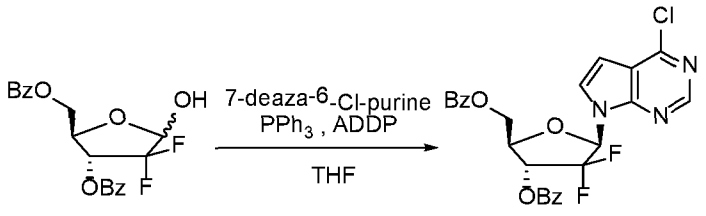


To a solution of N-(3-((2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)benzamide (0.68g, 1.7mmol) in pyridine (15mL) at 0°C was added DMTrCl (0.65g, 1.9mmol). It was stirred at rt for 1h. LCMS showed a peak with the desired mass (m/e = 695). It was partly concentrated (to 5mL), and EtOAc (20mL) and water (10mL) were added. The phases were separated, and the aqueous layer was extracted with EtOAc (2x10mL). The combined organic portions were washed with brine, dried ($MgSO_4$), concentrated and purified by silica column chromatography eluting with 0% to 60% EtOAc in Hex to give the product. LCMS (ES, m/z): 695.2 $[M + H]^+$. ^{1}H-NMR (500MHz, DMSO-d_6): δ 12.05 (s, 1H), 8.93 (s, 1H), 8.11 (d, *J* = 7.6Hz, 2H), 7.70 (m, 1H), 7.60 (t, *J* = 7.7Hz, 2H), 7.38-7.32 (m, 2H), 7.25-7.13 (m, 7H), 6.96 (d, *J* = 11.9Hz, 1H), 6.84-6.73 (m, 4H), 6.48 (d, *J* = 6.9Hz, 1H), 4.97 (dq, *J* = 16.7, 8.3Hz, 1H), 4.23 (m, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.42-3.36 (m, 1H), 3.32-3.28 (m, 1H).

Preparation 15: N-(7-((2R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3,3-difluoro-4-hydroxytetrahydrofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzamide



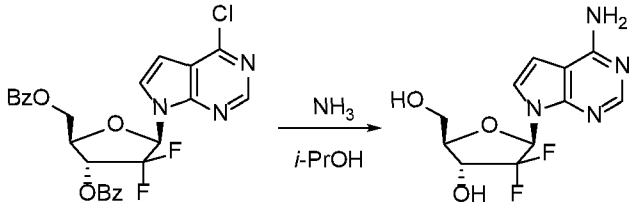
Step 1: ((2R,3R,5R)-3-(benzoyloxy)-5-(4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4,4-difluorotetrahydrofuran-2-yl)methyl benzoate



To a solution of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (4.06g, 26.4mmol) and ((2R,3R)-3-(benzoyloxy)-4,4-difluoro-5-hydroxytetrahydrofuran-2-yl)methyl benzoate (10.0g, 26.4mmol) and triphenylphosphine (20.80g, 79mmol) in THF (100mL) was added (*E*)-diazene-1,2-diylbis(piperidin-1-ylmethanone) (20.01g, 79mmol) dropwise. It was slowly warmed to rt.

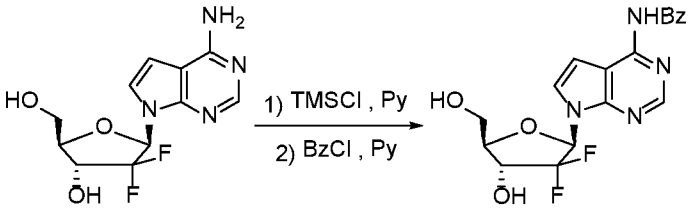
After stirring for 2h, the mixture was concentrated. The residue was purified by silica gel column chromatography using 0-20% EtOAc in Petroleum Ether to give the product. LCMS (ES, m/z): 514.3 $[M + H]^+$. ^{1}H-NMR (300MHz, DMSO-d_6): δ 8.71 (s, 1H), 8.08-7.87 (m, 5H), 7.76-7.42 (m, 6H), 6.98 (dd, J = 10.3, 7.9Hz, 1H), 6.84 (d, J = 3.8Hz, 1H), 6.29 (ddd, J = 10.0, 6.0, 3.8Hz, 1H), 5.44 (q, J = 5.5Hz, 1H), 4.79-4.60 (m, 2H).

Step 2: (2R,3R,5R)-5-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4,4-difluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol



A solution of ((2R,3R,5R)-3-(benzoyloxy)-5-(4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4,4-difluorotetrahydrofuran-2-yl)methyl benzoate (1.9g, 3.70mmol) in NH_3/2-propanol (saturated at -78°C, 100mL) was stirred at 90°C for 16h. It was cooled to rt, concentrated, and purified by silica gel column chromatography using 0-10% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 287.1 $[M + H]^+$. ^{1}H-NMR (300MHz, DMSO-d_6): δ 8.06 (s, 1H), 7.40-7.25 (m, 1H), 7.14 (s, 2H), 6.82 (d, J = 5.9Hz, 1H), 6.60 (d, J = 3.7Hz, 1H), 6.34 (dd, J = 16.2, 2.1Hz, 1H), 4.88 (t, J = 5.7Hz, 1H), 4.27 (ddd, J = 9.9, 6.2, 3.6Hz, 1H), 4.08 (dq, J = 6.8, 4.1, 3.5Hz, 1H), 3.78 (dt, J = 11.1, 5.4Hz, 1H), 3.62 (dt, J = 11.4, 5.8Hz, 1H).

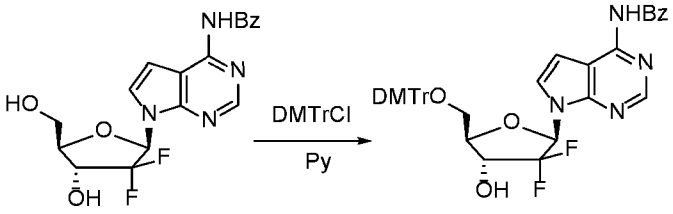
Step 3: N-(7-((2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzamide



To a solution of (2*R*,3*R*,5*R*)-5-(4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-4,4-difluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (900mg, 3.14mmol) in pyridine (15mL) at 0°C was added chlorotrimethylsilane (3.42g, 31.4mmol). It was warmed to rt over 1h and benzoyl chloride (663mg, 4.72mmol) was added dropwise. After 2h, NH_4OH (28%, 15.00mL) was added, and it was stirred for 0.5h. The resulting mixture was concentrated, and the residue was purified by silica gel column chromatography using 0-10% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 391.1 $[M + H]^+$. ^{1}H-NMR (300MHz, DMSO-d_6): δ 8.61 (s, 1H), 8.08-7.98 (m, 2H), 7.97-7.91 (m, 1H), 7.67-7.51 (m, 2H), 7.50-7.47 (m, 1H), 6.70 (d, J = 3.9Hz, 1H), 6.56 (dd,

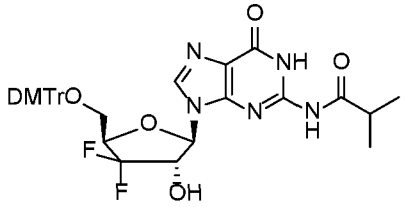
J = 15.6, 1.5Hz, 1H), 4.32 (dd, J = 9.4, 3.6Hz, 1H), 4.16 (d, J = 5.6Hz, 1H), 3.80 (dd, J = 11.5, 5.2Hz, 1H), 3.66 (dd, J = 11.5, 6.5Hz, 1H), 3.12 (s, 2H).

Step 4: N-(7-((2R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3,3-difluoro-4-hydroxytetrahydrofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzamide

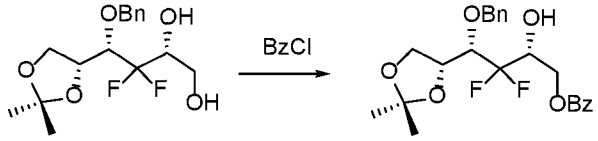


To a solution of *N*-(7-((2*S*,4*R*,5*R*)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)benzamide (1.1g, 2.82mmol) in pyridine (12mL) at 0°C was added 4,4'-(chloro(phenyl)methylene)-bis(methoxybenzene) (0.955g, 2.82mmol). It was warmed to RT and stirred for 3h. The mixture was concentrated. The product was purified by silica gel column chromatography using 0-10% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 693.3 $[M + H]^+$. ^{1}H-NMR (300MHz, DMSO-d_6): δ 11.25 (s, 1H), 8.67 (s, 1H), 8.09-8.01 (m, 2H), 7.60-7.48 (m, 2H), 7.47-7.36 (m, 3H), 7.33-7.18 (m, 7H), 6.91-6.78 (m, 4H), 6.75-6.57 (m, 3H), 5.76 (s, 1H), 4.55-4.33 (m, 2H), 3.74 (s, 6H), 3.44 (t, J = 8.7Hz, 1H), 3.35 (s, 1H).

Preparation 16: N-(9-((2R,3S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4,4-difluoro-3-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



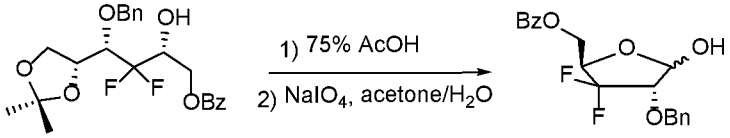
Step 1: (2R,4S)-4-(benzyloxy)-4-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-3,3-difluoro-2-hydroxybutyl benzoate



To a solution of (2R,4S)-4-(benzyloxy)-4-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-3,3-difluorobutane-1,2-diol (3.50g, 10.5mmol) in CH_2Cl_2 (52mL) and pyridine (26mL) at -70°C was added benzoyl chloride (1.48g, 10.5mmol) in CH_2Cl_2 (11mL) dropwise over 50min. After 2h, methanol (150mL) was added. The mixture was stirred at RT for 0.5h. Water (200mL) was

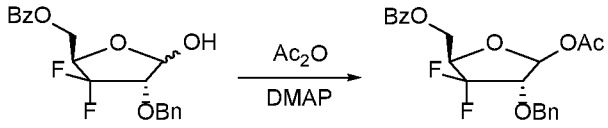
added. Layers were separated, and the aq layer was extracted with ether (4x150mL). The combined organic layers were washed with aq HCl (1 N, 2x150mL), sat aq $NaHCO_3$ (2x150mL) and brine (2x150mL). It was dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether = 1/10) to give the product. ^{1}H-NMR (400MHz, $CDCl_3$): δ 8.11-8.01 (m, 2 H), 7.61-7.55 (m, 1 H), 7.48-7.31 (m, 7H), 4.89-4.53 (m, 4H), 4.46-4.41 (m, 1H), 4.40-4.29 (m, 1H), 4.17-4.02 (m, 2 H), 3.98-3.84 (m, 0.5H), 3.74-3.66 (m, 0.5 H), 1.46 (s, 3H), 1.28 (s, 3H). ^{19}F-NMR: (376MHz, $CDCl_3$): δ -106.8 (d, J = 270.7Hz, 1F), -119.2 (d, J = 270.7Hz, 1F).

Step 2: ((2R,4S)-4-(benzyloxy)-3,3-difluoro-5-hydroxytetrahydrofuran-2-yl)methyl benzoate



(2R,4S)-4-(benzyloxy)-4-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-3,3-difluoro-2-hydroxybutyl benzoate (3.00g, 6.87mmol) was dissolved in aq AcOH (75%, 66mL). It was stirred at 50°C for 3h. It was partly concentrated. The residue was redissolved in acetone (33mL). To the solution at rt was added sodium periodate (1.20g, 5.61mmol) in water (33mL). After 1.5h, the solid formed was filtered off and washed with acetone. The filtrate was concentrated. Water and CH_2Cl_2 were added, and layers were separated. The aq layer was extracted with CH_2Cl_2 (4x150mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by silica gel column chromatography (ethyl acetate/petroleum ether = 1/5) to give the product. ^{1}H-NMR (400MHz, $CDCl_3$): δ 8.05-8.01 (m, 2 H), 7.59-7.52 (m, 1 H), 7.46-7.35 (m, 7H), 5.49-5.42 (m, 1H), 4.99-4.72 (m, 1H), 4.67-4.47 (m, 4H), 4.11-3.80 (m, 1H). ^{19}F-NMR: (376MHz, $CDCl_3$): δ -117.1 (d, *J* = 240.6Hz, 1F), -117.9 (d, *J* = 251.9Hz, 1F).

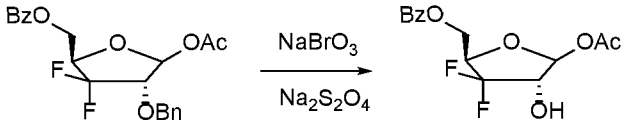
Step 3: ((2R,4S)-5-acetoxy-4-(benzyloxy)-3,3-difluorotetrahydrofuran-2-yl)methyl benzoate



To a solution of ((2R,4S)-4-(benzyloxy)-3,3-difluoro-5-hydroxytetrahydrofuran-2-yl)methyl benzoate (2.40g, 6.59mmol) in CH_2Cl_2 (66mL) at rt was added N,N-dimethylpyridin-4-amine (0.080g, 0.659mmol) and acetic anhydride (4.03g, 39.5mmol) dropwise. After 6h, it was quenched by addition of sat aq $NaHCO_3$ (30mL). Layers were separated, and the aq layer was extracted with CH_2Cl_2 (3x150mL). The combined organic layers were washed with water (2x150mL) and brine (2x100mL), dried (Na_2SO_4), concentrated and purified by silica gel

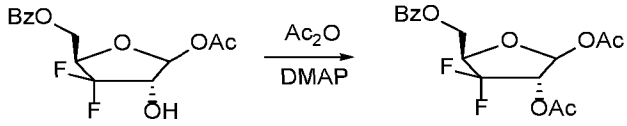
column chromatography (ethyl acetate/petroleum ether = 1/7) to give the product. ^{1}H-NMR (300MHz, $CDCl_3$): δ 8.08-7.89 (m, 2 H), 7.61-7.48 (m, 1 H), 7.47-7.25 (m, 7H), 6.15 (d, *J* = 6.0Hz, 1H), 4.89-4.75 (m, 1H), 4.73-4.40 (m, 4H), 4.18-4.02 (m, 1H), 1.98 (s, 3H). ^{19}F-NMR: (282MHz, $CDCl_3$): δ -116.5 (d, *J* = 248.2Hz, 1F), -120.9 (d, *J* = 248.2Hz, 1F).

Step 4: ((2R,4S)-5-acetoxy-3,3-difluoro-4-hydroxytetrahydrofuran-2-yl)methyl benzoate



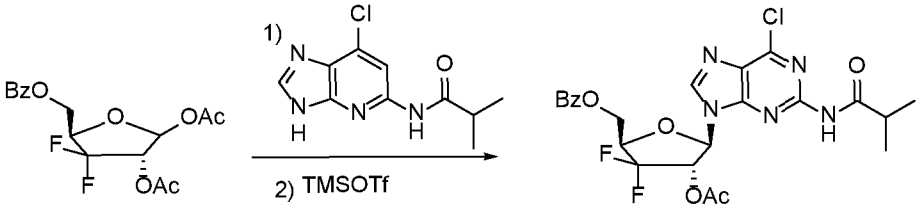
To a solution of ((2R,4S)-5-acetoxy-4-(benzyloxy)-3,3-difluorotetrahydrofuran-2-yl)methyl benzoate (2.50g, 6.15mmol) in EtOAc (60mL) was added sodium bromate (5.57g, 36.9mmol) in water (46mL). The mixture was stirred vigorously, and to it was added sodium dithionite (6.43g, 36.9mmol) in water (92mL) dropwise over 1h. After 5h, layers were separated, and the aq layer was extracted with EtOAc (5x150mL). The combined organic layers were washed with sat aq $Na_2S_2O_3$ (2x150mL) and brine (2x150mL), dried (Na_2SO_4), concentrated, and purified by silica gel column chromatography (ethyl acetate/petroleum ether = 1/5) to give the product. ^{1}H-NMR (300MHz, $CDCl_3$): δ 8.10-7.89 (m, 2 H), 7.63-7.50 (m, 1 H), 7.48-7.30 (m, 2H), 6.09 (d, *J* = 6.0Hz, 1H), 4.71-4.42 (m, 3H), 4.36-4.26 (m, 1H), 2.04 (s, 3H). ^{19}F-NMR: (282MHz, $CDCl_3$): δ -119.5 (d, *J* = 248.2Hz, 1F), -122.0 (d, *J* = 248.2Hz, 1F).

Step 5: (3S,5R)-5-((benzoyloxy)methyl)-4,4-difluorotetrahydrofuran-2,3-diyl diacetate



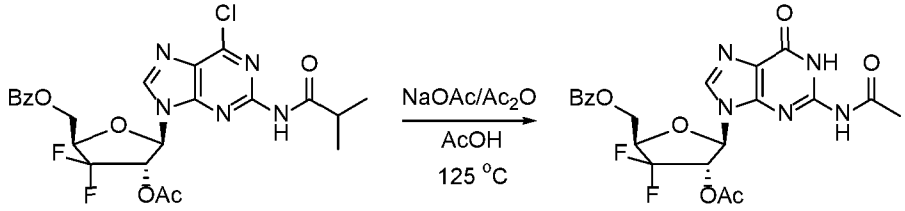
To a solution of ((2R,4S)-5-acetoxy-3,3-difluoro-4-hydroxytetrahydrofuran-2-yl)methyl benzoate (2.00g, 6.32mmol) in CH_2Cl_2 (84mL) at rt was added DMAP (0.08g, 0.632mmol) and acetic anhydride (3.87g, 37.9mmol) dropwise. After 6h, it was quenched by addition of sat aq $NaHCO_3$ (30mL). Layers were separated, and the aq layer was extracted with CH_2Cl_2 (3x140mL). The combined organic layers were washed with water (2x140mL) and brine (2x140mL), dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (ethyl acetate/petroleum ether = 1/6) to give the product. ^{1}H-NMR (400MHz, $CDCl_3$): δ 8.11-8.01 (m, 2 H), 7.63-7.54 (m, 1 H), 7.50-7.41 (m, 2H), 6.20 (d, *J* = 4.0Hz, 1H), 5.39 (d, *J* = 8.0Hz,1H), 4.69-4.48 (m, 3H), 2.23 (s, 3H), 2.08 (s, 3H). ^{19}F-NMR: (376MHz, $CDCl_3$): δ -117.6 (d, *J* = 251.9Hz, 1F), -119.5 (d, *J* = 251.9Hz, 1F).

Step 6: ((2R,4S,5R)-4-acetoxy-5-(6-chloro-2-isobutyramido-9H-purin-9-yl)-3,3-difluorotetrahydrofuran-2-yl)methyl benzoate



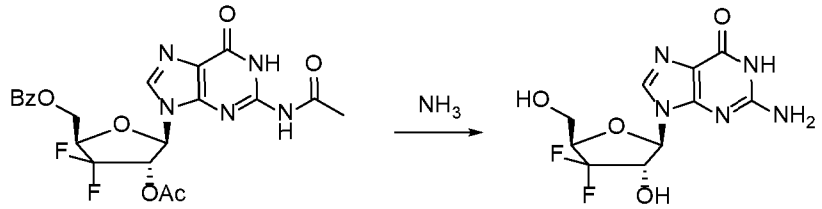
To a solution of (3S,5R)-5-((benzoyloxy)methyl)-4,4-difluorotetrahydrofuran-2,3-diyl diacetate (2.20g, 6.14mmol) and *N*-(6-chloro-9*H*-purin-2-yl)isobutyramide (1.77g, 7.37mmol) in ACN (80mL) at 0°C was added 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (2.80mL, 18.4mmol). After 0.5h, trimethylsilyl trifluoromethanesulfonate (6.82mL, 36.8mmol) was added dropwise to the reaction at 0°C. After 0.5h, it was heated at 80°C for 16h. The reaction was then quenched by the addition of water (150mL). Layers were separated, and the aq layer was extracted with EtOAc (3x150mL). The combined organic layers were washed with sat aq $NaHCO_3$ (2x150mL) and brine (2x150mL), dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (ethyl acetate/petroleum ether = 1/1) to give the product. ^{1}H-NMR (400MHz, $CDCl_3$): δ 8.10 (s, 1H), 8.08-7.98 (m, 2 H), 7.64-7.53 (m, 1 H), 7.51-7.40 (m, 2H), 6.25 (d, *J* = 4.0Hz, 1H), 5.98-5.93 (m,1H), 4.85-4.53 (m, 3H), 2.92-2.80 (m, 1H), 2.22 (s, 3H) 1.28 (d, *J* = 4.0Hz, 6H). ^{19}F-NMR: (376MHz, $CDCl_3$): δ -116.7 (d, *J* = 248.2Hz, 1F), -118.1 (d, *J* = 248.2Hz, 1F).

Step 7: ((2R,4S,5R)-5-(2-acetamido-6-oxo-1,6-dihydro-9H-purin-9-yl)-4-acetoxy-3,3-difluorotetrahydrofuran-2-yl)methyl benzoate



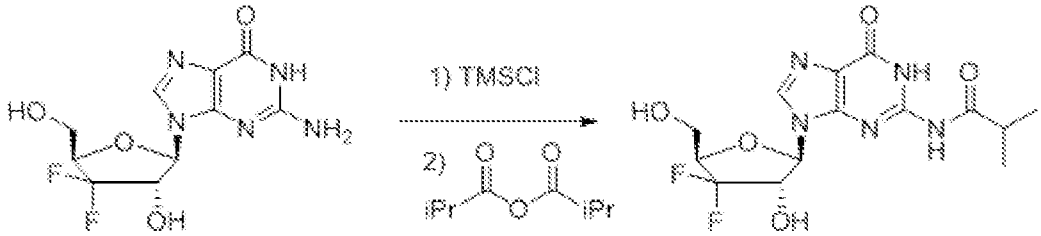
To a solution of ((2*R*,4*S*,5*R*)-4-acetoxy-5-(6-chloro-2-isobutyramido-9*H*-purin-9-yl)-3,3-difluorotetrahydrofuran-2-yl)methyl benzoate (1.80g, 3.35mmol) in AcOH (29mL) was added Sodium acetate (1.37g, 16.7mmol) and acetic anhydride (29mL). The reaction was stirred at 125°C for 2.5h. It was cooled to rt, and MeOH (50mL) was added. The mixture was concentrated under reduced pressure, and the residue was coevaporated with ethanol (2x50mL). DCM (150mL) and water (150mL) were added, and layers were separated. The organic phase was washed with sat aq $NaHCO_3$ (2x150mL), dried (Na_2SO_4), and concentrated to give the product. LCMS (ES, m/z): 492.1 $[M + H]^+$.

Step 8: 2-amino-9-((2R,3S,5R)-4,4-difluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one



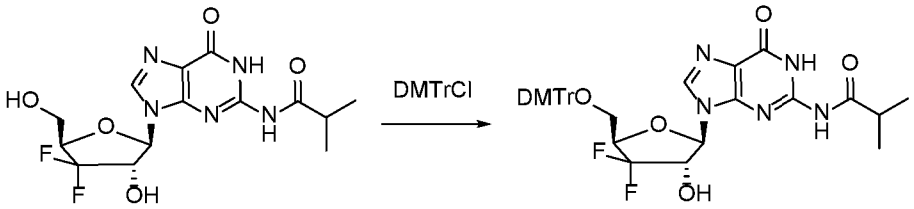
To ((2R,4S,5R)-5-(2-acetamido-6-oxo-1,6-dihydro-9H-purin-9-yl)-4-acetoxy-3,3-difluorotetrahydrofuran-2-yl)methyl benzoate (neat, 1.80g, 3.66mmol) was added NH_3 in MeOH (7 M, 90mL, MeOH). It was stirred at rt for 60h. It was concentrated and purified by reverse phase (C18) chromatography eluting with 5-20% MeCN in aq NH_4HCO_3 (5 mM) to give the product. ^{1}H-NMR (300MHz, DMSO-d_6): δ 10.7 (s, 1H), 7.95 (s, 1H), 6.56-6.44 (m, 3H), 5.62(d, J = 6.0Hz, 1H), 5.32 (t, J = 5.4Hz, 1H), 4.90-4.77 (m, 1H), 4.23-4.08 (m, 1H), 3.68-3.52 (m, 2H). ^{19}F-NMR: (282MHz, DMSO-d_6): δ -113.1 (d, J = 234.1Hz, 1F), -121.8 (d, J = 234.1Hz, 1F).

Step 9: N-(9-((2R,3S,5R)-4,4-difluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



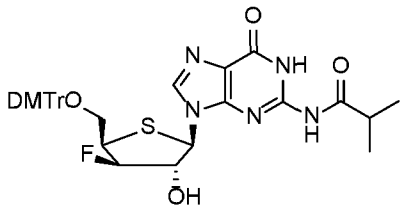
2-amino-9-((2R,3S,5R)-4,4-difluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one (800mg, 2.64mmol) was co-evaporated with pyridine (3x3mL). It was re-suspended in pyridine (13mL). To the mixture at 0°C was added chlorotrimethylsilane (1.686mL, 13.19mmol) dropwise. It was warmed to rt and stirred for 2h. The reaction was cooled to 0°C, and isobutyric anhydride (0.656mL, 3.96mmol) was added dropwise. It was warmed to rt, stirred for 2h, and then water (4mL) and NH_4OH (8mL) were added to the reaction. After 30min, it was concentrated. The residue was purified by flash column chromatography with 0-10% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 374.1 [M + H]$^+$. ^{1}H-NMR: (300MHz, DMSO-d_6) δ 12.11 (s, 1H), 11.69 (s, 1H), 8.30 (s, 1H), 6.58 (d, J = 6.0Hz, 1H), 5.74 (dd, J = 9.0, 3.0Hz, 1H), 5.33 (t, J = 6.0Hz, 1H), 4.96-4.83 (m, 1H), 4.26-4.17 (m, 1H), 3.72-3.62 (m, 2H), 2.80-2.71 (m, 1H), 1.11 (d, J = 9.0Hz, 6H).

Step 10: N-(9-((2R,3S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4,4-difluoro-3-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide

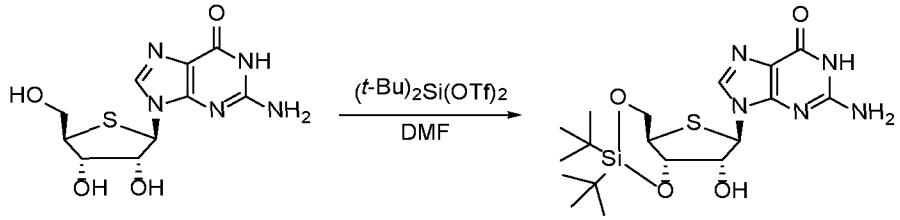


N-(9-((2R,3S,5R)-4,4-difluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (640mg, 1.714mmol) was co-evaporated with pyridine (3x3mL) and then re-suspended in pyridine (5.7mL). To the suspension was added 4,4'-(chloro(phenyl)methylene)bis(methoxybenzene) (639mg, 1.886mmol), and it was stirred for 16h. Then, it was concentrated and then, co-evaporated with toluene (3x20mL). The crude was purified by silica column chromatography eluting with 1 to 30% MeOH in CH_2Cl_2 (containing 1% Et_3N) to give the product. LCMS (ES, m/z): 676.3 $[M + H]^+$. ^{1}H-NMR: (300MHz, DMSO-d_6) δ 12.11 (s, 1H), 11.61 (s, 1H), 8.15 (s, 1H), 7.34 (J = 6.0, 3.0Hz, 2H), 7.28-7.19 (m, 7H), 6.85-6.80 (m, 4H), 6.71 (d, J = 6.0Hz, 1H), 5.78 (d, J = 6.0Hz, 1H), 5.13-5.05 (m, 1H), 4.46-4.39 (m, 1H), 3.71 (s, 6H), 3.46-3.40 (m, 1H), 3.22-3.18 (m, 1H), 2.78-2.70 (m, 1H), 1.11 (d, J = 9.0Hz, 6H).

Preparation 17: N-(9-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-fluoro-3-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



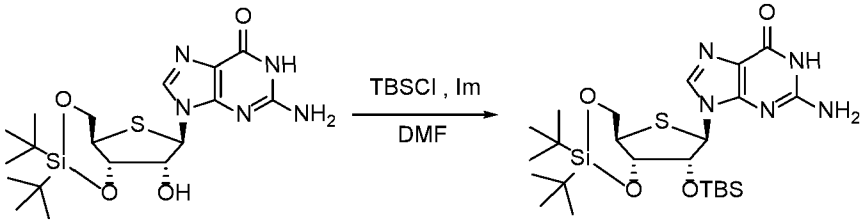
Step 1: 2-amino-9-((4aR,6R,7R,7aR)-2,2-di-tert-butyl-7-hydroxytetrahydro-4H-thieno[3,2-d][1,3,2]dioxasilin-6-yl)-1,9-dihydro-6H-purin-6-one



To a stirred suspension of 2-amino-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-1*H*-purin-6(9*H*)-one (15g, 50.1mmol) in DMF (150mL) at 0°C under Ar was injected di-*tert*-butylsilanediyl bis(trifluoromethanesulfonate)

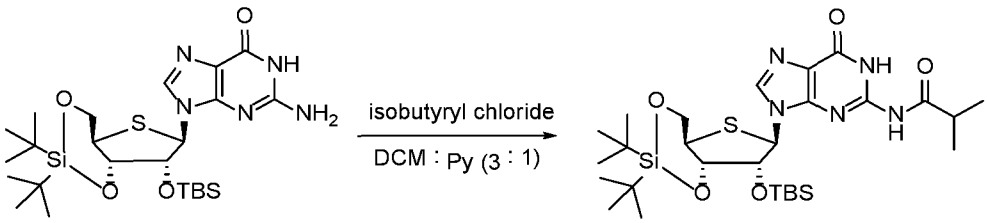
(26.5g, 60.1mmol). The resulting solution was stirred at rt for 1h. It was used for the next reaction step directly without purification. LCMS (ES, m/z): 440.2 $[M + H]^+$.

Step 2: 2-amino-9-((4aR,6R,7R,7aR)-2,2-di-tert-butyl-7-((tert-butyldimethylsilyl)oxy) tetrahydro-4H-thieno[3,2-d][1,3,2]dioxasilin-6-yl)-1,9-dihydro-6H-purin-6-one



To the reaction mixture from the previous step at 0 °C was added 1*H*-imidazole (17.05g, 251mmol) in one portion. The mixture was stirred rt for 0.5h. *tert*-butylchlorodimethylsilane (15.10g, 100mmol) was added to the mixture, and it was stirred at 60°C for 16h. Then, the volatile components were removed under reduced pressure. The solid was suspended in cold methanol (75mL), filtered, and washed with cold methanol (2x30mL). The solid was kept under reduced pressure to give the product. LCMS (ES, m/z): 554.4 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ 10.80 (s, 1H), 7.98 (s, 1H), 6.49 (s, 2H), 5.53 (s, 1H), 4.46 (d, J = 3.2Hz, 1H), 4.42 (d, J = 9.9Hz, 1H), 4.34 (dd, J = 9.9, 4.7Hz, 1H), 4.21 (t, J = 10.5Hz, 1H), 3.70-3.64 (m, 1H), 1.04 (s, 9H), 1.00 (s, 9H), 0.92 (s, 9H), 0.19 (s, 3H), 0.11 (s, 3H).

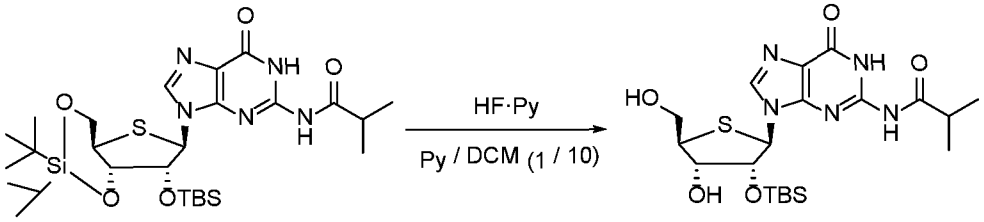
Step 3: N-(9-((4aR,6R,7R,7aR)-2,2-di-tert-butyl-7-((tert-butyldimethylsilyl)oxy)tetrahydro-4H-thieno[3,2-d][1,3,2]dioxasilin-6-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



2-amino-9-((4a*R*,6*R*,7*R*,7a*S*)-2,2-di-*tert*-butyl-7-((*tert*-butyldimethylsilyl)oxy)tetrahydro-4*H*-thieno[3,2-d][1,3,2]dioxasilin-6-yl)-1*H*-purin-6(9*H*)-one (29.1g, 52.5mmol) was co-evaporated with dry pyridine (3x50mL) and re-dissolved in pyridine (70mL) and dichloromethane (210mL). The mixture was charged with Ar and cooled to 0°C. To the mixture was added isobutyryl chloride (11.20g, 105mmol). It was stirred at rt for 4h. It was concentrated under reduced pressure. The solid was suspended in cold methanol (100mL), filtered, and washed with cold methanol (3x50mL). The solid was kept under reduced pressure to give the product. LCMS (ES, m/z): 624.1 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ 12.13 (s, 1H), 11.39 (s, 1H), 8.32 (s, 1H), 5.61 (s, 1H), 4.66 (d, J = 3.4Hz, 1H), 4.48 (d, J = 9.9Hz, 1H),

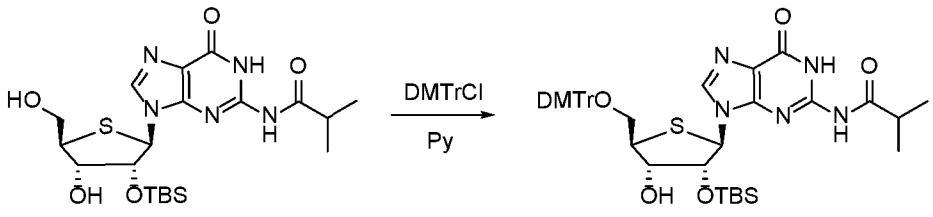
4.38-4.33 (m, 1H), 4.21 (t, J = 9.9Hz, 1H), 3.76-3.70 (m, 1H), 2.84-2.80 (m, 1H), 1.13 (d, J = 6.7Hz, 6H), 1.06 (s, 9H), 1.01 (s, 9H), 0.91 (s, 9H), 0.16 (s, 3H), 0.11 (s, 3H).

Step 4: N-(9-((2R,3R,4S,5R)-3-((tert-butyldimethylsilyl)oxy)-4-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



HF-Pyridine (26.6g, 188mmol) at 0°C was diluted with pyridine (29mL). The resulting solution was added slowly to a stirred suspension of *N*-(9-((4a*R*,6*R*,7*R*,7a*S*)-2,2-di-*tert*-butyl-7-((*tert*-butyldimethylsilyl)oxy)tetrahydro-4*H*-thieno[3,2-*d*][1,3,2]dioxasilin-6-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)isobutyramide (29.3g, 47.0mmol) in CH_2Cl_2 (290mL) at 0°C. It was stirred at 0°C for 2h. The reaction mixture was diluted with CH_2Cl_2 (500mL). It was washed with water (500mL) and sat aq $NaHCO_3$ (500mL). The organic layer was dried (Na_2SO_4) and concentrated to give the product. LCMS (ES, m/z): 484.4 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ 12.05 (s, 1H), 11.73 (s, 1H), 8.43 (s, 1H), 5.89 (d, J = 7.9Hz, 1H), 5.34 (d, J = 3.9Hz, 1H), 5.27 (t, J = 5.6Hz, 1H), 4.60 (dd, J = 8.1, 3.2Hz, 1H), 4.19-4.17 (m, 1H), 3.80-3.74 (m, 1H), 3.66-3.60 (m, 1H), 3.30 (t, J = 8.0Hz, 1H), 2.80-2.73 (m, 1H), 1.12 (d, J = 6.7Hz, 6H), 0.68 (s, 9H), -0.06 (s, 3H), -0.29 (s, 3H).

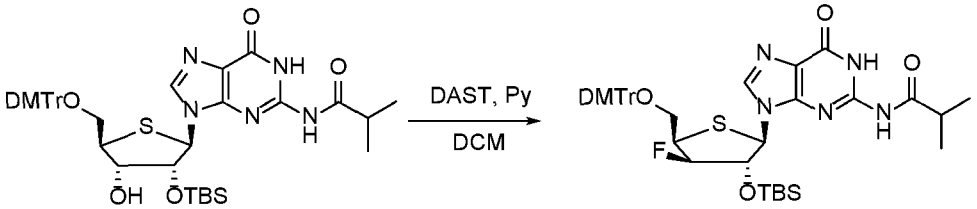
Step 5: N-(9-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-((tert-butyldimethylsilyl)oxy)-4-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



N-(9-((2*R*,3*R*,4*S*,5*R*)-3-((*tert*-butyldimethylsilyl)oxy)-4-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)isobutyramide (21g, 43.4mmol) was co-evaporated with pyridine (3x50mL) and dissolved in pyridine (210mL). 4,4'-(chloro(phenyl)methylene)bis(methoxybenzene) (16.2g, 47.8mmol) was added, and it was stirred at rt for 3h and then concentrated under reduced pressure and co-evaporated with toluene (3x50mL). The crude was purified by silica gel chromatography eluting with 0-40% EtOAc in CH_2Cl_2 (containing 0.1% Et_3N) to give the product. LCMS (ES, m/z): 786.4 $[M + H]^+$. ^{1}H-NMR

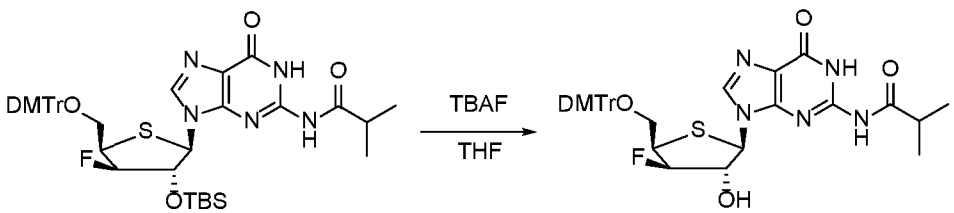
(300MHz, DMSO-d_6): δ 12.06 (s, 1H), 11.71 (s, 1H), 8.19 (s, 1H), 7.44-7.42 (m, 2H), 7.37-7.22 (m, 7H), 6.92 (d, J = 8.5Hz, 4H), 5.87 (d, J = 7.2Hz, 1H), 5.44 (d, J = 4.4Hz, 1H), 4.40 (dd, J = 7.3, 3.3Hz, 1H), 4.19 (d, J = 5.9Hz, 1H), 3.75 (s, 6H), 3.54-3.35 (m, 2H), 3.34-3.28 (m, 1H), 2.83 – 2.71 (m, 1H), 1.11 (d, J = 6.7Hz, 6H), 0.70 (s, 9H), -0.08 (s, 3H), -0.29 (s, 3H).

Step 6: N-(9-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-((tert-butyldimethylsilyl)oxy)-4-fluorotetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



To a solution of *N*-(9-((2*R*,3*R*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)-methyl)-3-((*tert*-butyldimethylsilyl)oxy)-4-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)isobutyramide (22g, 28.0mmol) in CH_2Cl_2 (220mL) at 0°C was added pyridine (18.11mL, 224mmol) and DAST (14.79mL, 112mmol) dropwise. The reaction was allowed to warm to rt and stirred for 7h. It was then cooled to 0°C and quenched by slow addition of sat aq $NaHCO_3$ (500mL). More CH_2Cl_2 (500mL) was added, and the phases were separated. The organic phase was washed with sat aq $NaHCO_3$ (3x200mL) and brine (300mL), dried (Na_2SO_4), concentrated, and purified by reverse phase (C18) chromatography eluting with 45-95% of ACN in aq NH_4HCO_3 (5 mM) to give the product. LCMS (ES, m/z): 788.2 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ 12.06 (s, 1H), 11.56 (s, 1H), 8.02 (s, 1H), 7.44-7.42 (m, 2H), 7.34-7.23 (m, 7H), 6.92-6.89 (m, 4H), 5.71 (d, J = 4.9Hz, 1H), 5.14 (dt, J = 51.0, 5.6Hz, 1H), 5.01-4.96 (m, 1H), 3.88-3.85 (m, 1H), 3.75 (s, 6H), 3.57 (t, J = 8.8Hz, 1H), 3.50 (dd, J = 10.0, 5.3Hz, 1H), 2.81-2.74 (m, 1H), 1.12 (d, J = 6.8Hz, 6H), 0.77 (s, 9H), 0.00 (s, 3H), -0.16 (s, 3H). F-NMR: (376MHz, DMSO-d_6, *ppm*) δ -193.99 (s, 1F).

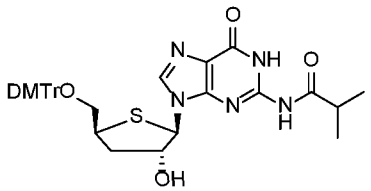
Step 7: N-(9-((2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



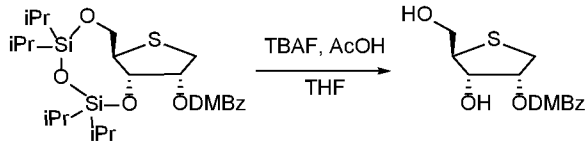
To a solution of *N*-(9-((2*R*,3*R*,4*R*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)-methoxy) methyl)-3-((*tert*-butyldimethylsilyl)oxy)-4-fluorotetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-

1*H*-purin-2-yl)isobutyramide (7.2g, 6.85mmol) in THF (70mL) at rt was added TBAF (1.0M in THF, 8.22mL, 8.22mmol) dropwise. It was stirred at rt for 30min, the solution was concentrated, and CH_2Cl_2 (300mL) was added. The mixture was washed with $NaHCO_3$ (3x200mL) and brine (200mL), and the organic phase was separated, dried (Na_2SO_4), concentrated and purified by reverse phase (C18) chromatography eluting with 0-95% of ACN in aq NH_4HCO_3 (5mM) to give the product. LCMS (ES, m/z): 674.3 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ 12.02 (br, 1H), 7.85 (s, 1H), 7.44-7.41 (m, 2H), 7.36-7.24 (m, 7H), 6.94-6.90 (m, 4H), 6.33 (bs, 1H), 5.78 (d, *J* = 2.7Hz, 1H), 5.19 (dt, *J* = 50.2, 4.0Hz, 1H), 4.80-4.75 (m, 1H), 4.10-4.02 (m, 1H), 3.76 (s, 6H), 3.55 (dd, *J* = 9.3, 5.6Hz, 1H), 3.44 (t, *J* = 8.9Hz, 1H), 2.79-2.71 (m, 1H), 1.12 (d, *J* = 6.8Hz, 6H). F-NMR: (376MHz, DMSO-d_6) δ -194.75 (s).

Preparation 18: N-(9-((2R,3R,5S)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide

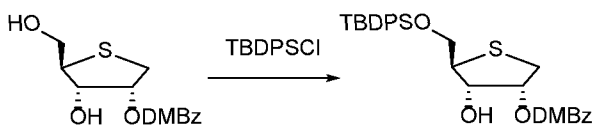


Step 1: (3R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate



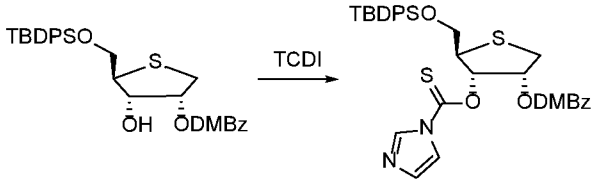
To a solution of (6aR,9R,9aS)-2,2,4,4-tetraisopropyltetrahydro-6H-thieno[3,2-f][1,3,5,2,4]trioxadisilocin-9-yl 2,4-dimethoxybenzoate (60g, 108mmol) in THF (500mL) were added AcOH (13.59g, 226mmol) and TBAF in THF (1 M, 226mL, 226mmol). After 1h, it was concentrated under reduced pressure and purified by silica gel column chromatography eluting with 0-20% EtOAc in CH_2Cl_2 to give the product. LCMS (ES, m/z): 315.1 $[M + H]^+$. ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.7Hz, 1H), 6.58-6.46 (m, 2H), 5.51 (dt, *J* = 5.0, 3.7Hz, 1H), 4.31 (td, *J* = 6.9, 3.7Hz, 1H), 3.88 (d, *J* = 12.0Hz, 8H), 3.58 (dt, *J* = 7.1, 4.7Hz, 1H), 3.26 (dd, *J* = 12.2, 5.0Hz, 1H), 3.15-3.01 (m, 2H), 2.31 (s, 1H).

Step 2: (3R,4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-hydroxytetrahydrothiophen-3-yl 2,4-dimethoxybenzoate



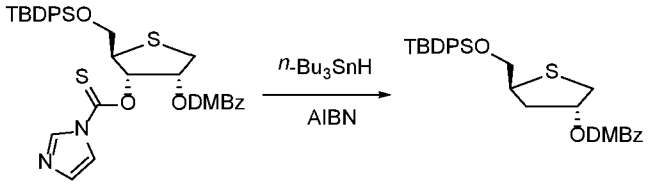
To a solution of (3R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate (33g, 105mmol) in pyridine (300mL) was added *tert*-butylchlorodiphenylsilane (43.3g, 157mmol). It was stirred at rt for 4h. Then, water (300mL) was added. Layers were separated, and the aq layer was extracted with CH_2Cl_2 (3x300mL). The combined organic layer was washed with brine (300mL), dried (Na_2SO_4), concentrated, and purified by silica gel column chromatography eluting with 1 to 10% EtOAc in petroleum ether to give the product. LCMS (ES, m/z): 575.3 $[M + Na]^+$. ^{1}H-NMR (400MHz, DMSO-d_6) δ 7.83 (d, J = 8.7Hz, 1H), 7.68 (t, J = 5.5Hz, 5H), 7.54-7.38 (m, 6H), 6.69-6.60 (m, 2H), 5.40 (d, J = 5.6Hz, 1H), 5.35-5.29 (m, 1H), 4.12 (s, 1H), 4.05 (d, J = 7.3Hz, 1H), 3.85 (d, J = 7.2Hz, 6H), 3.78-3.66 (m, 1H), 3.55 (d, J = 7.2Hz, 1H), 3.14 (dd, J = 11.0, 5.0Hz, 1H), 2.86-2.77 (m, 1H), 1.03 (s, 9H).

Step 3: (3R,4S,5R)-4-((1H-imidazole-1-carbonothioyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate



To a solution of (3R,4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-hydroxytetrahydrothiophen-3-yl 2,4-dimethoxybenzoate (48g, 87mmol) in dichloroethane (500mL) was added di(1*H*-imidazol-1-yl)methanethione (20.12g, 113mmol). It was heated at 85°C under Ar for 1h. Then, it was concentrated and used in the next step without purification. LCMS (ES, m/z): 663.2 $[M + H]^+$.

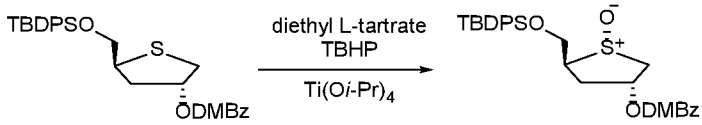
Step 4: (3R,5S)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate



To a solution of (3R,4S,5R)-4-((1H-imidazole-1-carbonothioyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate (crude, 57.6g, 87mmol) in THF (40mL) and toluene (200mL) was added tributylstannane (139g, 478mmol). It was heated at 95°C, and 2, 2'-(diazene-1,2-diyl)bis(2-methylpropanenitrile) (1.427g, 8.69mmol) in toluene (200mL) was added over 30min. After 1h, the resulting mixture was concentrated and purified by silica gel column chromatography eluting with 0 to 10% EtOAc in petroleum ether to

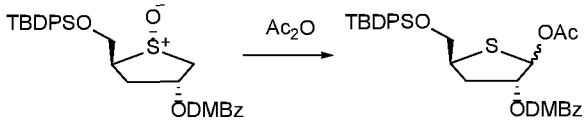
give the product. LCMS (ES, m/z): 537.3 $[M + H]^+$. ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.88 (d, J = 8.6Hz, 1H), 7.77-7.67 (m, 4H), 7.50-7.36 (m, 6H), 6.58-6.48 (m, 2H), 5.70 (p, J = 3.8Hz, 1H), 3.95-3.74 (m, 10H), 3.28 (dd, J = 12.0, 4.6Hz, 1H), 3.10-3.02 (m, 1H), 2.49-2.39 (m, 1H), 1.92 (ddd, J = 13.3, 8.6, 4.1Hz, 1H), 1.09 (s, 9H).

Step 5: (1R,3R,5S)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-1-oxidotetrahydrothiophen-3-yl 2,4-dimethoxybenzoate



To a solution of $Ti(OiPr)_4$ (23.29mL, 78mmol) in CH_2Cl_2 (130mL) under Ar was added diethyl (L)-tartrate (38.3mL, 224mmol) dropwise. After 10min, the mixture was cooled to -20°C, and then TBHP in decane (~5.5M, 27.1mL, 149mmol) was added dropwise. After 5min, a solution of (3R,5S)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate (40g, 74.5mmol) in CH_2Cl_2 (130mL) was added to the reaction at -20°C. The resulting mixture was stirred at -20°C for 16h. It was quenched by the addition of ice water (300mL) and allowed to warm up to rt. The precipitate was filtered off and washed with EtOAc (3x300mL). The filtrate was washed with water (3x200mL). The aq layer was extracted with EtOAc (400mL). The combined organic layer was dried (Na_2SO_4), concentrated and purified by flash chromatography eluting with 0 to 70% EtOAc in petroleum ether to give the product (mixture of two isomers). LCMS (ES, m/z): 553.2 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6) δ 7.81 (d, J = 8.7Hz, 1H), 7.75-7.61 (m, 4H), 7.54-7.39 (m, 6H), 6.67-6.56 (m, 2H), 5.66 (q, J = 3.8Hz, 1H), 4.12 (dt, J = 10.5, 4.4Hz, 1H), 3.92-3.79 (m, 8H), 3.58-3.42 (m, 1H), 3.20-3.09 (m, 1H), 2.97 (d, J = 15.0Hz, 1H), 2.05 (ddd, J = 14.5, 10.5, 4.3Hz, 1H), 1.02 (s, 9H).

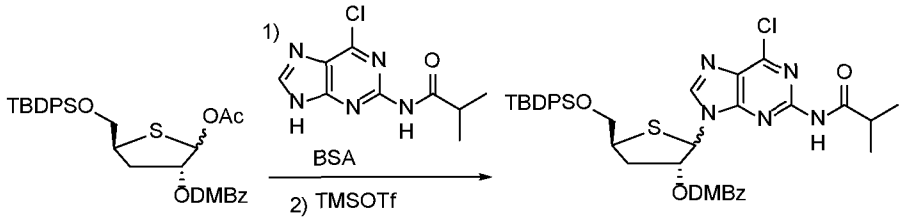
Step 6: (3R,5S)-2-acetoxy-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate



A solution of (3R,5S)-2-acetoxy-5-(((tert-butyldiphenylsilyl)oxy)methyl)-tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate (21g, 34.2mmol) in acetic anhydride (210mL) was heated at 110°C. After stirring of 3.5h, the reaction mixture was cooled to rt and concentrated. The residue was purified by silica gel column chromatography eluting with 0% to 20% EtOAc in petroleum ether to give the product. LCMS (ES, m/z): 535.3 $[M - OAc]^+$. ^{1}H-NMR (400MHz, DMSO-d_6) δ 7.76-7.61 (m, 5H), 7.46 (dq, J = 7.6, 4.5, 3.7Hz, 6H), 6.68-6.58

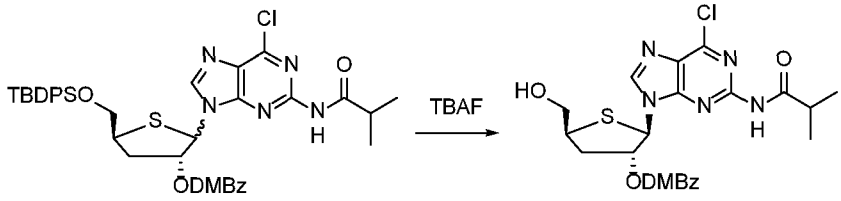
(m, 2H), 6.22 (d, J = 4.3Hz, 0.65H), 5.94 (s, 0.28H), 5.51-5.46 (m, 0.33H), 5.38 (ddd, J = 11.1, 7.2, 4.3Hz, 0.65H), 3.96-3.61 (m, 9H), 2.40-2.21 (m, 2H), 2.03 (d, J = 1.6Hz, 3H), 1.01 (s, 9H).

Step 7: (3R,5S)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(6-chloro-2-isobutyramido-9H-purin-9-yl)tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate



To a solution of *N*-(6-chloro-9*H*-purin-2-yl)isobutyramide (10.27g, 42.9mmol) in toluene (600mL) at 0°C was added trimethylsilyl *N*-(trimethylsilyl)acetimidate (23.26g, 114mmol). It was heated at 80°C for 1h and was cooled to 0°C again. To the reaction was then added a solution of (3*R*, 5*S*)-2-acetoxy-5-(((*tert*-butyldiphenylsilyl)oxy)methyl)-tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate (17g, 28.6mmol) in toluene (600mL) and trimethylsilyl trifluoromethanesulfonate (19.06g, 86mmol). It was heated to 80°C and stirred under Ar for 12h. At that time, the reaction was cooled to rt, and sat aq $NaHCO_3$ (400mL) was added. Layers were separated, and the aq layer was extracted with EtOAc (4x1000mL). The combined organic phase was dried (Na_2SO_4), concentrated and purified by silica gel column chromatography eluting with 10% to 40% EtOAc in petroleum ether to give the product (mixture of α and β isomers). LCMS (ES, m/z): 774.3 $[M + H]^+$. ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.48 (s, 0.32H), 8.35 (s, 0.69H), 7.97-7.83 (m, 1.67H), 7.70 (dq, J = 8.4, 1.5Hz, 4H), 7.54 (d, J = 8.7Hz, 0.33H), 7.49-7.36 (m, 6H), 6.57-6.36 (m, 2.5H), 6.18 (d, J = 2.4Hz, 0.7H), 5.87-5.80 (m, 0.35H), 5.73 (q, J = 3.3Hz, 0.75H), 4.23-4.01 (m, 1.2H), 3.98-3.74 (m, 7.8H), 3.11 (s, 0.73H), 2.95 (s, 0.37H), 2.57-2.36 (m, 1.75H), 2.30-2.20 (m, 0.34H), 1.23 (d, J = 6.8Hz, 2.19H), 1.19 (dd, J = 6.8, 3.5Hz, 4.38H), 1.09 (d, J = 1.7Hz, 9H).

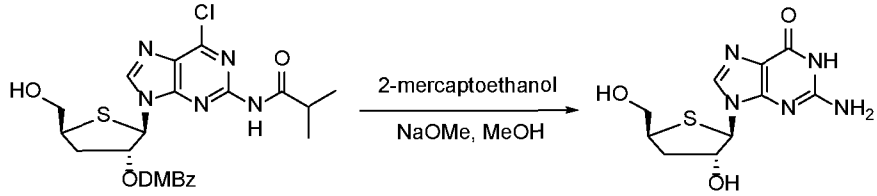
Step 8: (2R,3R,5S)-2-(6-chloro-2-isobutyramido-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate



To a solution of (3R,5S)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(6-chloro-2-isobutyramido-9H-purin-9-yl)tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate (20g, 25.8mmol) in THF (135mL) was added TBAF in THF (1 M, 31mL, 31mmol) dropwise. After 1h, the

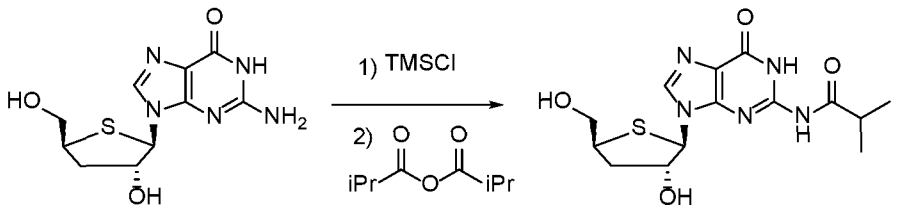
reaction mixture was concentrated and purified by column chromatography using 1% to 10% MeOH in CH_2Cl_2 as the eluent to give a mixture of two isomers. It was re-purified by reverse phase (C18) chromatography eluting with 10 to 45% ACN in aq NH_4CO_3 (5mM) to give the product. LCMS (ES, m/z): 536.2 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6) δ 10.80 (s, 1H), 8.83 (s, 1H), 7.72 (d, J = 8.7Hz, 1H), 6.65-6.55 (m, 2H), 6.18 (d, J = 2.5Hz, 1H), 5.80 (q, J = 3.5Hz, 1H), 5.22 (t, J = 5.1Hz, 1H), 3.93-3.67 (m, 9H), 2.85 (p, J = 6.9Hz, 1H), 2.70 (ddd, J = 13.4, 8.5, 4.5Hz, 1H), 2.36 (dt, J = 14.1, 5.0Hz, 1H), 1.06 (dd, J = 6.8, 3.3Hz, 6H).

Step 9: 2-amino-9-((2R,3R,5S)-3-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-1,9-dihydro-6H-purin-6-one



To a solution of (2R,3R,5S)-2-(6-chloro-2-isobutyramido-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrothiophen-3-yl 2,4-dimethoxybenzoate (6.0g, 11.19mmol) in MeOH (300mL) were added 2-mercaptoethanol (3.50g, 44.8mmol) and NaOMe (10.08g, 56mmol, 30% in MeOH). It was heated at 60°C for 16h, cooled to rt, and conc. HCl (4mL) was added. The resulting mixture was concentrated, and water (100mL) and EtOAc (100mL) were added. Layers were separated, and the aq layer was extracted with EtOAc (3x100mL). The aq layer was basified with $NaHCO_3$ (solid) to ~pH 8 and stirred at rt for 1h. The precipitate was filtered and kept under reduced pressure to give the product. LCMS (ES, m/z): 284.1 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6) δ 10.57 (s, 1H), 7.98 (s, 1H), 6.46 (s, 2H), 5.57 (dd, J = 10.9, 3.9Hz, 2H), 5.12 (t, J = 5.4Hz, 1H), 4.48 (p, J = 4.1Hz, 1H), 3.78-3.53 (m, 3H), 2.11-1.99 (m, 2H).

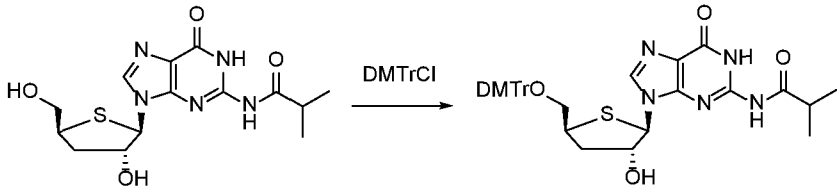
Step 10: N-(9-((2R,3R,5S)-3-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



2-amino-9-((2R,3R,5S)-3-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-1,9-dihydro-6H-purin-6-one (580mg, 2.047mmol) was co-evaporated with pyridine (3x20mL) and then re-dissolved in pyridine (20mL). The mixture was cooled to 0°C and then treated with chlorotrimethylsilane (1557mg, 14.33mmol). It was warmed to rt and stirred for 2h. Then, the

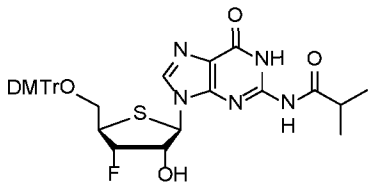
reaction was cooled to 0°C again, and isobutyric anhydride (486mg, 3.07mmol) was added dropwise. It was warmed to rt and stirred for 2h. The reaction was quenched by the addition of methanol (5mL). After 5min, NH_4OH (ca 29%, 10mL) was added. The mixture was stirred at rt for 30min. Then, it was concentrated and purified by column chromatography on silica gel eluting with 10% to 20% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 354.1 $[M + H]^+$. ^{1}H-NMR (300MHz, CD_3OD) δ: 8.40 (s, 1H), 5.83-5.82 (m, 1H), 4.62-4.59 (m, 1H), 3.89-3.80 (m, 2H), 3.79-3.74 (m, 1H), 2.73-2.64 (m, 1H), 2.19-2.11(m, 2H), 1.20 (d, J = 6.8Hz, 6H).

Step 11: N-(9-((2R,3R,5S)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide

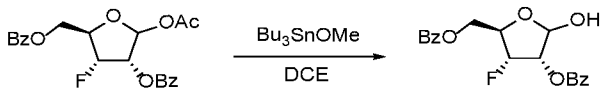


N-(9-((2R,3R,5S)-3-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (510mg, 1.443mmol) was co-evaporated with pyridine (3x5mL) and then re-suspended in pyridine (7mL). To the suspension was added 4,4'-(chloro(phenyl)methylene)bis(methoxybenzene) (538mg, 1.587mmol), and the mixture was stirred at rt for 2h. At that time the mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel eluting with 1% to 4% MeOH in CH_2Cl_2 (containing 1% Et_3N) to give the product. LCMS (ES, m/z): 656.0 $[M + H]^+$. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.02 (s, 1H), 7.51- 7.43 (m, 2H), 7.40-7.17 (m, 7H), 6.91-6.82 (m, 4H), 5.85-5.84 (d, J = 2.3Hz, 1H), 4.59-4.57 (m, 1H), 4.02-3.95 (m, 1H), 3.78(s, 6H), 3.52-3.34 (m, 3H), 2.72-2.68 (m, 1H), 1.95-191(m, 1H), 1.38 (d, J = 6.8Hz, 6H).

Preparation 19: N-(9-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-fluoro-3-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide

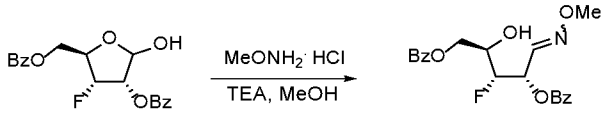


Step 1: ((2R,3R,4S)-4-(benzoyloxy)-3-fluoro-5-hydroxytetrahydrofuran-2-yl)methyl benzoate



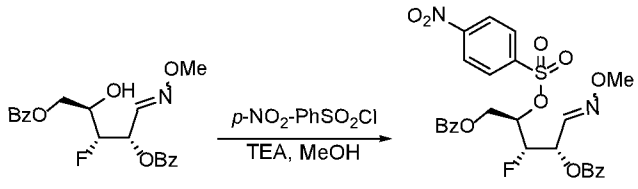
To a stirred solution of ((2R,3R,4S)-5-acetoxy-4-(benzoyloxy)-3-fluorotetrahydrofuran-2-yl)methyl benzoate (20.0g, 49.7mmol) in dry 1,2-dichloroethane (200 mL) was added tri-N-butyltin methoxide (28.8mL, 99mmol). The resulting mixture was stirred at 80°C for 3h and then concentrated *in vacuo*. The residue was diluted in 500mL of ethyl acetate and washed with sat aq. NH_4Cl (500mL) and brine (500mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by a silica gel column, eluting with 0-40% EtOAc/Hexane. LCMS (ES, m/z): 343.2 $[M+H-H_2O]^+$. ^1H NMR (500MHz, Chloroform-*d*) δ 8.18 – 8.00 (m, 7H), 7.68 – 7.57 (m, 3H), 7.61 – 7.35 (m, 7H), 5.73 (dd, *J* = 8.3, 4.6Hz, 1H), 5.65 (dt, *J* = 3.9, 2.0Hz, 1H), 5.54 (t, *J* = 4.7Hz, 0H), 5.50 – 5.40 (m, 2H), 5.36 – 5.24 (m, 1H), 4.87 (dtd, *J* = 25.5, 4.0, 1.5Hz, 1H), 4.74 – 4.46 (m, 4H), 4.15 (q, *J* = 7.2Hz, 1H), 3.39 (d, *J* = 4.1Hz, 1H), 3.30 (dd, *J* = 8.6, 3.4Hz, 1H), 2.07 (s, 2H), 1.41 – 1.23 (m, 3H).

Step 2: (2R,3R,4S)-3-fluoro-2-hydroxy-5-(methoxyimino)pentane-1,4-diyl dibenzoate



To a stirred solution of ((2R,3R,4S)-4-(benzoyloxy)-3-fluoro-5-hydroxytetrahydrofuran-2-yl)methyl benzoate (17.9g, 49.7mmol) in dry MeOH (100 mL) was added O-methylhydroxylamine hydrochloride (6.23g, 74.6mmol), followed by triethylamine (10.39mL, 74.6mmol). The reaction mixture was stirred at rt for 2h and then concentrated *in vacuo*. The residue was diluted in 500mL of ethyl acetate and washed with sat aq. NH_4Cl (500mL) and brine (500mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was used for the next step directly without further purification. LCMS (ES, m/z): 390.2 $[M+H]^+$.

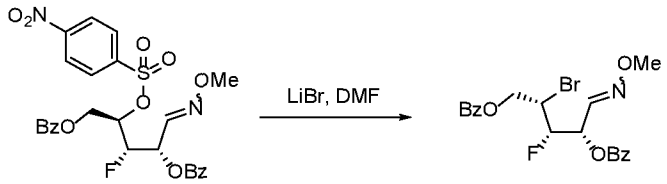
Step 3: (2R,3S,4S)-3-fluoro-5-(methoxyimino)-2-(((4-nitrophenyl)sulfonyl)oxy)pentane-1,4-diyl dibenzoate



To a stirred solution of (2R,3R,4S)-3-fluoro-2-hydroxy-5-(methoxyimino)pentane-1,4-diyl dibenzoate (19.4g, 49.7mmol) in dry EtOAc (100 mL) was added 4-nitrobenzenesulfonyl chloride (16.5g, 74.6mmol), followed by triethylamine (10.4mL, 74.6mmol). The reaction mixture was stirred at rt for 18h and was then diluted with 200mL of ethyl acetate, washed with

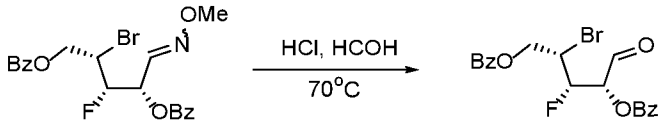
water (300mL) and brine (300mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by a silica gel column, eluting with 0-30% EtOAc/Hexane. LCMS (ES, m/z): 575.3 $[M+H]^+$. ^1H NMR (500MHz, Chloroform-*d*) δ 8.25 – 7.99 (m, 7H), 7.94 – 7.80 (m, 2H), 7.68 – 7.55 (m, 2H), 7.57 – 7.46 (m, 3H), 7.50 – 7.38 (m, 3H), 6.55 (ddd, *J* = 17.2, 5.3, 2.8Hz, 0H), 5.89 (ddd, *J* = 17.7, 6.2, 4.1Hz, 1H), 5.46 – 5.37 (m, 1H), 5.40 – 5.28 (m, 1H), 5.21 (t, *J* = 4.3Hz, 0H), 4.84 (tdd, *J* = 12.8, 2.6, 1.5Hz, 1H), 4.53 (dddd, *J* = 23.1, 13.0, 7.0, 1.8Hz, 1H), 4.14 (q, *J* = 7.2Hz, 1H), 3.99 (d, *J* = 36.3Hz, 4H), 2.06 (s, 2H), 1.44 – 1.23 (m, 2H).

Step 4: (2S,3S,4S)-2-bromo-3-fluoro-5-(methoxyimino)pentane-1,4-diyl dibenzoate



To a stirred solution of (2R,3S,4S)-3-fluoro-5-(methoxyimino)-2-(((4-nitrophenyl)sulfonyl)oxy)pentane-1,4-diyl dibenzoate (21.3g, 37.1mmol) in dry DMF (100mL) was added freshly opened lithium bromide powder (16.1g, 185mmol). The resulting mixture was stirred at 60°C for 18h. The reaction mixture was diluted in 300mL of ethyl acetate and washed with water (500mL) and brine (500mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by a silica gel column, eluting with 0-25% EtOAc/Hexane to give product. LCMS (ES, m/z): 452.1 $[M+H]^+$. ^1H NMR (500MHz, Chloroform-*d*) δ 8.15 – 8.03 (m, 11H), 7.69 – 7.56 (m, 8H), 7.56 – 7.41 (m, 12H), 6.93 – 6.86 (m, 1H), 6.45 (ddd, *J* = 14.7, 5.9, 4.7Hz, 0H), 5.93 (ddd, *J* = 11.8, 6.7, 5.9Hz, 2H), 5.32 (s, 1H), 5.29 – 5.10 (m, 3H), 4.89 – 4.77 (m, 3H), 4.80 – 4.67 (m, 3H), 4.60 – 4.40 (m, 3H), 3.95 (d, *J* = 16.9Hz, 2H), 3.89 (s, 6H), 1.32 – 1.22 (m, 1H).

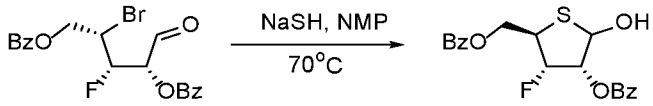
Step 5: (2S,3S,4S)-2-bromo-3-fluoro-5-oxopentane-1,4-diyl dibenzoate



To a stirred solution of (2S,3S,4S)-2-bromo-3-fluoro-5-(methoxyimino)pentane-1,4-diyl dibenzoate (20.0g, 44.2mmol) in THF (200mL) was added 37% aqueous solution of formaldehyde (32.9mL, 442mmol) and 1N HCl (44.2mL, 44.2mmol). The resulting mixture was stirred at 55°C for 5h. The reaction mixture was concentrated *in vacuo* to remove most of the THF. The residue was diluted in 300mL of ethyl acetate and washed with water (300mL) and brine (300mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered, and

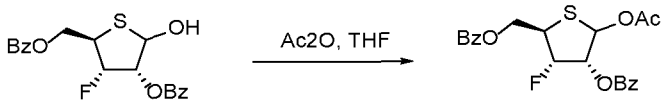
concentrated *in vacuo*. The residue was used for the next step without further purification. LCMS (ES, m/z): 423.2 $[M+H]^+$.

Step 6: ((2R,3S,4R)-4-(benzoyloxy)-3-fluoro-5-hydroxytetrahydrothiophen-2-yl)methyl benzoate



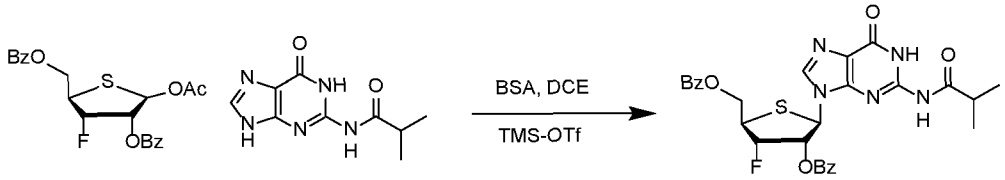
To a stirred solution of (2S,3S,4S)-2-bromo-3-fluoro-5-oxopentane-1,4-diyl dibenzoate (18.7g, 44.2mmol) in NMP (150mL) at 0°C was added sodium hydrosulfide (5.0g, 89.0mmol). The resulting mixture was stirred at 0°C for 30min and then at rt for 30min. The reaction mixture was diluted in 300mL of ethyl acetate and washed with water (300mL) and brine (300mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was used for the next step without further purification. LCMS (ES, m/z): 359.2 $[M+H-H_2O]^+$.

Step 6: ((2R,3S,4R)-5-acetoxy-4-(benzoyloxy)-3-fluorotetrahydrothiophen-2-yl)methyl benzoate



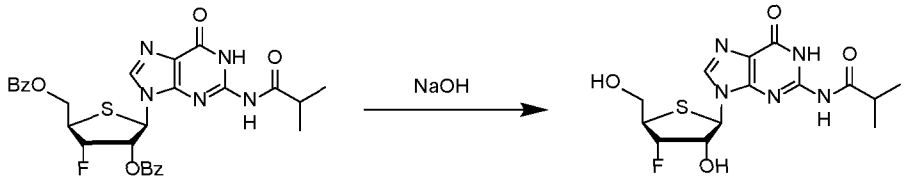
To a stirred solution of ((2R,3S,4R)-4-(benzoyloxy)-3-fluoro-5-hydroxytetrahydrothiophen-2-yl)methyl benzoate (16.6g, 44.2mmol) in dry THF (150 mL) at 0°C was added acetic anhydride (8.3mL, 133mmol) and trimethylamine (18.5mL, 133mmol). The resulting mixture was stirred at 0°C for 30min and then at rt for 2h. The reaction was quenched by addition of MeOH, and the reaction mixture was concentrated *in vacuo* to remove most of the THF. The residue was diluted in 300mL of ethyl acetate and washed with water (300mL) and brine (300mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by a silica gel column, eluting with 0-25% EtOAc/Hexane to give product. LCMS (ES, m/z): 441.2 $[M+Na]^+$. ^1H NMR (500MHz, Chloroform-*d*) δ 8.15 – 8.02 (m, 4H), 7.68 – 7.57 (m, 2H), 7.55 – 7.42 (m, 4H), 6.02 (t, J = 2.4Hz, 1H), 5.88 (ddd, J = 6.1, 3.6, 2.4Hz, 1H), 5.50 – 5.42 (m, 0H), 5.35 (dd, J = 7.3, 3.6Hz, 0H), 5.32 (s, 0H), 4.80 – 4.69 (m, 1H), 4.56 (dd, J = 11.6, 5.9Hz, 1H), 4.26 – 4.10 (m, 2H), 2.25 – 2.11 (m, 1H), 2.08 (d, J = 10.0Hz, 3H), 1.29 (t, J = 7.1Hz, 1H).

Step 7: ((2R,3S,4R,5R)-4-(benzoyloxy)-3-fluoro-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-2-yl)methyl benzoate



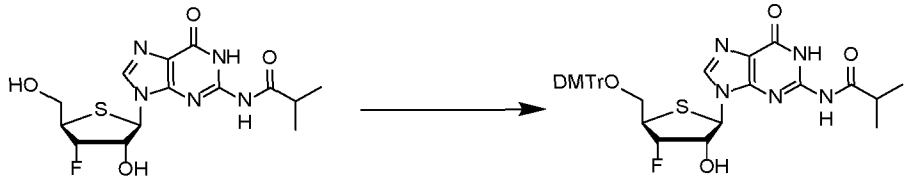
To a suspension of N-(6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (15.86g, 71.7mmol) in $ClCH_2CH_2Cl$ (300mL) was added (Z)-trimethylsilyl N-(trimethylsilyl)acetimidate (35.1mL, 143mmol). The suspension was stirred at 70°C overnight and was then cooled to -15°C. To this mixture was added ((2R,3S,4R)-5-acetoxy-4-(benzoyloxy)-3-fluorotetrahydrothiophen-2-yl)methyl benzoate (10g, 23.90mmol), followed by TMS-OTf (8.64mL, 47.8mmol). The reaction mixture was stirred at -15°C for 2h, then at rt for 5h and finally at 70°C for 5d. The reaction mixture was allowed to cool to RT and was then filtered. The filtrate was washed with sat. aq. $NaHCO_3$, brine and then dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column, eluting with 0-60% EtOAc/Hexane. Subsequent recrystallization from EtOAc gave product. LCMS (ES, m/z): 580.5 $[M+H]^+$. ^1H NMR (500MHz, Chloroform-*d*) δ 12.19 (s, 0H), 9.56 (s, 1H), 8.12 – 8.06 (m, 1H), 8.02 – 7.96 (m, 1H), 7.66 (s, 0H), 7.71 – 7.58 (m, 1H), 7.57 – 7.50 (m, 1H), 7.54 – 7.42 (m, 1H), 6.59 (ddd, *J* = 25.3, 7.4, 3.1Hz, 1H), 6.20 (d, *J* = 7.4Hz, 0H), 5.68 – 5.59 (m, 1H), 4.87 (ddd, *J* = 11.7, 7.7, 1.5Hz, 1H), 4.26 – 4.11 (m, 1H), 2.96 (hept, *J* = 6.8Hz, 1H), 1.38 (dd, *J* = 19.0, 6.9Hz, 3H).

Step 8: N-(9-((2R,3R,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



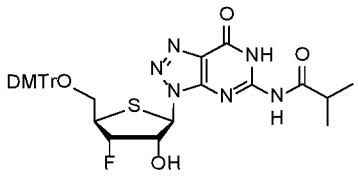
To a solution of ((2R,3S,4R,5R)-4-(benzoyloxy)-3-fluoro-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-2-yl)methyl benzoate (0.96g, 1.66mmol) in THF (5mL)/MeOH (4mL)/H_2O (1mL) at 0°C was added 2N sodium hydroxide (1.8mL, 3.6mmol). The reaction mixture was stirred at 0°C for 30min and then neutralized with acetic acid (0.38mL, 6.6mmol). The product was collected by filtration and carried on to the next step without further purification. LCMS (ES, m/z): 372.3 $[M+H]^+$.

Step 9: N-(9-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide

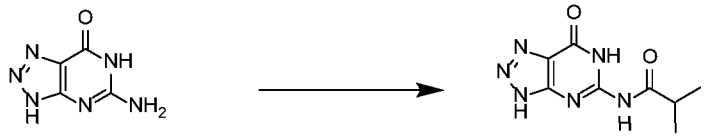


To a solution of N-(9-((2R,3R,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl)-tetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (0.62g, 1.66mmol) in pyridine (25mL) at 0°C was added 4,4'-dimethoxytrityl chloride (0.84g, 2.48mmol). The reaction mixture was stirred at 0°C for 3h. The reaction was quenched with H_2O (1mL), and the mixture was concentrated. The residue was diluted in 100mL of ethyl acetate and washed with saturated aq. $NaHCO_3$ (100mL) and brine (100mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by a silica gel column, eluting with 0-100% EtOAc/Hexane containing 0.1% Et_3N to give product. LCMS (ES, m/z): 674.6 $[M+H]^+$. ^1H NMR (500MHz, Chloroform-*d*) δ 11.96 (s, 0H), 8.35 (s, 0H), 7.70 (s, 0H), 7.63 – 7.56 (m, 1H), 7.50 – 7.43 (m, 2H), 7.38 – 7.24 (m, 1H), 6.93 – 6.85 (m, 2H), 5.92 (d, J = 8.3Hz, 0H), 5.35 – 5.23 (m, 1H), 4.15 (q, J = 7.1Hz, 1H), 3.85 – 3.69 (m, 3H), 3.46 (dd, J = 10.2, 5.4Hz, 0H), 3.35 (dd, J = 10.2, 5.2Hz, 0H), 2.09 (d, J = 19.4Hz, 2H), 1.34 – 1.22 (m, 2H), 1.01 (d, J = 6.8Hz, 1H), 0.91 (d, J = 6.9Hz, 1H).

Preparation 20: N-(3-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-fluoro-3-hydroxytetrahydrothiophen-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide



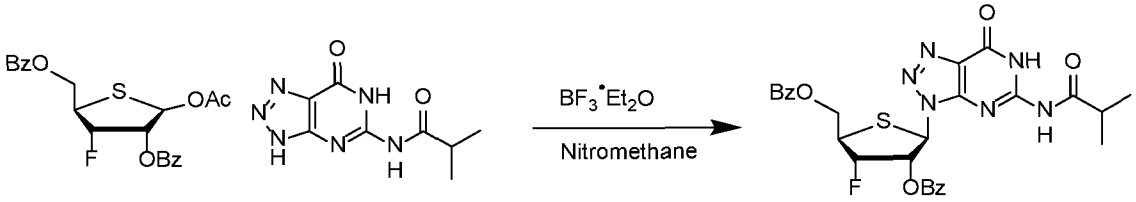
Step 1: N-(7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide



To a suspension of 5-amino-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (5.0g, 32.9mmol) in anhydrous DMF (60mL) was added isobutyric anhydride (12.5mL, 76.0mmol) dropwise. The reaction mixture was refluxed at 150°C for 1h. The reaction was quenched with MeOH (6.6mL, 164mmol) and concentrated *in vacuo*. The residue was taken up in DCM (50mL)/Hexane (100mL) and was stirred at vigorously at rt for 15min. The product was

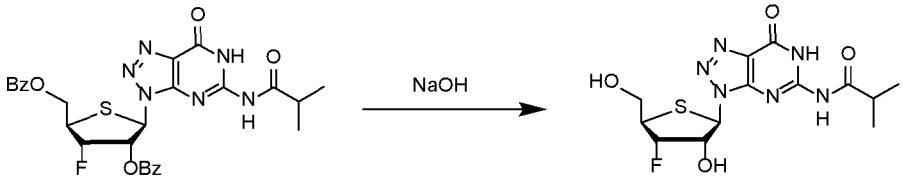
collected by filtration. LCMS (ES, m/z): 223.2 $[M+H]^+$. ^1H NMR (500MHz, DMSO-d_6) δ 16.04 (s, 1H), 12.19 (s, 1H), 11.78 (s, 1H), 2.78 (hept, J = 6.7Hz, 1H), 1.13 (d, J = 6.8Hz, 6H).

Step 2: ((2R,3S,4R,5R)-4-(benzoyloxy)-3-fluoro-5-(5-isobutyramido-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)tetrahydrothiophen-2-yl)methyl benzoate



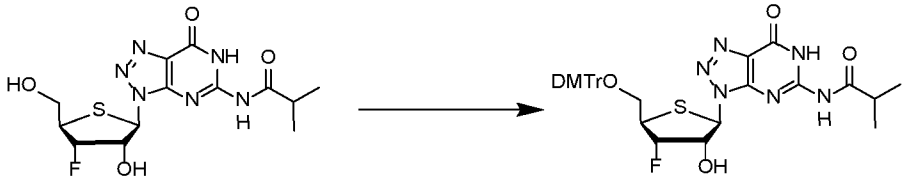
To a mixture of ((2R,3S,4R)-5-acetoxy-4-(benzoyloxy)-3-fluorotetrahydrothiophen-2-yl)methyl benzoate (1.5g, 3.6mmol) and N-(7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide (0.96g, 4.3mmol) in nitromethane (20mL) was added BF_3•Et_2O (0.54mL, 4.3mmol) and the resulting mixture was heated at 100°C under microwave irradiation for 1h. The reaction mixture was cooled, diluted in 100mL of ethyl acetate and washed with saturated aq. $NaHCO_3$ (100mL) and brine (100mL). The organic portion was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by a silica gel column, eluting with 0-35% EtOAc/Hexane. LCMS (ES, m/z): 581.4 $[M+H]^+$. ^1H NMR (500MHz, Chloroform-*d*) δ 12.27 (s, 2H), 9.73 (s, 2H), 8.19 – 7.93 (m, 8H), 7.86 – 7.80 (m, 0H), 7.71 – 7.55 (m, 4H), 7.57 – 7.39 (m, 8H), 7.40 – 7.33 (m, 0H), 6.76 (d, J = 6.7Hz, 2H), 6.62 – 6.48 (m, 2H), 5.79 (t, J = 2.9Hz, 1H), 5.69 (t, J = 2.9Hz, 1H), 5.62 – 5.54 (m, 2H), 4.89 (ddd, J = 11.6, 7.8, 1.3Hz, 2H), 4.78 – 4.65 (m, 1H), 4.31 – 4.18 (m, 2H), 4.15 (q, J = 7.1Hz, 3H), 2.95 (hept, J = 6.9Hz, 2H), 2.81 – 2.68 (m, 1H), 2.07 (s, 4H), 1.39 (dd, J = 17.5, 6.9Hz, 10H), 1.34 – 1.24 (m, 8H).

Step 3: N-(3-((2R,3R,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide



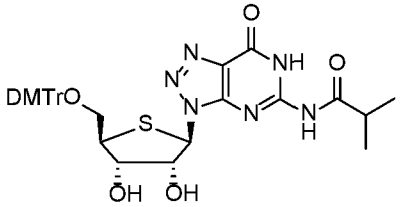
To a solution of ((2R,3S,4R,5R)-4-(benzoyloxy)-3-fluoro-5-(5-isobutyramido-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)tetrahydrothiophen-2-yl)methyl benzoate (4.5g, 7.8mmol) in THF (35mL)/MeOH (28mL)/H (7mL) at 0°C was added 2N sodium hydroxide (8.6mL, 17.2mmol). The reaction mixture was stirred at 0°C for 1h and then neutralized with acetic acid (2.3mL, 39.0mmol). Product was collected by filtration and carried on to the next step without further purification. LCMS (ES, m/z): 373.3 $[M+H]^+$.

Step 4: N-(3-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrothiophen-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide

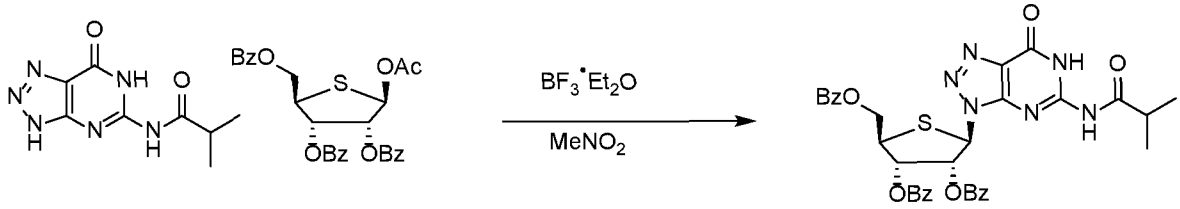


To a solution of N-(3-((2R,3R,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl)-tetrahydrothiophen-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide (1.8g, 4.8mmol) in pyridine (30mL) at 0°C was added 4,4'-dimethoxytrityl chloride (1.8g, 5.3mmol). The reaction mixture was stirred at 0°C for 1h. The reaction was quenched with H_2O (1mL), and the mixture was concentrated. The residue was diluted in 100mL of ethyl acetate and washed with saturated aq. $NaHCO_3$ (100mL) and brine (100mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by a silica gel column, eluting with 0-100% EtOAc/Hexane containing 0.1% Et_3N to give product. LCMS (ES, m/z): 675.5 $[M+H]^+$. ^1H NMR (500MHz, Chloroform-*d*) δ 12.16 (s, 1H), 8.48 (s, 0H), 7.57 – 7.50 (m, 1H), 7.49 – 7.37 (m, 2H), 7.39 – 7.29 (m, 0H), 7.31 (s, 1H), 7.32 – 7.14 (m, 1H), 6.91 – 6.81 (m, 2H), 6.22 – 6.16 (m, 0H), 5.42 – 5.31 (m, 1H), 3.77 (d, *J* = 36.0Hz, 7H), 3.46 (dd, *J* = 10.2, 5.6Hz, 0H), 3.36 (dd, *J* = 10.2, 5.4Hz, 0H), 2.17 (p, *J* = 6.9Hz, 0H), 1.06 (dd, *J* = 26.1, 6.9Hz, 3H).

Preparation 21: N-(3-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-3,4-dihydroxytetrahydrothiophen-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide

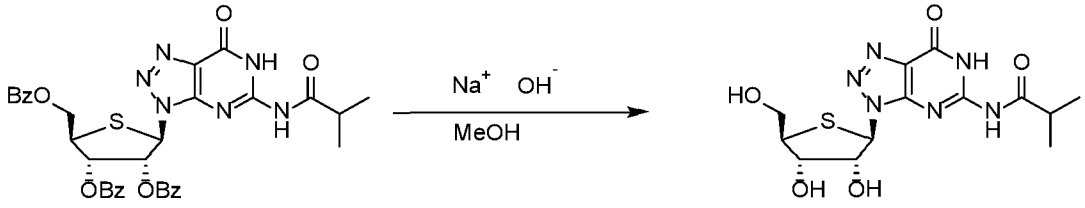


Step 1: (2R,3S,4R,5R)-2-((benzoyloxy)methyl)-5-(5-isobutyramido-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)tetrahydrothiophene-3,4-diyl dibenzoate



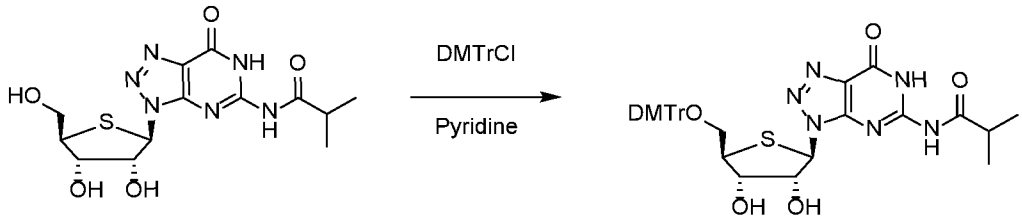
BF_3•OEt_2 (3.65mL, 28.8mmol) was added dropwise to a mixture of (2R,3R,4S,5R)-2-acetoxy-5-((benzoyloxy)methyl)tetrahydrothiophene-3,4-diyl dibenzoate (10.0g, 19.2mmol) and N-(7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5 yl)isobutyramide (5.98g, 26.9mmol) in $MeNO_2$ (180mL) at ambient temperature. Upon completion of addition, the mixture was heated at 120°C in a microwave reactor for 45min. The sample was cooled to rt and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with EtOAc/isohexane (10-90%) to give desired product. LCMS (ES, m/z): 683.5 $[M+H]^+$. ^{1}H NMR (500MHz, Chloroform-*d*) δ 12.24 (s, 1H), 9.77 (s, 1H), 8.08 – 7.95 (m, 4H), 7.94 – 7.87 (m, 2H), 7.70 – 7.35 (m, 9H), 6.84 (d, *J* = 5.9Hz, 1H), 6.67 (dd, *J* = 5.9, 3.9Hz, 1H), 6.49 (t, *J* = 3.7Hz, 1H), 5.52 (dd, *J* = 11.4, 7.8Hz, 1H), 5.04 (dd, *J* = 11.4, 7.8Hz, 1H), 4.28 (m, 1H), 2.97 (hept, *J* = 6.9Hz, 1H), 1.42 (dd, 6.9Hz, 6H).

Step 2: N-(3-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide



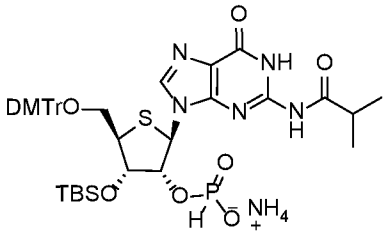
To a stirred solution of the product from Step 1 (5.3g, 7.8mmol) dissloved in Pyridine (8mL) and MeOH (32mL) at 25°C was added sodium hydroxide (1.24g, 31.1mmol) in one portion. The mixture was stirred at 25°C for 15min before the addition of acetic acid (1.8mL, 31.1mmol). The mixture was concetrated *in vacuo,* and the residue was purified by flash column chromatography eluting with EtOAc/isohexane (70-100%) to give the desired product. LCMS (ES, m/z): 371.3 $[M+H]^+$. ^{1}H NMR (500MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.96 (s, 1H), 5.91 (d, *J* = 5.6Hz, 1H), 5.73 (d, *J* = 5.5Hz, 1H), 5.43 (d, *J* = 5.0Hz, 1H), 5.12 (m, 1H), 4.81 (m,br, 1H), 4.40 (m, 1H), 3.89 – 3.77 (m, 1H), 3.53 (m, 1H), 3.46 – 3.36 (m, 1H), 2.79 (m,1H), 1.14 (dd, *J* = 6.7, 1.2Hz, 6H).

Step 3: N-(3-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3,4-dihydroxytetrahydrothiophen-2-yl)-7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-yl)isobutyramide

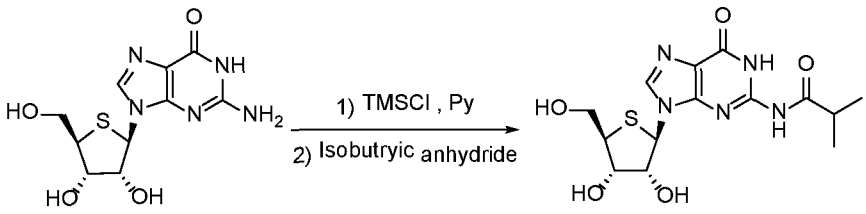


To the product from step 2 (2.3g, 6.2mmol) was added pyridine (62mL) at ambient temperature. To this mixture was added DMTrCl (2.3g, 6.8mmol). After 1h, water (2mL) was added, and it was concentrated *in vacuo*. Ethyl acetate (15mL), water (5mL) and brine (1mL) were added. Layers were separated, and the aqueous layer was extracted with ethyl acetate twice (20mLx2). The combined organics were dried over $MgSO_4$, concentrated *in vacuo* and purified by flash column chromatography, eluting with 0 to 80% EtOAc in Hexane to give the desired product. LCMS (ES, m/z): 673.4 $[M+H]^+$. ^1H NMR (500MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.96 (s, 1H), 7.50 – 7.36 (m, 2H), 7.35 – 7.10 (m, 9H), 6.96 – 6.82 (m, 4H), 5.88 (d, J = 4.1Hz, 1H), 5.83 (d, J = 5.1Hz, 1H), 5.42 (d, J = 5.7Hz, 1H), 4.66 (q, J = 4.1Hz, 1H), 4.45 (td, J = 5.9, 3.5Hz, 1H), 3.81 – 3.69 (6H), 3.65 (ddd, J = 8.4, 6.0, 4.3Hz, 1H), 3.46 – 3.35 (m, 1H), 3.19 (dd, J = 9.4, 7.9Hz, 1H), 2.78 (h, J = 6.8Hz, 1H), 1.22 – 1.05 (m, 6H).

Preparation 22: ammonium (2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl phosphonate



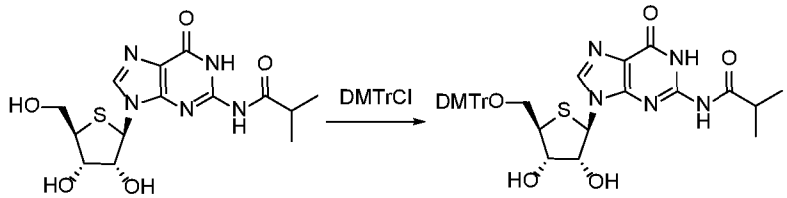
Step 1: N-(9-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



2-amino-9-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)-1,9-dihydro-6H-purin-6-one (1.7g, 5.7mmol) was co-evaporated with pyridine (3x5mL) and then, re-dissolved in pyridine (34mL). To the mixture at 0°C was added chlorotrimethylsilane

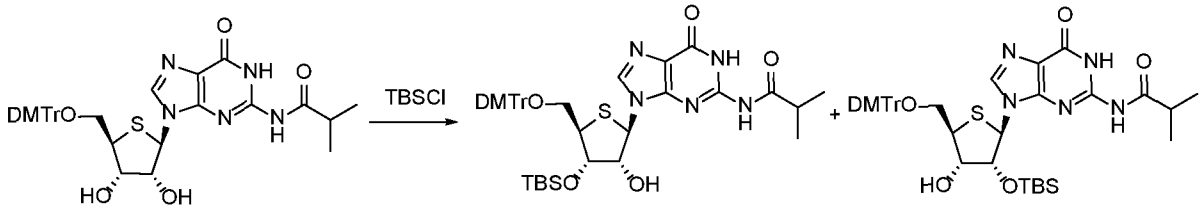
(4.32g, 39.8mmol) dropwise. It was stirred at rt for 1h and then, cooled to 0°C again. Isobutyric anhydride (1.348g, 8.52mmol) was added dropwise, and it was stirred at rt for 3h. It was quenched by the addition of water (8.5mL). After 5min, NH_4OH (ca. 29%, 17mL) was added, and the mixture was stirred for 30min. It was concentrated and purified by column chromatography eluted with 1 to 30% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 396.9 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ 9.52 (br s, 2 H), 8.39 (s, 1H), 5.79 (d, J = 7.1Hz, 1H), 5.59 (s, 1H), 5.40 (s, 1H), 5.22 (s, 1H), 4.55 (d, J = 6.7Hz, 1H), 4.21 (s, 1H), 3.77 (t, J = 9.3Hz, 1H), 3.61 (s, 1H), 3.30 (dt, J = 6.4, 3.3Hz, 1H), 2.78 (p, J = 6.9Hz, 1H), 1.13 (d, J = 6.8Hz, 6H).

Step 2: N-(9-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3,4-dihydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



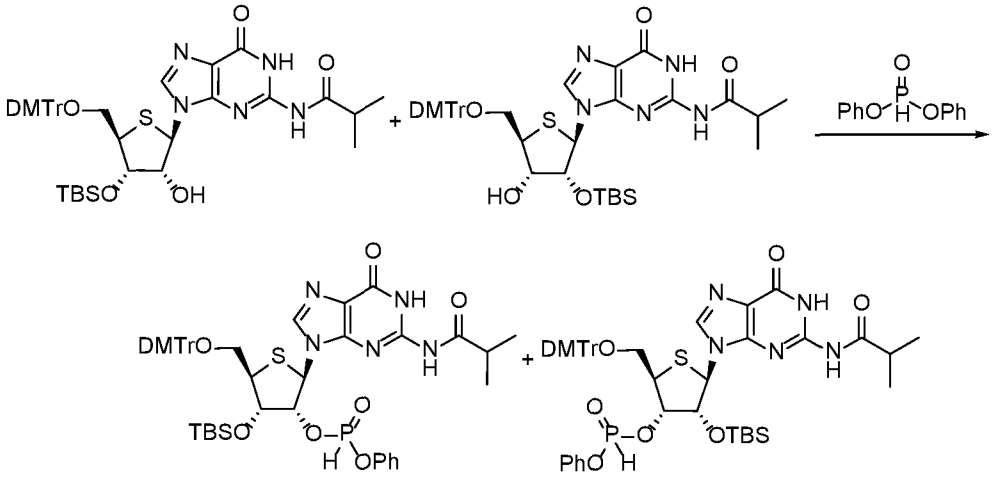
To a mixture of N-(9-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (480mg, 1.299mmol) in pyridine (10mL) was added 4,4'-(chloro(phenyl)methylene)-bis(methoxybenzene) (484mg, 1.43mmol). It was stirred at rt for 16h and then, concentrated. The crude was purified by column chromatography on silica gel eluted with 1 to 30% MeOH in CH_2Cl_2 (containing 1% Et_3N) to give the product. LCMS (ES, m/z): 672.2 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6 + D_2O): δ 8.08 (s, 1H), 7.39 (d, J = 7.2Hz, 2H), 7.32 (t, J = 7.6Hz, 2H), 7.26 (dt, J = 9.1, 3.3Hz, 5H), 6.94-6.87 (m, 4H), 5.75 (d, J = 5.9Hz, 1H), 4.39 (dd, J = 5.9, 3.5Hz, 1H), 4.14 (t, J = 3.9Hz, 1H), 3.74 (s, 6H), 3.49-3.37 (m, 2H), 3.33 (dd, J = 14.5, 7.3Hz, 1H), 2.87-2.67 (m, 1H), 1.11 (dd, J = 6.8, 1.6Hz, 6H).

Step 3: N-(9-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-3-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide and N-(9-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-((tert-butyldimethylsilyl)oxy)-4-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide



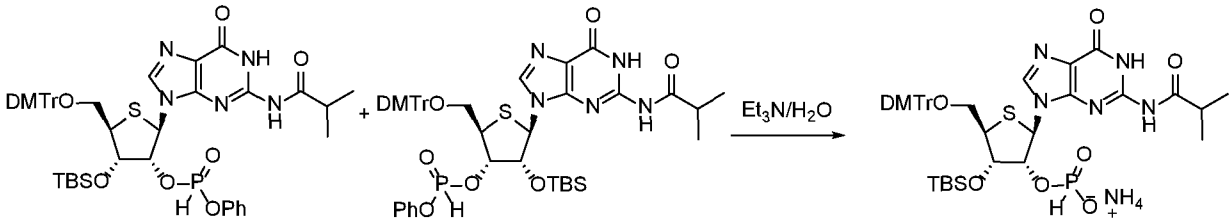
To a solution of N-(9-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-3,4-dihydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (580mg, 0.863mmol) in DMF (5mL) at rt was added 1*H*-imidazole (147mg, 2.16mmol) and *tert*-butylchlorodimethylsilane (156mg, 1.04mmol). After 6h, the mixture was diluted with EtOAc (50mL) and washed with sat aq $NaHCO_3$ (2x20mL) and brine (20mL). It was dried (Na_2SO_4), concentrated, and purified by reverse phase (C18) chromatography eluted with 0 to 95% ACN in water to give the products. LCMS (ES, m/z): 786.3 $[M + H]^+$.

Step 4: (2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl phenyl phosphonate and (2R,3S,4R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl phenyl phosphonate



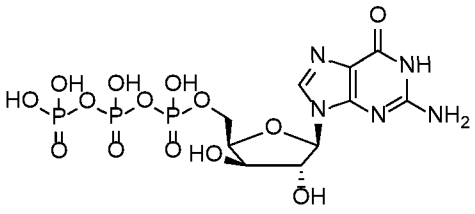
To a solution of a mixture of N-(9-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-3-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide and N-(9-((2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-((tert-butyldimethylsilyl)oxy)-4-hydroxytetrahydrothiophen-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (220mg, 0.280mmol) in pyridine (2mL) at 0°C was added diphenyl phosphonate (98mg, 0.420mmol). The resulting mixture was stirred at rt for 20min. It was used in the next reaction step without purification. LCMS (ES, m/z): 926.2 $[M + H]^+$.

Step 5: ammonium (2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl phosphonate

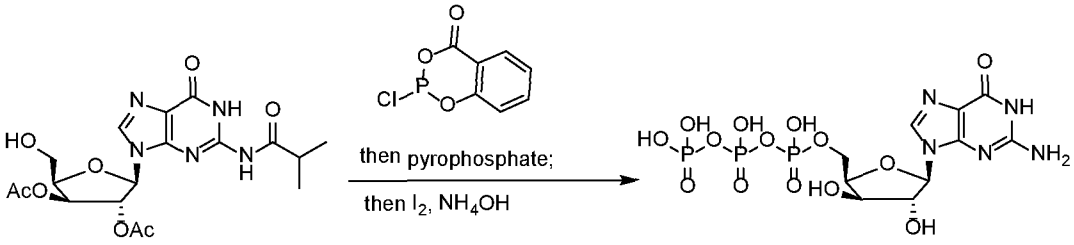


To the reaction mixture from Step 4 at 0°C was added Et_3N (0.28mL, 2.0mmol) and water (0.28mL). It was stirred at rt for 30min. It was concentrated, and the residue was partitioned between CH_2Cl_2 (40mL) and aq $NaHCO_3$ (5%, 30mL). The organic layer was washed with aq $NaHCO_3$ (5%, 2x30mL), dried (Na_2SO_4), concentrated and purified by silica gel column chromatography using 0-10% MeOH in $CHCl_3$ containing 1% Et_3N to give a mixture. The mixture was further purified by prep-HPLC Prep-HPLC (XBridge Shield RP18 OBD Column, 19×150 mm) eluted with 46 to 79% ACN in aq NH_4HCO_3 (10 mM) over 7min to give the product. LCMS (ES, m/z): 850.2 $[M + H]^+$. ^{1}H-NMR (400MHz, CD_3OD): δ 8.18 (s, 1H), 7.68 (s, 0.5H), 7.59-7.49 (m, 2H), 7.45-7.36 (m, 4H), 7.37-7.30 (m, 2H), 7.28-7.22 (m, 1H), 6.95-6.87 (m, 4H), 6.16-6.07 (m, 2H), 4.88-4.87 (m, 1H), 4.69 (dd, J = 7.3, 3.3Hz, 1H), 3.81 (s, 6H), 3.51 (dd, J = 4.9, 1.9Hz, 2H), 3.37 (s, 1H), 2.67 (p, J = 6.9Hz, 1H), 1.21 (dd, J = 6.9, 0.9Hz, 6H), 0.77 (s, 9H), 0.01 (s, 3H), -0.28 (s, 3H). ^{31}P-NMR (162MHz, DMSO-d_6): δ -0.74 (s, 1P).

Preparation 23: 2-amino-9-[5-*O*-(hydroxy{[hydroxy(phosphonooxy)phosphoryl]oxy}-phosphoryl)-β-D-xylofuranosyl]-1,9-dihydro-6*H*-purin-6-one

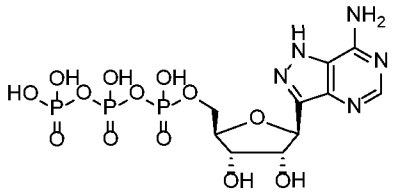


Step 1: 2-amino-9-[5-O-(hydroxy{[hydroxy(phosphonooxy)phosphoryl]oxy}-phosphoryl)-β-D-xylofuranosyl]-1,9-dihydro-6H-purin-6-one

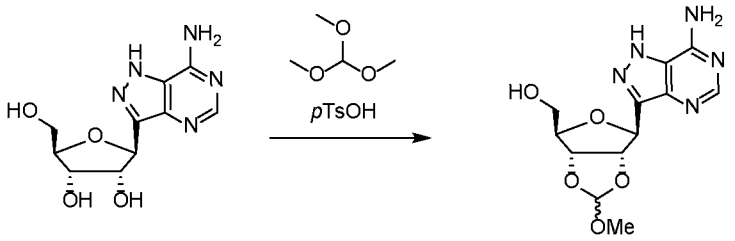


To a stirred solution of 9-(2,3-di-O-acetyl-β-D-xylofuranosyl)-2-[(2-methylpropanoyl)-amino]-1,9-dihydro-6H-purin-6-one (100mg, 0.229mmol) in pyridine (0.25mL) and 1,4-dioxane (0.75mL) was added a freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (50mg, 0.247mmol) in 1,4-dioxane (0.25mL). The reaction mixture was stirred at ambient temperature for 10min, and then a solution of tributylammonium pyrophosphate (189mg, 0.344mmol) in DMF (0.69mL) was added, followed by addition of tributylamine (0.23mL, 0.968mmol) in one portion at ambient temperature. The reaction mixture was stirred for 10min at ambient temperature, and then a solution of iodine (29.0mg, 0.114mmol) in pyridine (0.50mL) and water (0.05mL) was added. The reaction mixture was stirred for 15min, excess iodine was quenched with 5% aq $NaHSO_3$ (3mL), and the reaction mixture was evaporated to dryness. The residue was dissolved in 10mL H_2O, and after standing at rt for 30min, 28% aq ammonium hydroxide (5mL) was added. The reaction mixture was stirred at 50°C for 5h. LCMS indicated full conversion to desired product, and the mixture was filtered and lyophilyzed. The product was purified using mass-directed reverse phase HPLC with a Waters SunFire C18 OBD Prep Column, 100Å, 5µm, 19mmx150mm, [Waters Part# 186002568] using a gradient solvent system with MeCN and 100 mM aq triethylammonium acetate. Lyophilization of the product fractions furnished 2-amino-9-[5-*O*-(hydroxy{[hydroxy(phosphonooxy)phosphoryl]oxy}phosphoryl)-β-D-xylofuranosyl]-1,9-dihydro-6*H*-purin-6-one as the tetra-triethylamine salt. LCMS (ES, m/z): 522 $[M - H]^-$.

Preparation 24: ((2R,3S,4R,5S)-5-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate

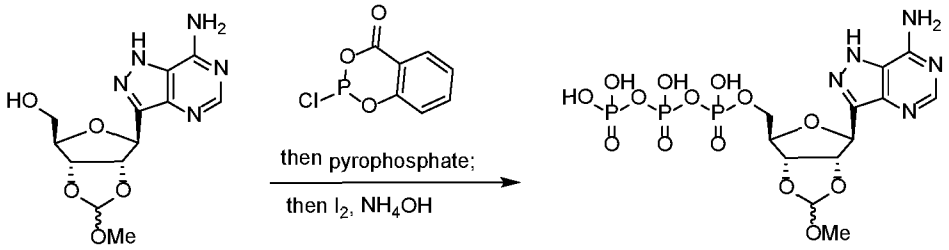


Step 1: ((3aR,4R,6S,6aS)-6-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-2-methoxytetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol



To the stirred suspension of (2S,3R,4S,5R)-2-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (30mg, 0.105mmol) in 1,4-dioxane (0.3mL) was added trimethyl orthoformate (0.22mL, 2.011mmol) in one portion at ambient temperature, followed by p-toluenesulfonic acid monohydrate (22mg, 0.116mmol). The reaction mixture was stirred at ambient temperature for 16h. LCMS indicated significant conversion to desired product, and the crude mixture was carefully quenched by adding triethylamine (0.05mL) at 0°C. Following concentration, the residue was purified by flash column chromatography on 12 gram silica gel using a gradient solvent system with MeOH and CH_2Cl_2. Concentration of the product fractions furnished ((3aR,4R,6S,6aS)-6-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-2-methoxytetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol. LCMS (ES, m/z): 310 $[M + H]^+$.

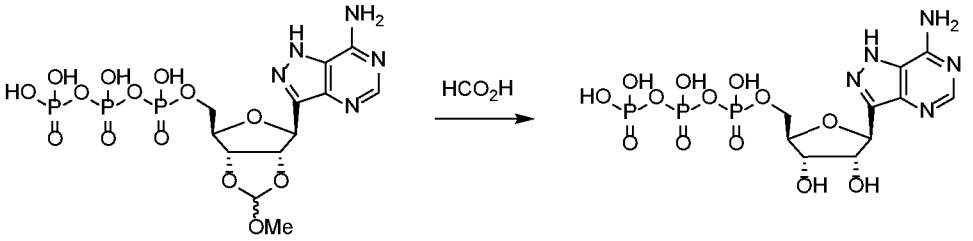
Step 2: ((3aR,4R,6S,6aS)-6-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-2-methoxytetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl tetrahydrogen triphosphate



To the stirred suspension of ((3aR,4R,6S,6aS)-6-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-2-methoxytetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol (16mg, 0.052mmol) in pyridine (0.05mL) and 1,4-dioxane (0.15mL) was added DMF (0.05mL) to form a homogeneous solution. To this solution was added a freshly prepared solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (12mg, 0.059mmol) in 1,4-dioxane (0.05mL) at ambient temperature. The reaction mixture was stirred at ambient temperature for 15min, and then a solution of tributylammonium pyrophosphate (43mg, 0.078mmol) in DMF (0.10mL) was added, followed by tributylamine (0.052mL, 0.219mmol). The reaction mixture was stirred at ambient temperature for 15min, and then a solution of iodine (6.58mg, 0.026mmol) in pyridine (0.10mL) and water (0.01mL) was added. The reaction mixture was stirred at ambient temperature for 15min and excess iodine was quenched with 5% aqueous $NaHSO_3$ (0.5mL). LCMS indicated

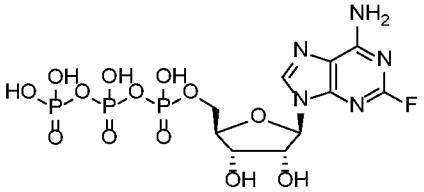
significant conversion to desired product, and the reaction mixture was concentrated to yield ((3aR,4R,6S,6aS)-6-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-2-methoxytetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl tetrahydrogen triphosphate, which was used directly in the next reaction step without additional purification. LCMS (ES, m/z): 548 [M - H]$^-$.

Step 3: ((2R,3S,4R,5S)-5-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate

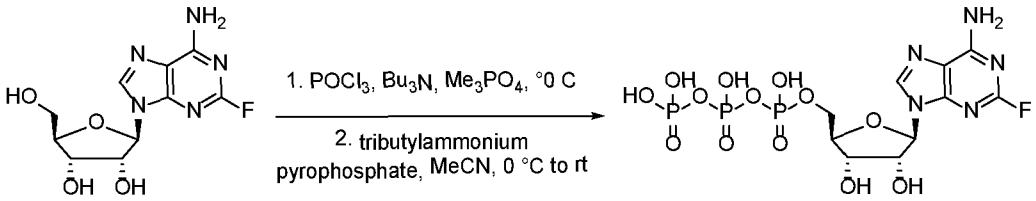


To the stirred solution of crude ((3aR,4R,6S,6aS)-6-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-2-methoxytetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl tetrahydrogen triphosphate (49.4mg, 0.090mmol) in water (0.15mL) and DMF (0.15mL) was added formic acid (0.4mL, 10.60mmol) in one portion. The reaction mixture was stirred at ambient temperature for 18h. LCMS indicated significant conversion to desired product, and the mixture was filtered and lyophilyzed. The product was purified using mass-directed reverse phase HPLC with a Waters SunFire C18 OBD Prep Column, 100Å, 5µm, 19mmx150mm, [Waters Part# 186002568] using a gradient solvent system with MeCN and 100 mM aqueous triethylammonium acetate. Lyophilization of the product fractions furnished ((2R,3S,4R,5S)-5-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate. LCMS (ES, m/z): 506 [M - H]$^-$.

Preparation 25: ((2R,3S,4R,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate

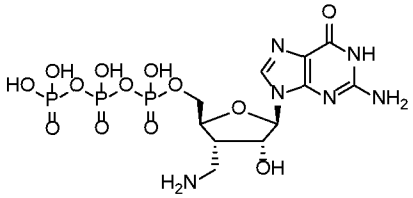


Step 1: ((2R,3S,4R,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate



To a mixture of 2-fluoroadenosine (200mg, 0.701mmol) in trimethylphosphate (1.948mL, 16.83mmol) was added tributylamine (0.500mL, 2.104mmol), and the mixture was stirred 15min at rt and then cooled in an ice/brine bath. Then $POCl_3$ (0.137mL, 1.472mmol) was added dropwise with bath temperature maintained at -5 to 0°C. After 1.25h, a 0°C mixture of tributylammonium pyrophosphate (327mg, 0.596mmol), MeCN (2.8mL) and tributylamine (1.000mL, 4.21mmol) were added, and the mixture was allowed to warm to rt, followed by 16h stirring at rt. The mixture was purified directly by reverse phase HPLC using a gradient of 1-20% MeCN with 100 mM aqueous triethylammonium acetate to furnish ((2R,3S,4R,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphatemg. LCMS (ES, m/z): 524 $[M - H]^-$.

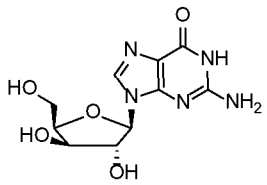
Preparation 26: 3'-(aminomethyl)-3'-deoxyguanosine 5'-(tetrahydrogen triphosphate)



The title compound was prepared according to published procedures (WO2015161137).

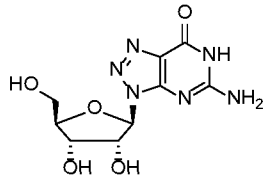
The Preparations below were used as shown or were further modifiec through additional synthetic manipulations analogous to those described in Preparations 1 – 26.

Preparation 27: 2-amino-9-(β-D-xylofuranosyl)-1,9-dihydro-6*H*-purin-6-one



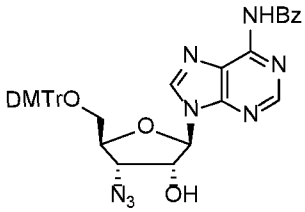
The title compound was prepared according to published procedures (*Journal of Medicinal Chemistry* **1987**, *30*(6), 982-991).

Preparation 28: 5-amino-3-(β-D-ribofuranosyl)-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one



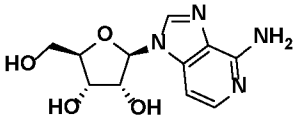
The title compound was prepared according to published procedures (*Journal of Organic Chemistry* **2007**, *72*(1), 173-179).

Preparation 29: 9-{3-azido-5-*O*-[bis(4-methoxyphenyl)(phenyl)methyl]-3-deoxy-β-D-ribofuranosyl}-N-(phenylcarbonyl)-9*H*-purin-6-amine



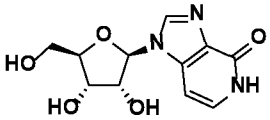
The title compound was prepared according to published procedures (*Bulletin of the Korean Chemical Society* **2004**, *25*(2), 243-248 and *Nucleosides, Nucleotides & Nucleic Acids* **2005** *24*(10-12), 1707-1727).

Preparation 30: 1-(β-D-ribofuranosyl)-1*H*-imidazo[4,5-c]pyridin-4-amine



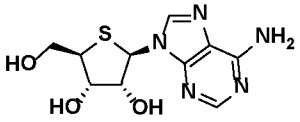
The title compound was prepared according to published procedures (*Tetrahedron* **1993**, *49*(3), 557-570).

Preparation 31: 1-(β-D-ribofuranosyl)-1,5-dihydro-4*H*-imidazo[4,5-c]pyridin-4-one



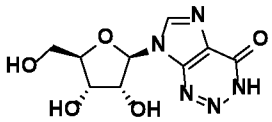
The title compound was prepared according to published procedures (*Tetrahedron* **1993**, *49*(3), 557-570)

Preparation 32: 4'-thioadenosine



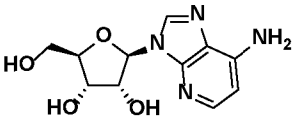
The title compound was prepared according to published procedures (*Journal of Medicinal Chemistry* **2006**, *49*(5), 1624-1634).

Preparation 33: 7-(β-D-ribofuranosyl)-3,7-dihydro-4*H*-imidazo[4,5-d][1,2,3]triazin-4-one



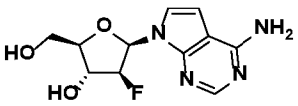
The title compound was prepared according to published procedures (*Organic & Biomolecular Chemistry* **2014**, *12*(23), 3813-3815).

Preparation 34: 3-(β-D-ribofuranosyl)-3*H*-imidazo[4,5-b]pyridin-7-amine



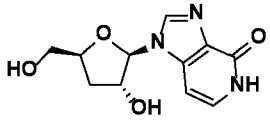
The title compound was prepared according to published procedures (*Biochemistry* **2005**, *44*(37), 12445-12453).

Preparation 35: 7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-d]pyrimidin-4-amine



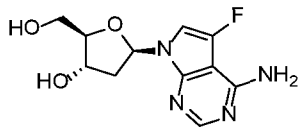
The title compound was prepared according to published procedures (*Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry* **1995** (12), 1543-50).

Preparation 36: 1-(3-deoxy-β-D-erythro-pentofuranosyl)-1,5-dihydro-4*H*-imidazo[4,5-c]pyridin-4-one



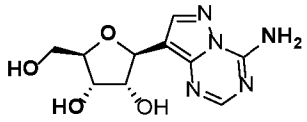
The title compound was prepared according to published procedures (*Chemical & Pharmaceutical Bulletin* **1996**, *44*(2), 288-295).

Preparation 37: 7-(2-deoxy-β-D-erythro-pentofuranosyl)-5-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-4-amine



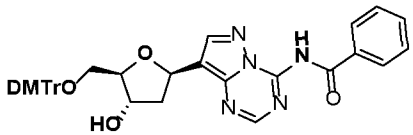
The title compound was prepared according to published procedures (*Synthesis* **2006** (12), 2005-2012).

Preparation 38: (2S,3R,4S,5R)-2-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol



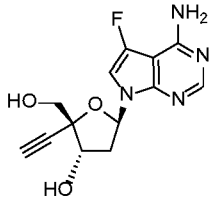
The title compound was prepared according to published procedures (WO2015148746).

Preparation 39: N-(8-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)pyrazolo[1,5-a][1,3,5]triazin-4-yl)benzamide



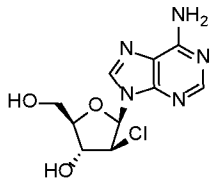
The title compound was prepared according to published procedures (WO2015148746).

Preparation 40: 7-(2-deoxy-4-ethynyl-β-D-erythro-pentofuranosyl)-5-fluoro-7*H*-pyrrolo[2,3-d]pyrimidin-4-amine



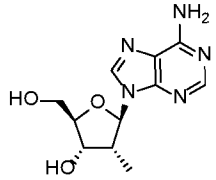
The title compound was prepared according to published procedures (WO2015148746).

Preparation 41: 9-(2-chloro-2-deoxy-β-D-arabinofuranosyl)-9H-purin-6-amine



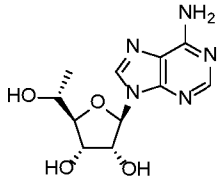
The title compound was prepared according to published procedures (*Journal of the American Chemical Society* **1996**, *118*(46), 11341-11348).

Preparation 42: 2'-deoxy-2'-methyladenosine



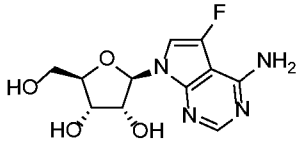
The title compound was prepared according to published procedures (*Synthesis* **2005** (17), 2865-2870).

Preparation 43: (2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((R)-1-hydroxyethyl)tetrahydrofuran-3,4-diol



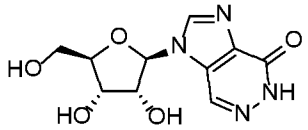
The title compound was prepared according to published procedures (*Bioorganicheskaya Khimiya* **1989**, *15*(7), 969-975).

Preparation 44: 5-fluoro-7-(β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine



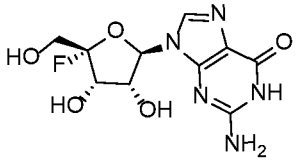
The title compound was prepared according to published procedures (*Nucleosides, Nucleotides, & Nucleic Acids* **2004**, *23*(1-2), 161-170).

Preparation 45: 1-(β-D-ribofuranosyl)-1,5-dihydro-4H-imidazo[4,5-d]pyridazin-4-one



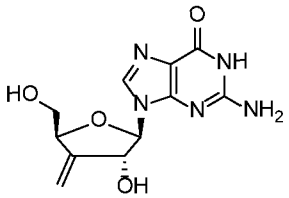
The title compound was prepared according to published procedures (*Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry (1972-1999)*, **1989** (10), 1769-1774).

Preparation 46: 2-amino-9-[(2R,3R,4S,5S)-5-fluoro-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl]-1,9-dihydro-6H-purin-6-one



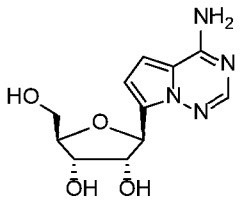
The title compound was prepared according to published procedures (WO2014099941)

Preparation 47: 2-amino-9-((2R,3R,5S)-3-hydroxy-5-(hydroxymethyl)-4-methylenetetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one



The title compound was prepared according to published procedures (*Journal of Medicinal Chemistry* **1992**, *35*, 2283-2293).

Preparation 48: (2S,3R,4S,5R)-2-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol

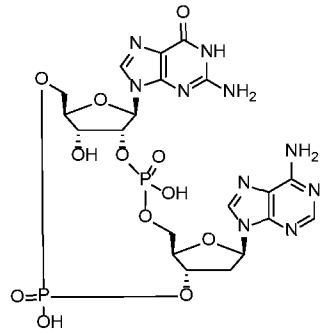


The title compound was prepared according to published procedures (*Tetrahedron Letters* **1994**, *35*(30), 5339).

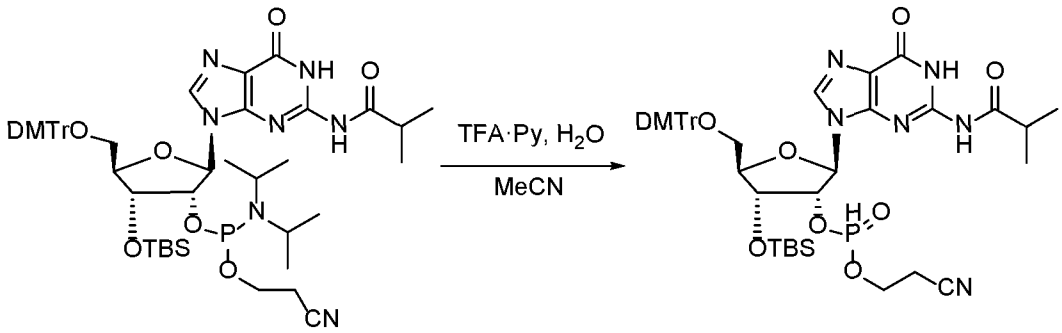
The following experimental procedures detail the preparation of specific examples of the instant disclosure. The compounds of the examples are drawn in their neutral forms in the procedures and tables below. In some cases, the compounds were isolated as salts depending on the method used for their final purification and/or intrinsic molecular properties. The examples are for illustrative purposes only and are not intended to limit the scope of the instant disclosure in any way.

EXAMPLES

Example 1: (5R,7R,8R,12aR,14R,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-16-hydroxyoctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide



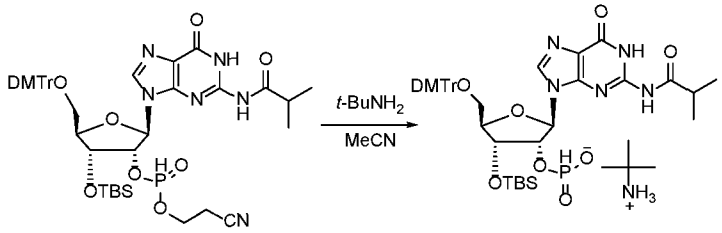
Step 1: (2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl (2-cyanoethyl) phosphonate



To a solution of (2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (3g, 3.09mmol) in ACN (15mL) was added water (0.111mL, 6.18mmol) and pyridin-1-ium 2,2,2-trifluoroacetate (0.717, 3.71mmol). The resulting mixture was stirred at RT, and the reaction progress was monitored by

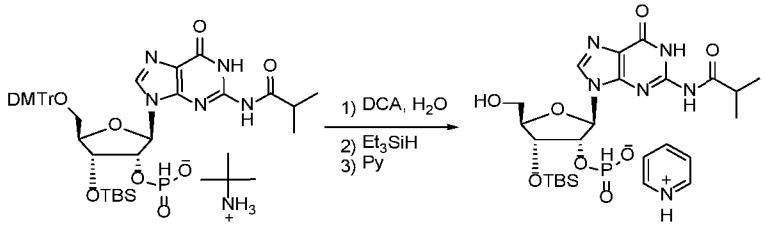
LCMS/TLC. After the phosphoramidite was consumed, the reaction mixture containing the product was used in the next step without purification. LCMS (ES, m/z): 887.4 $[M + H]^+$.

Step 2: 2-methylpropan-2-aminium (2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl) methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



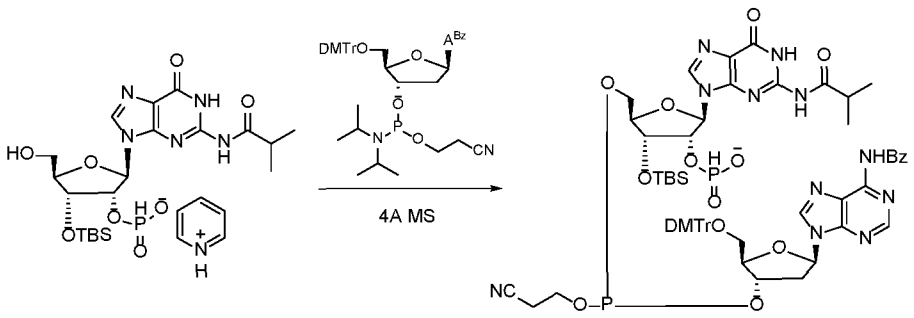
To the reaction mixture from Step 1 (assumed to contain 3.09mmol of (2*R*,3*S*,4*S*,5*R*)-5-((bis(4-methoxyphenyl) (phenyl)methoxy)methyl)-4-(*tert*-butyldimethylsilyloxy)-2-(2-isobutyramido-6-oxo-1,6-dihydropurin-9-yl)-tetrahydrofuran-3-yl 2-cyanoethyl phosphonate) was added *tert*-butylamine (15.0mL, 142mmol) in one portion, and the resulting solution was stirred at rt for 40 min. It was concentrated, and the residue was co-evaporated with ACN (2x15mL) to give the product, which was used for the next step without further purification. LCMS (ES, m/z): 832.3 $[M - H]^-$.

Step 3: pyridin-1-ium (2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



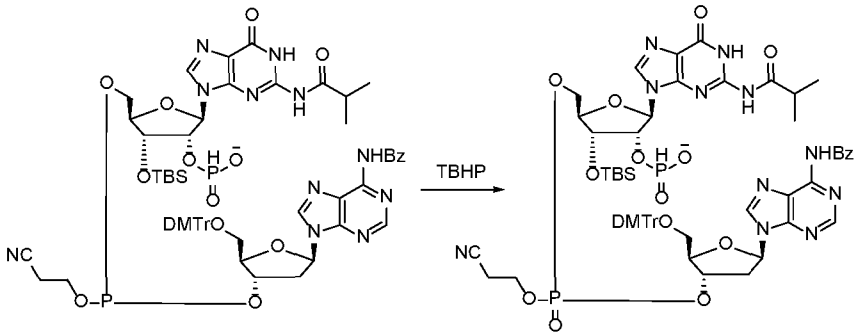
To a stirred solution of crude 2-methylpropan-2-aminium (2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (~4.2g, ~3.09mmol, from step 2) in CH_2Cl_2 (37mL) were added water (0.558mL, 31.0mmol) and dichloroacetic acid in CH_2Cl_2 (6%, 37mL, 31.5mmol). It was stirred for 40min. Then, triethylsilane (60mL) was added, and the solution was stirred for 1.5h. Pyridine (4.5mL) was added to the reaction. It was concentrated. The residue was triturated with MTBE (50ml) and Hexane (50mL), and the supernatant was decanted. This process was repeated twice. The crude mixture was kept over P_2O_5 under reduced pressure for 20h to give a crude mixture containing the product. LCMS (ES, m/z): 532.2 $[M + H]^+$.

Step 4: (2R,3R,4R,5R)-5-((((((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphanyl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



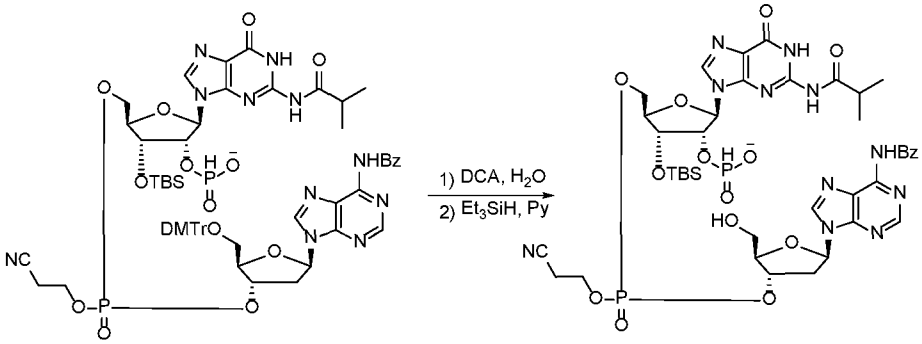
To a stirred solution of pyridin-1-ium (2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (680mg, crude, ~0.722mmol) in ACN (5mL) under Ar was added activated 4Å molecular sieves (100mg). The resulting mixture was stirred at rt over 30min. (2*R*, 3*S*, 5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-2-((bis(4-methoxyphenyl) (phenyl)methoxy)methyl) tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (0.805g, 0.939mmol) was co-evaporated with ACN (3x1mL), re-dissolved in ACN (5mL), and dried by adding activated 4Å molecular sieve (100mg). After 30min, it was added to the previously prepared mixture containing pyridin-1-ium (2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate. The mixture was stirred at rt for 1h. The reaction mixture containing the product was used in the next reaction step immediately without purification. LCMS (ES, m/z): 1288.4 $[M + H]^+$.

Step 5: (2R,3R,4R,5R)-5-((((((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



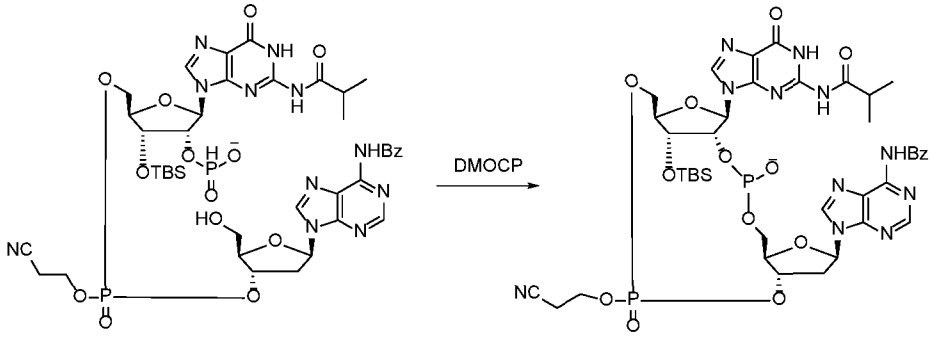
To the reaction mixture containing the crude (2R,3R,4R,5R)-5-((((((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphanyl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (~1mmol, with excess pyridinium 2,2-dichloroacetate) was added *tert*-butyl hydroperoxide in decane (5.5M, 0.64mL, 3.5mmol) dropwise. It was stirred at rt for 1h. Then, the solution was cooled to 0°C, and $NaHSO_3$ (250mg) in water (5mL) was added slowly. After 5min, the mixture was concentrated, and the residue was purified by reverse phase (C18) chromatography eluted with 5 to 45% ACN in aq NH_4HCO_3 (0.04%) to give the product. LCMS (ES, m/z): 1305.6 $[M + H]^+$.

Step 6: (2R,3R,4R,5R)-5-((((((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-(hydroxymethyl) tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-((tert-butyldimethylsilyl) oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



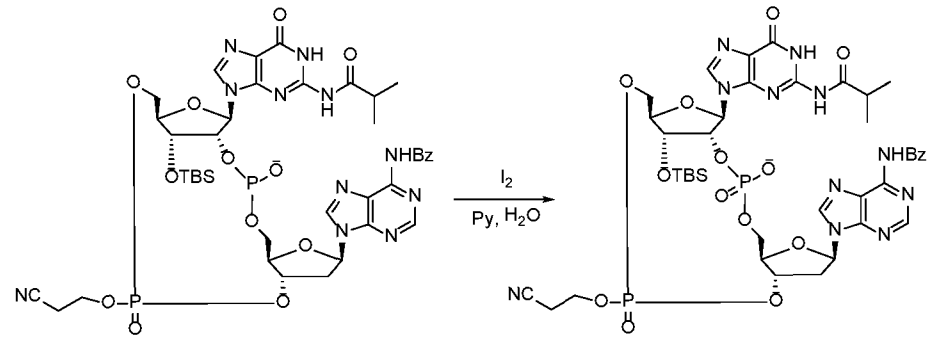
To a stirred solution of (2R,3R,4R,5R)-5-((((((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (340mg, 0.239mmol) in CH_2Cl_2 (4mL) were added water (44.5mg, 2.468mmol) and dichloroacetic acid (0.280g, 2.17mmol) in CH_2Cl (5ml). It was stirred at rt for 30min. Et_3SiH (4mL) was then added, and the mixture was stirred for 1.5h. Pyridine (3mL) was added to the reaction, and it was concentrated to give a crude product, which was used for the next step without purification. LCMS (ES, m/z): 1002.4 $[M + H]^+$.

Step 7: (5R,7R,8R,12aR,14R,15aS,16R)-16-{[tert-butyl(dimethyl)silyl]oxy}-2-(2-cyanoethoxy)-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl) amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-10-olate 2-oxide



Crude (2R,3R,4R,5R)-5-((((((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (1.5g, ~0.24mmol) was co-evaporated with pyridine (3x5mL) and then re-dissolved in pyridine (4mL). To the reaction was added 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide (160mg, 0.865mmol) in one portion. The resulting mixture was stirred at rt for 1h. It was used for the next reaction step directly without purification. LCMS (ES, m/z): 984.3 $[M + H]^+$.

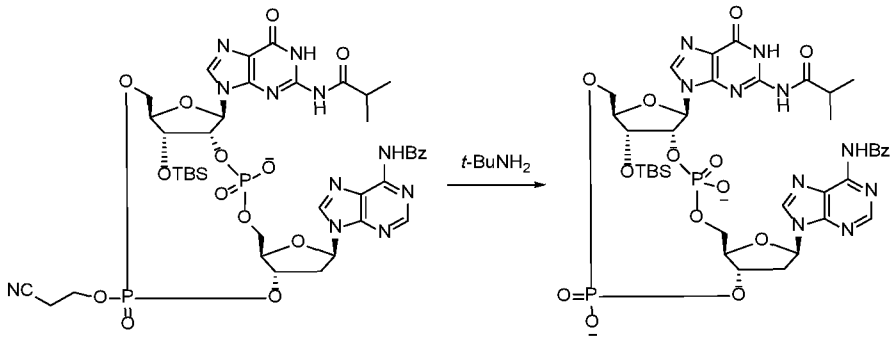
Step 8: (5R,7R,8R,12aR,14R,15aS,16R)-16-{[tert-butyl(dimethyl)silyl]oxy}-2-(2-cyanoethoxy)-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-10-olate 2,10-dioxide



To the stirred mixture containing (5R,7R,8R,12aR,14R,15aS,16R)-16-{[tert-butyl(dimethyl)silyl]oxy}-2-(2-cyanoethoxy)-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-10-olate 2-oxide was added water (156mg, 8.65mmol) and iodine (81mg, 0.321mmol). After 10min, the mixture was poured into a solution of $NaHSO_3$ (52mg) in water (36ml), and it was stirred for 5min. It was cooled to 0°C, and $NaHCO_3$ (1.04g) was slowly added. After 5min, EtOAc (50mL) and Et_2O (50ml) were added. Layers were separated, and the aq layer was extracted with EtOAc

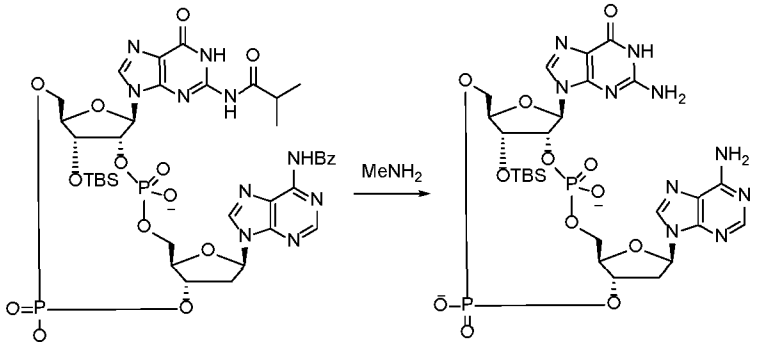
(1x30ml). The organic layers were combined, concentrated, and purified by silica gel column chromatography eluted with 0-20% MeOH in CH_2Cl_2 to give the product. LCMS (ES, m/z): 998.3 $[M + H]^+$.

Step 9: (5R,7R,8R,12aR,14R,15aS,16R)-16-{[tert-butyl(dimethyl)silyl]oxy}-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide



To a stirred solution of (5R,7R,8R,12aR,14R,15aS,16R)-16-{[tert-butyl(dimethyl)silyl] oxy}-2-(2-cyanoethoxy)-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l] [1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-10-olate 2,10-dioxide (160mg) in ACN (2mL) was added *tert*-butylamine (2mL) at rt. After 30min, it was concentrated to give the crude product, which was used for the next step without purification. LCMS (ES, m/z): 945.2 $[M+H]^+$.

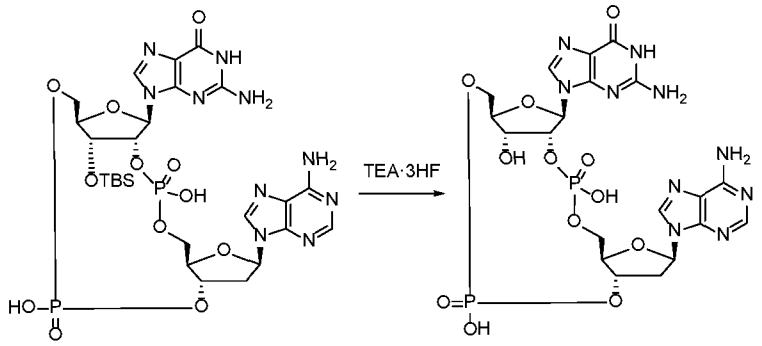
Step 10: (5R,7R,8R,12aR,14R,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-16-{[tert-butyl(dimethyl)silyl]oxy}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide



Crude (5R,7R,8R,12aR,14R,15aS,16R)-16-{[tert-butyl(dimethyl)silyl]oxy}-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide (220mg) was dissolved in a

solution of $MeNH_2$ in EtOH (30%, 4mL), and it was stirred at rt for 5h. Then, the volatile component was removed under reduced pressure to give a crude product, which was used for the next reaction step without purification. LCMS (ES, m/z): 773.2 $[M + H]^+$ and 771.3 $[M - H]^-$.

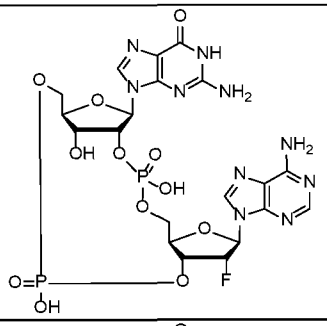
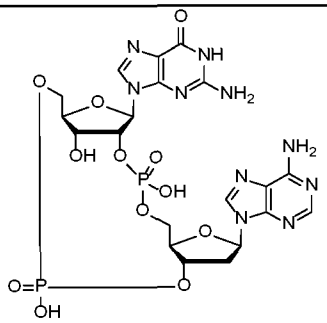
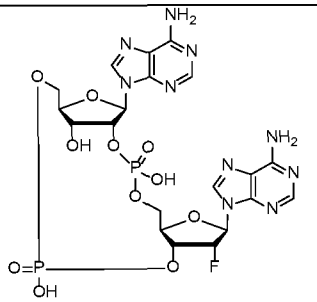
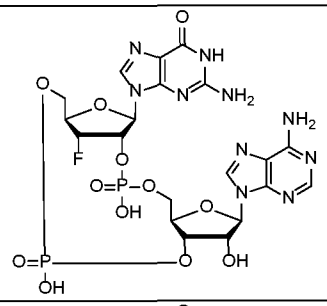
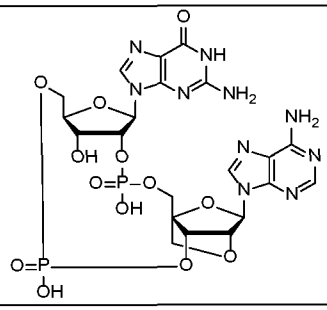
Step 11: (5R,7R,8R,12aR,14R,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-16-hydroxyoctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide

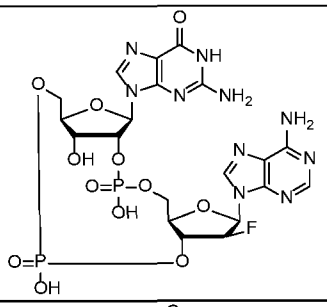
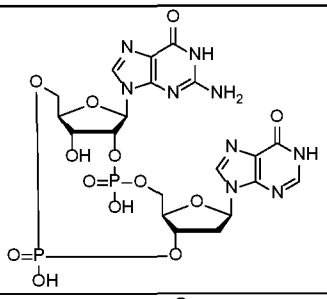
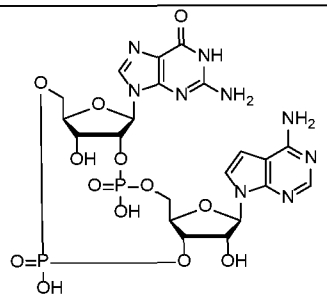
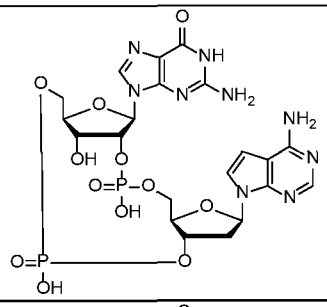
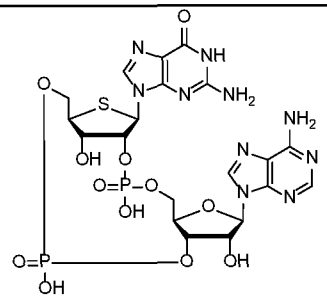
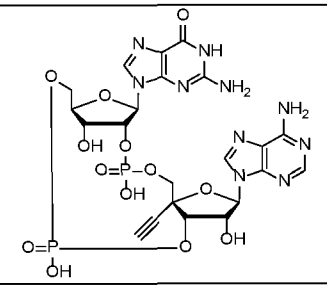


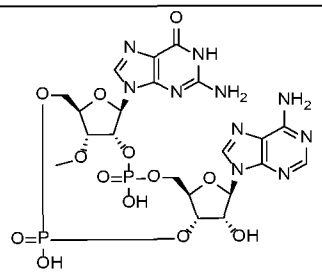
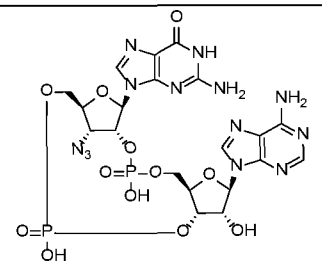
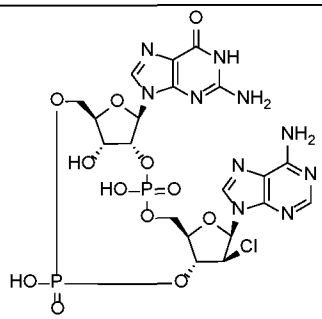
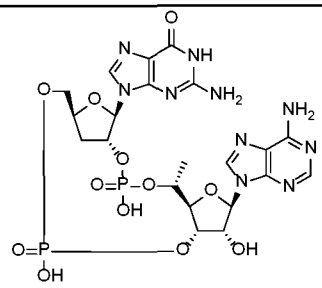
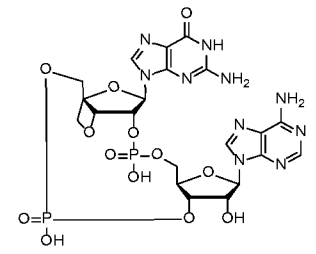
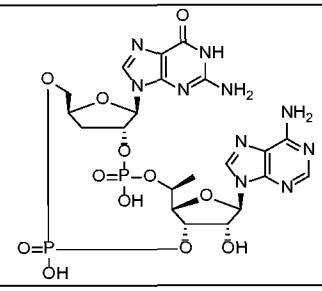
The crude from Step 10 was co-evaporated with pyridine (2.5ml) and Et_3N (2.5mL) three times. It was dissolved in pyridine (2mL). To the solution was added Et_3N (1.51g, 14.9mmol) and triethylamine trihydrofluoride (1.2g, 7.45mmol) dropwise. The mixture was heated at 50°C for 5h. Then, it was concentrated and purified by preparative-HPLC (T3 Prep Column, 100Å, 5μm, 19mm×250mm) eluted with 0 to 10% ACN in aq NH_4HCO_3 (50mM) to give the product. LCMS (ES, m/z): 657.1 $[M - H]^-$. ^{1}H-NMR: (300MHz, DMSO-d_6 + D_2O): δ 8.35 (s, 1H), 8.16 (s, 1H), 8.03 (s, 1H), 6.34 (dd, J = 8.8, 5.9Hz, 1H), 5.85 (d, J = 8.3Hz, 1H), 5.12-4.98 (m, 2H), 4.36 (d, J = 3.9Hz, 1H), 4.22 (t, J = 7.2Hz, 1H), 4.09 (s, 1H), 3.96-3.79 (m, 4H), 3.09-2.99 (m, 1H), 2.64-2.51 (m, 1H). ^{31}P-NMR: (121MHz, DMSO-d_6 + D_2O): δ -1.65 (s), -2.36 (s).

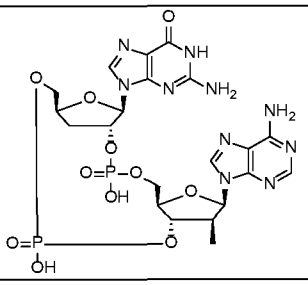
Examples 2 through 19, shown in Table 1 below, were prepared according to procedures analogous to those outlined in Example 1 above using the appropriate nucleoside monomers, decribed as Preparations or as obtained from commercial sources.

Table 1

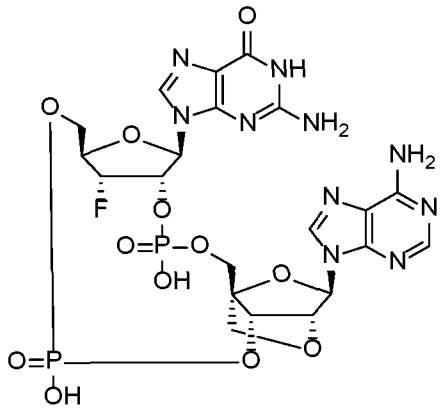
Ex.	Structure	Name	Mass [M-H]⁻
2		(5R,7R,8R,12aR,14R,15R,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-15-fluoro-16-hydroxyoctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide	675
3		(5R,7R,8R,12aR,14R,15aS,16R)-7,14-bis(6-amino-9H-purin-9-yl)-16-hydroxyoctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide	657
4		(5R,7R,8R,12aR,14R,15R,15aR,16R)-7,14-bis(6-amino-9H-purin-9-yl)-15-fluoro-16-hydroxyoctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide	659
5		(5R,7R,8S,12aR,14R,15R,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-16-fluoro-15-hydroxyoctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide	675
6		(5*R*,7*R*,8*R*,12a*R*,14*R*,15*R*,15a*S*,18*R*)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-14-(6-amino-9*H*-purin-9-yl)-18-hydroxyhexahydro-14*H*-15,12a-(epoxymethano)-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10(12*H*)-diolate 2,10-dioxide	685

Ex.	Structure	Name	Mass $[M-H]^-$
7		(5*R*,7*R*,8*R*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-14-(6-amino-9*H*-purin-9-yl)-15-fluoro-16-hydroxyoctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide	675
8		(5*R*,7*R*,8*R*,12a*R*,14*R*,15a*S*,16*R*)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-16-hydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)octahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide	660 $[M+H]^+$
9		(5*R*,7*R*,8*R*,12a*R*,14*R*,15*R*,15a*S*,16*R*)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-14-(4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-15,16-dihydroxyoctahydro-12*H*-5,8-methanofuro[3,2-*l*] [1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide	672
10		(5*R*,7*R*,8*R*,12a*R*,14*R*,15a*S*,16*R*)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-14-(4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-16-hydroxyoctahydro-12*H*-5,8-methanofuro[3,2-*l*] [1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide	656
11		(5*R*,7*R*,8*R*,12a*R*,14*R*,15*R*,15a*S*,16*S*)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-14-(6-amino-9*H*-purin-9-yl)-15,16-dihydroxyoctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,9,11,6,2,10] tetraoxathiadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide	689
12		(5*R*,7*R*,8*R*,12a*R*,14*R*,15*R*,15a*S*,16*R*)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-14-(6-amino-9*H*-purin-9-yl)-12a-ethynyl-15,16-dihydroxyoctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-dioxide	699 $[M+H]^+$

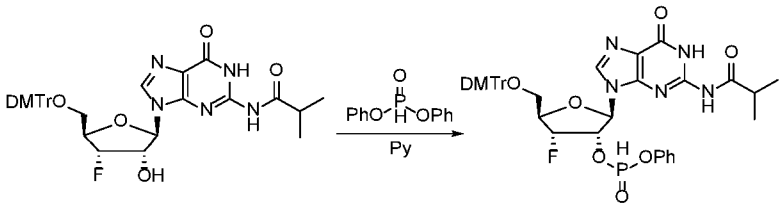
Ex.	Structure	Name	Mass [M-H]⁻
13		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-16-methoxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	687
14		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-azido-2,10,15-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	698
15		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-chloro-2,10,16-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	691
16		2-amino-9-[(5S,7R,8R,12R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-12-methyl-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	671
17		2-amino-9-[(2aR,5S,6aS,7R,8R,9aR,12S,14R,14aS,15R)-8-(6-amino-9H-purin-9-yl)-5,7,12-trihydroxy-5,12-dioxidohexahydro-6aH-2a,14-(epoxymethano)furo[3,2-d]oxeto[2,3-k][1,3,7,9,2,8]tetraoxadiphosphacyclotridecin-15(2H,3H)-yl]-1,9-dihydro-6H-purin-6-one	685
18		2-amino-9-[(5S,7R,8R,12S,12aS,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-12-methyl-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	671

Ex.	Structure	Name	Mass [M-H]⁻
19		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dihydroxy-15-methyl-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	655

Example 20: (5*R*,7*R*,8*S*,12a*R*,14*R*,15*R*,15a*S*,18*R*)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-14-(6-amino-9*H*-purin-9-yl)-18-fluorohexahydro-14*H*-15,12a-(epoxymethano)-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10(12*H*)-diolate 2,10-dioxide

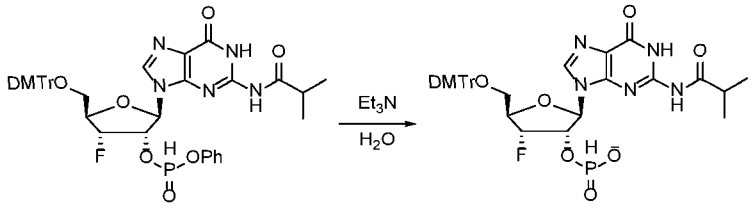


Step 1: (2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phenyl phosphonate



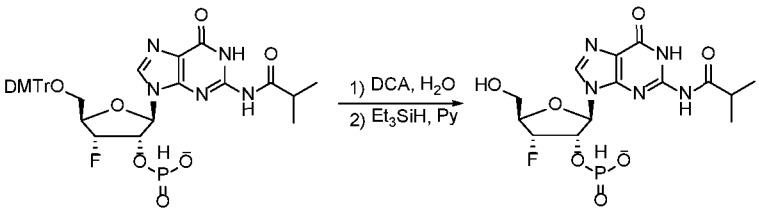
To a stirred solution of N-(9-((2R,3S,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy) methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (1g, 1.520mmol) in pyridine (7.6mL) under Ar was added diphenyl phosphonate (1.068g, 4.56mmol), and it was stirred at rt for 20min. The reaction mixture containing the product was used for the next reaction step without purification.

Step 2: (2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



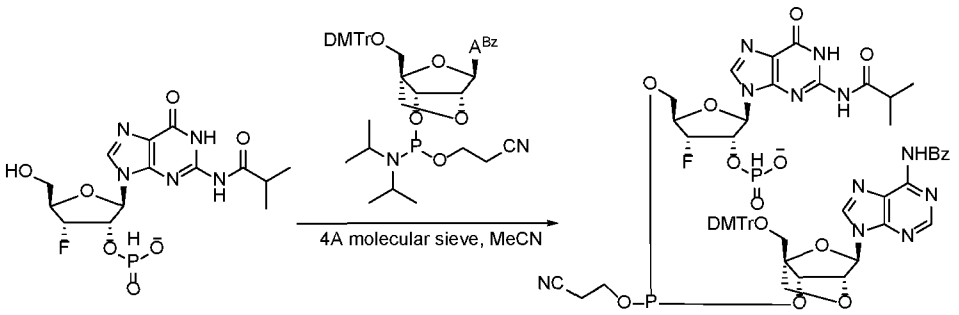
To the reaction mixture from Step 1 was added water (1.5ml) and Et_3N (1.5mL). The mixture was stirred at rt for 20min. Then, it was concentrated, and the residue was partitioned between CH_2Cl_2 (50mL) and aq $NaHCO_3$ (5%, 20mL). Layers were separated. The organic layer was washed with aq $NaHCO_3$ (5%, 2x20mL), dried (Na_2SO_4), concentrated and purified by silica gel column chromatography using 0 to 7% MeOH in CH_2Cl_2 (1% Et_3N) to give the product. LCMS (ES, m/z): 722 $[M + H]^+$. ^{31}P-NMR: (162MHz, CD_3OD): δ 2.73 (s, 1P).

Step 3: (2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



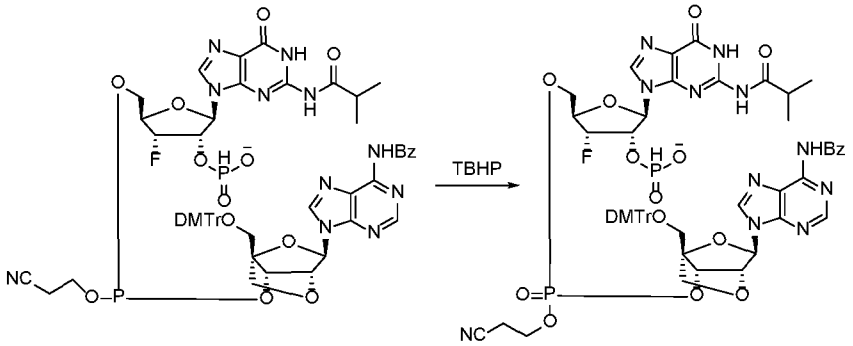
To a stirred solution of the product of Step 2 (0.9g, 0.999mmol) in CH_2Cl_2 (6mL) were added water (0.180g, 9.99mmol) and 2,2-dichloroacetic acid (1.16g, 8.99mmol) in CH_2Cl_2 (10ml). The mixture was stirred at rt for 15min. Et_3SiH (10mL) was added, and it was stirred for 1h. Then, pyridine (2mL) was added, and it was concentrated. The residue was purified by reverse phase (C18) chromatography eluted with 0 to 30% ACN in aq NH_4HCO_3 (0.04%) to give the product. LCMS (ES, m/z): 722 $[M - H]^-$. 417.9. ^{1}H-NMR: (400MHz, CD_3OD): δ 8.31 (s, 1H), 7.49 (d, J = 1.6Hz, 0.5H), 6.15 (d, J = 6.8Hz, 1H), 5.91 (d, J = 1.6Hz, 0.5H), 5.42-5.32 (m, 1.5H), 5.21 (dd, J = 4.5, 1.9Hz, 0.5H), 4.45-4.32 (m, 1H), 3.81 (d, J = 3.5Hz, 2H), 3.20 (q, J = 7.4Hz, 1H), 2.73 (p, J = 6.9Hz, 1H), 1.30 (t, J = 7.3Hz, 1.5H), 1.23 (d, J = 6.9Hz, 6H). ^{19}F-NMR: (376MHz, CD_3OD): δ -200.96 (s, 1F). ^{31}P-NMR: (162MHz, CD_3OD): δ 2.41 (s, 1P).

Step 4: (2R,3S,4R,5R)-5-((((((1R,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphanyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



(2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (600mg, 0.676mmol) was co-evaporated with ACN (3x5mL), re-dissolved in ACN (3mL), dried by addition of activated 4Å molecular sieves (150mg) and kept under Ar. (1R,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl (2-cyanoethyl) diisopropylphosphoramidite (available from Exiqon (EQ-0063-1000), 235.5mg, 0.56mmol) and pyridinium 2,2,2-trifluoroacetate (162mg, 0.84mmol) were co-evaporated with ACN (3x5mL), re-dissolved in ACN (5mL), and dried by addition activated 4Å molecular sieve (150mg). After 30min, it was added to the previously prepared mixture containing (2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate. The mixture was stirred at rt for 1h. The reaction mixture containing the product was used in the next reaction step immediately without purification. LCMS (ES, m/z): 1202.3 $[M - H]^-$.

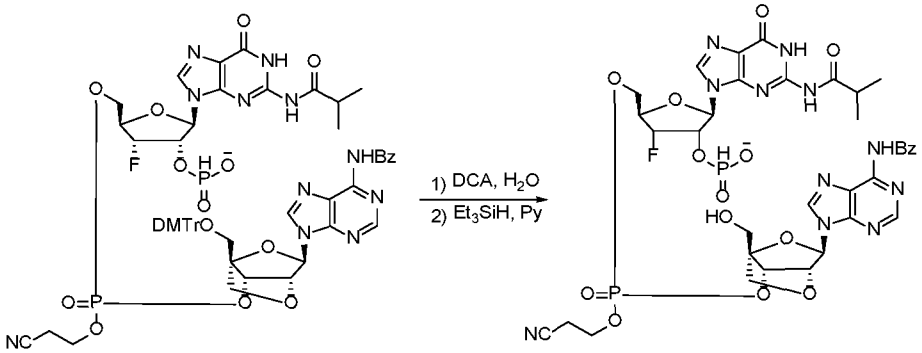
Step 5: (2R,3S,4R,5R)-5-((((((1R,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



To the stirred reaction mixture from Step 4 was added *tert*-butyl hydroperoxide in decane (5.5M, 0.31mL, 1.71mmol) dropwise. The resulting mixture was stirred at rt for 1h. After 30min, the solution was cooled to 0°C, and $NaHSO_3$ (150mg) in water (5mL) was added slowly.

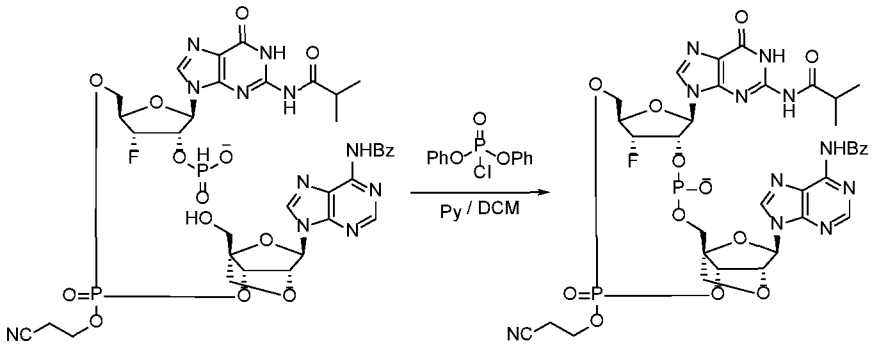
After 5min, the mixture was concentrated, and the residue was purified by reverse phase (C18) chromatography eluted with 0 to 75% ACN in aq NH_4HCO_3 (5mM) to give the product. LCMS (ES, m/z): 1220.1 $[M + H]^+$. ^{19}F-NMR (376MHz, CD_3OD): δ -200.38, -202.45 (2s, 1F). ^{31}P-NMR: (162MHz, CD_3OD): δ 2.57, 2.49 (2s, 1P); -3.52, -4.21 (2s, 1P).

Step 6: (2R,3S,4R,5R)-5-((((((1S,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



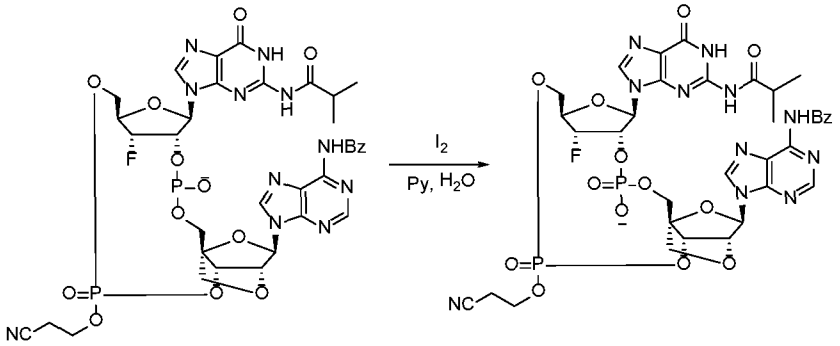
To a solution of (2R,3S,4R,5R)-5-((((((1R,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (190mg, 0.16mmol) in CH_2Cl_2 (2.5mL) was added water (28.8mg, 1.6mmol) and dichloroacetic acid in CH_2Cl_2 (0.6M, 2.5mL). The mixture was stirred at rt for 10min, and then Et_3SiH (4.5mL) was added. After 1h, pyridine (0.5mL) was added. After 10min, the resulting mixture was concentrated to give the product, which was used for the next reaction step without purification. LCMS (ES, m/z): 917.9 $[M + H]^+$. ^{31}P-NMR: (162MHz, CD_3OD): δ 2.51, 2.34 (2s, 1P); -3.46, -3.82 (2s, 1P).

Step 7: (5R,7R,8S,12aR,14R,15R,15aS,18R)-2-(2-cyanoethoxy)-18-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}hexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-10(12H)-olate 2,10-dioxide



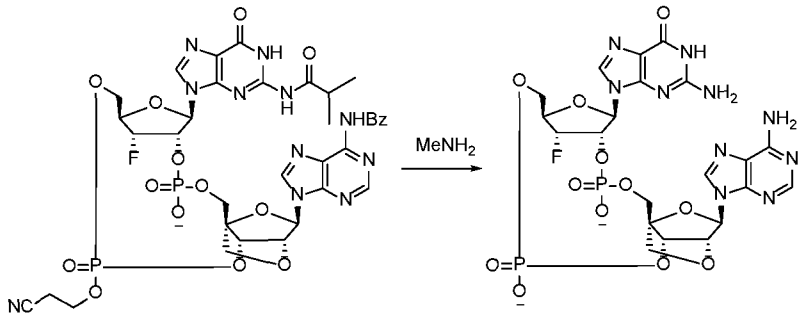
To pyridine (16mL) under Ar was added diphenyl chlorophosphate (0.66mL, 3.2mmol). It was cooled at -40°C and then, a solution of crude from Step 6 in CH_2Cl_2 (16mL) was added dropwise over 20min. The resulting mixture was stirred at -40°C for 40min. The reaction mixture was used in the next step without purification. LCMS (ES, m/z): 898.2 $[M - H]^-$.

Step 8: (5R,7R,8S,12aR,14R,15R,15aS,18R)-2-(2-cyanoethoxy)-18-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}hexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-10(12H)-olate 2,10-dioxide [3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10(12H)-diolate 2,10-dioxide



To the solution from Step 7 at 0°C was added I_2 in pyridine/water (9/1) (3%, 1.76mL) over 5min. The mixture was stirred at rt for 40min. Then, it was treated with a solution of $Na_2S_2O_3 \cdot 5H_2O$ (150mg) in water (2mL). After 5min, the mixture was concentrated under reduced pressure. The residue was purified by reverse phase (C18) chromatography eluted with 0 to 45% ACN in aq NH_4HCO_3 (0.04%) to give the product. LCMS (ES, m/z): 915.8 $[M + H]^+$. ^{19}F-NMR (376MHz, CD_3OD): δ -198.70, -203.36 (2s, 1F). ^{31}P-NMR (162MHz, CD_3OD): δ -0.96, -1.75 (2s, 1P); -3.64, -4.71 (2s, 1P).

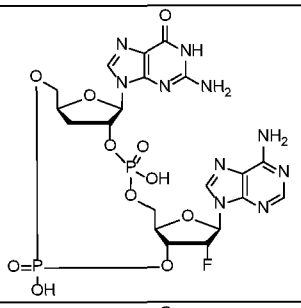
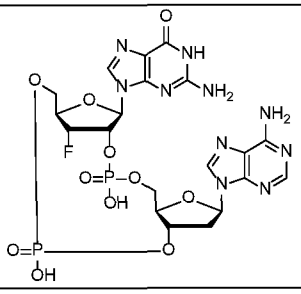
Step 9: (5R,7R,8S,12aR,14R,15R,15aS,18R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-18-fluorohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro [3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10(12H)-diolate 2,10-dioxide

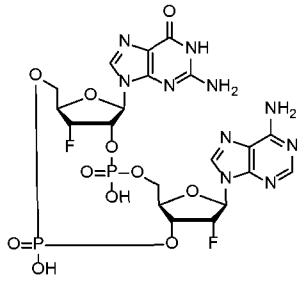
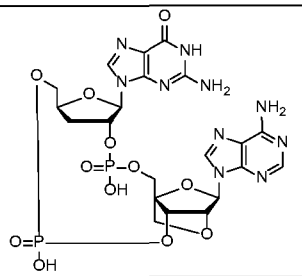
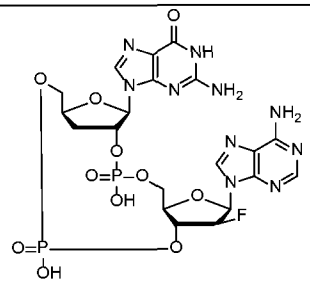
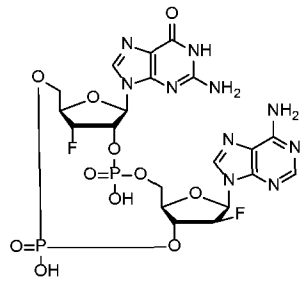
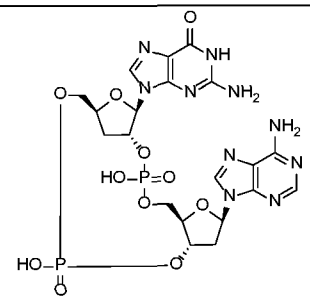
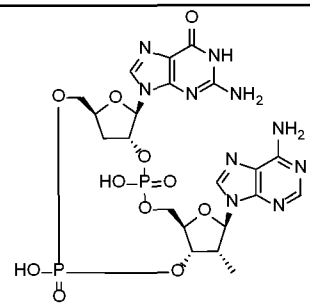


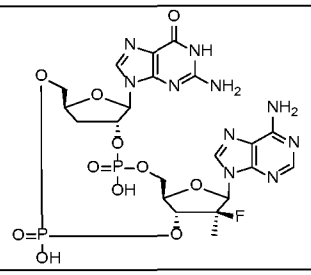
(5R,7R,8S,12aR,14R,15R,15aS,18R)-2-(2-cyanoethoxy)-18-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}hexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-10(12H)-olate 2,10-dioxide (110mg, 0.12mmol) was dissolved in a solution of $MeNH_2$ in EtOH (30%, 15mL), and the resulting solution was stirred at rt for 3h. Then, it was concentrated, and the residue was purified by preparative-HPLC (Atlantis Prep T3 Column, 19×250mm) eluted with 0 to 9% ACN in aq NH_4HCO_3 (50mM) to give the product. LCMS (ES, m/z): 686.9 $[M - H]^-$. ^{1}H-NMR (400MHz, D_2O): δ 8.14 (s, 1H), 7.85 (s, 1H), 7.80 (s, 1H), 6.01 (s, 1H), 5.99 (d, J = 8.5Hz, 1H), 5.84-5.66 (m, 1H), 5.44 (d, J = 3.6Hz, 0.5H), 5.31 (d, J = 3.6Hz, 0.5H), 4.96 (d, J = 3.9Hz, 1H), 4.84 (s, 1H), 4.65-4.53 (m, 1H), 4.33-4.15 (m, 4H), 4.10 (d, J = 8.2Hz, 1H), 3.96 (d, J = 8.2Hz, 1H). ^{19}F-NMR (376MHz, D_2O): δ -199.02 (s, 1F). ^{31}P-NMR (162MHz, D_2O): δ -1.89 (s, 1P), -2.49 (s, 1P).

Examples 21 through 29, as shown in Table 2 below, were prepared according to procedures analogous to those outlined in Example 20 above using the appropriate monomers, decribed as Preparations or as obtained from commercial sources, in the coupling step.

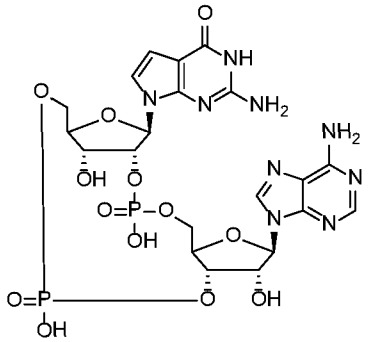
Table 2

Ex.	Structure	Name	Mass [M-H]
21		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aR)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10-dihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	659
22		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10-dihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	659

Ex.	Structure	Name	Mass [M-H]
23		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	677
24		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dihydroxy-2,10-dioxidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one	669
25		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10-dihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	659
26		2-amino-9-[(2S,5R,7R,8S,10S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	677
27		2-amino-9-[(5S,7R,8R,12aR,14R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	641
28		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dihydroxy-15-methyl-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	655

Ex.	Structure	Name	Mass [M-H]
29		2-amino-9-[(5S,8R,12aR,15S,15aR)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10-dihydroxy-15-methyl-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	673

Example 30: 2-amino-7-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-3,7-dihydro-4H-pyrrolo[2,3-d] pyrimidin-4-one



cGAS buffer consisted of 40mM Tris-HCL, pH 7.5, 100uM NaCl, 10mM $MgCl_2$. cGAS enzyme was purchased from Novoprotein (Novoprotein code: SGCAS), having been expressed in *E. coli* and purified using a HIS tag. The calculated molecular weight was 55.3kDa, and the sequence was:

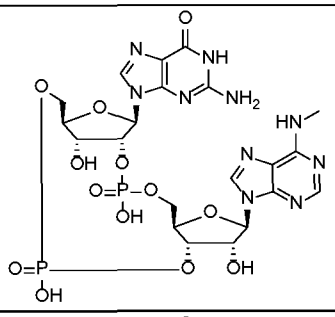
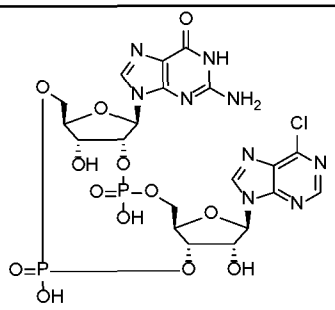
MAHHHHHHGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAF
AKRQGKEMDSLTFLYDGIEIQADQTPEDLDMEDNDIIEAHREQIGGENLYFQGGASKLR
AVLEKLKLSRDDISTAAGMVKGVVDHLLLRLKCDSAFRGVGLLNTGSYYEHVKISAPN
EFDVMFKLEVPRIQLEEYSNTRAYYFVKFKRNPKENPLSQFLEGEILSASKMLSKFRKIIK
EEINDIKDTDVIMKRKRGGSPAVTLLISEKISVDITLALESKSSWPASTQEGLRIQNWLSA
KVRKQLRLKPFYLVPKHAKEGNGFQEETWRLSFSHIEKEILNNHGKSKTCCENKEEKCC
RKDCLKLMKYLLEQLKERFKDKKHLDKFSSYHVKTAFFHVCTQNPQDSQWDRKDLGL
CFDNCVTYFLQCLRTEKLENYFIPEFNLFSSNLIDKRSKEFLTKQIEYERNNEFPVFDEF
(SEQ. ID. NO. 1)

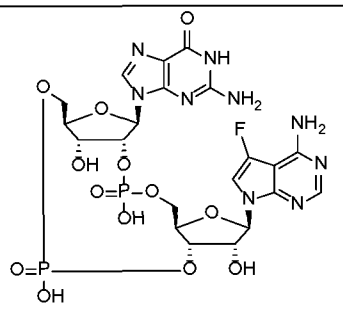
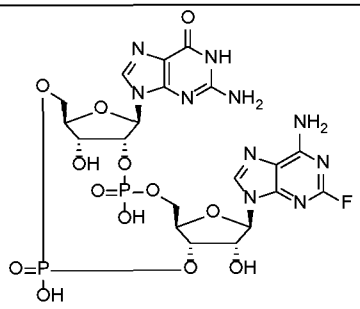
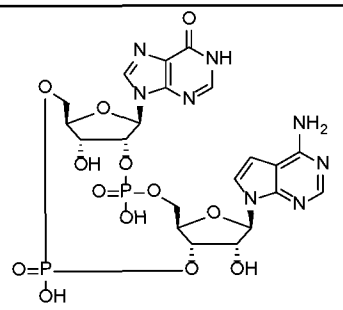
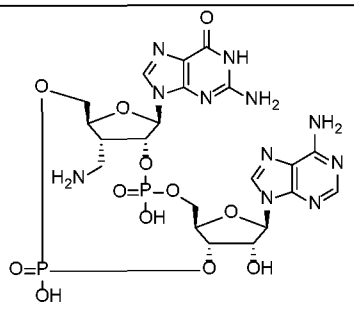
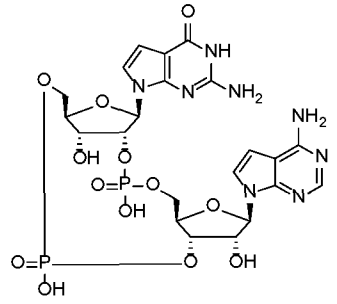
To a vial were added Herring DNA (CAS# 9007-49-2, 0.3mg/mL in cGAS buffer; 14.8mL) and cGAS enzyme (3.1mg/mL in cGAS buffer; 0.78mL), and the mixture was incubated at RT for 15min. 7-Deaza-GTP (TriLink catalog # N-1044; 5mM in cGAS buffer,

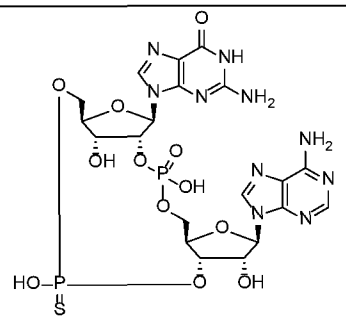
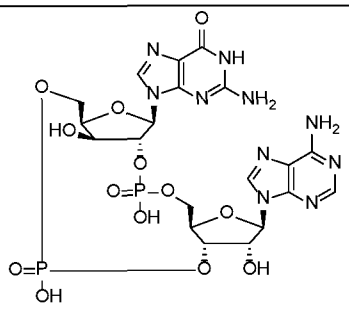
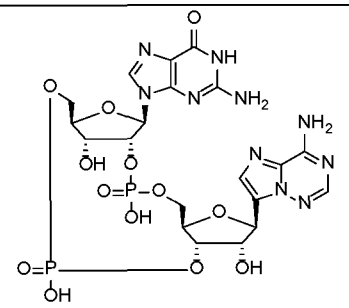
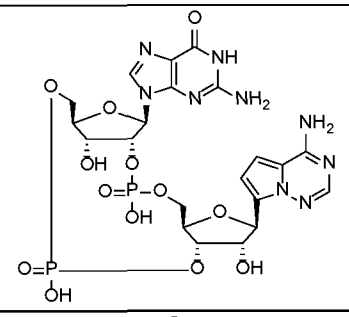
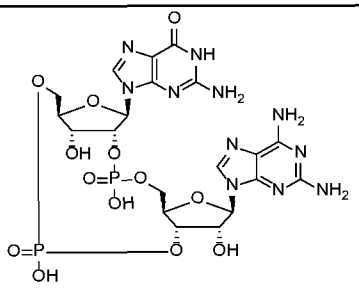
1.95mL, 9.75μmol) and ATP (5mM in cGAS buffer, 1.95mL, 9.75μmol) were added, and the mixture was incubated on a Radleys Metz heater shaker set to maintain 37°C while shaking at 250rpm for 16h, after which the mixture was filtered and lyopholyzed. The product was purified using mass-directed reverse phase HPLC with a Waters SunFire C18 OBD Prep Column, 100Å, 5μm, 19mmx150mm, [Waters Part# 186002568] using a gradient solvent system with MeCN and 100mM aqueous triethylammonium acetate. Lyopholization of the product fractions furnished the title compound. LCMS (ES, m/z): 672 $[M - H]^-$. ^{1}H NMR (600MHz, DMSO-d_6) δ 10.33 (s, 1H), 8.37 (s, 1H), 8.10 (s, 1H), 7.56 (s, 1H), 7.24 (s, 2H), 6.94 (d, J = 3.4Hz, 1H), 6.28 – 6.24 (m, 3H), 6.01 (d, J = 8.1Hz, 1H), 5.86 (d, J = 7.9Hz, 1H), 5.61 (s, 1H), 5.01 – 4.97 (m, 1H), 4.86 – 4.83 (m, 1H), 4.70 (s, 1H), 4.25 (s, 1H), 4.21 (dd, J = 10.4, 4.8Hz, 1H), 4.03-3.92 (4, 3H), 3.80 – 3.75 (m, 1H), 3.69 (d, J = 12.1Hz, 1H), 2.76 (s, 12H), 1.02 (S, 18H).

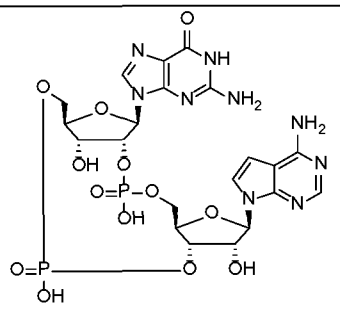
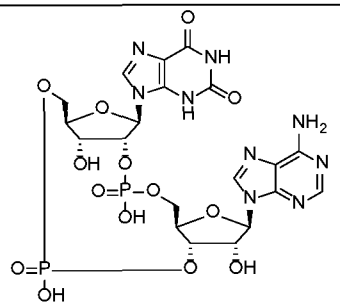
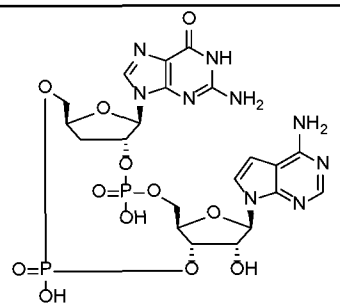
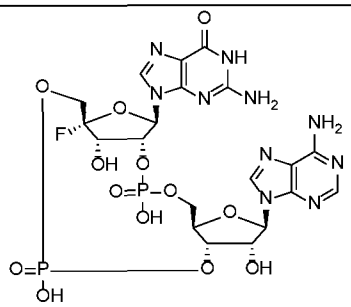
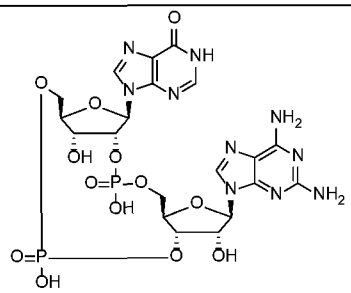
Examples 31 to 65 in Table 3 below were made using procedures analogous to those described above for Example 30 using the appropriate nucleoside triphosphate monomers. Where necessary, the triphosphates were formed according to methods similar to those described for Preparations 23 to 26 or by submission of the requisite 5'-OH nucleoside monomer to NuBlocks LLC (Oceanside, CA). Example 38 was made using ATP and α-thio-GTP (BIOLOG Life Science Institute, catalog # G014/G015).

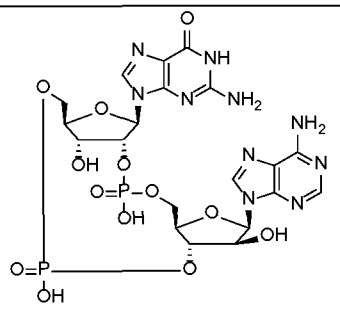
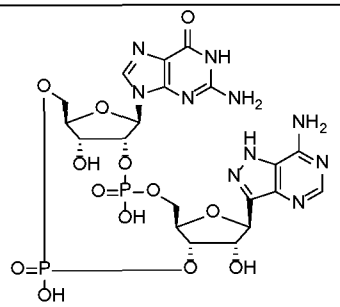
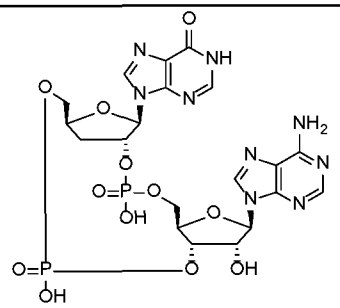
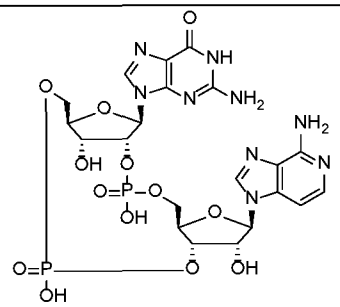
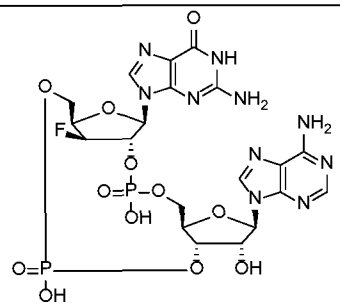
Table 3

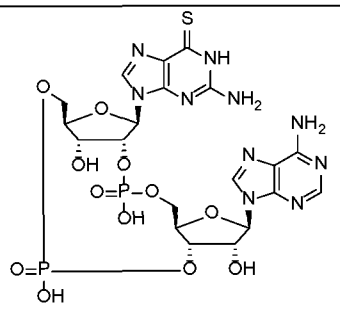
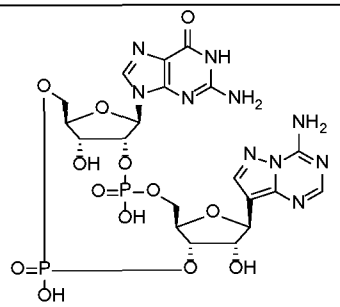
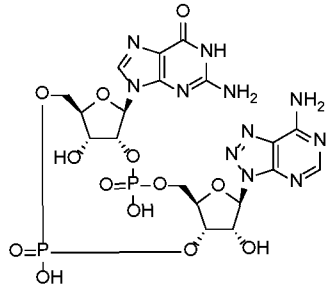
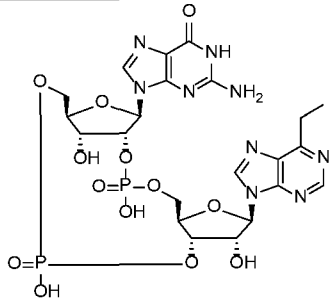
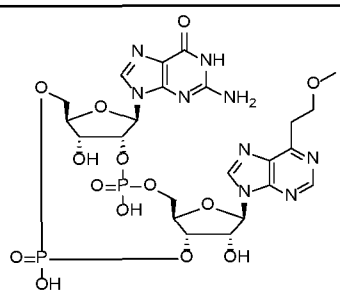
Ex.	Structure	Name	Mass $[M-H]^-$
31		2-amino-9-{(5R,7R,8R,12aR,14R,15R,15aS,16R)-2,10,15,16-tetrahydroxy-14-[6-(methylamino)-9H-purin-9-yl]-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl}-1,9-dihydro-6H-purin-6-one	687
32		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-chloro-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	692

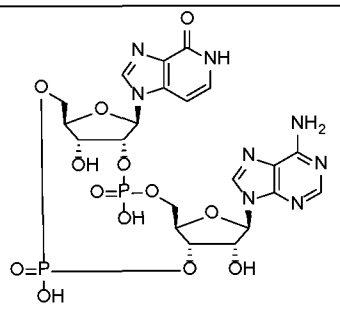
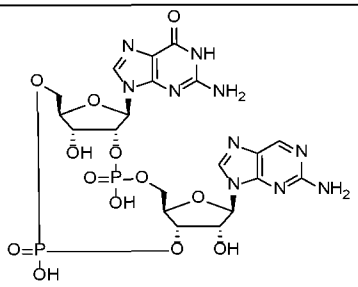
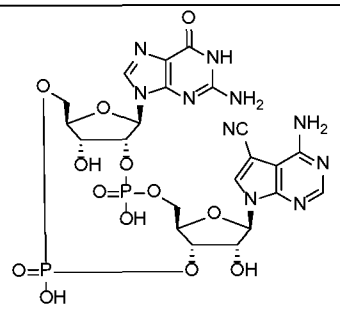
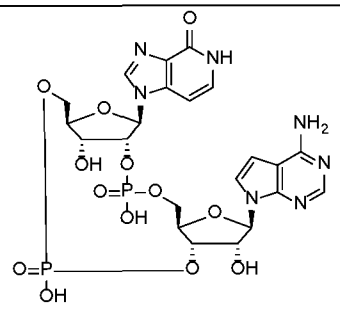
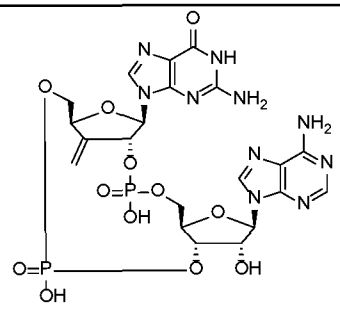
Ex.	Structure	Name	Mass [M-H]⁻
33		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(4-amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	690
34		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-2-fluoro-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	691
35		9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	657
36		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS,16R)-16-(aminomethyl)-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	686
37		2-amino-7-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one	671

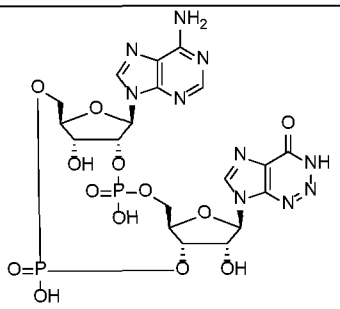
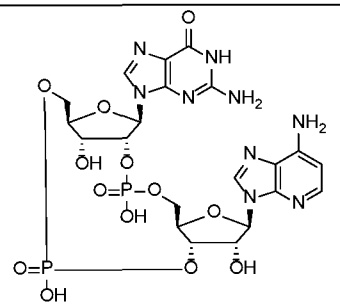
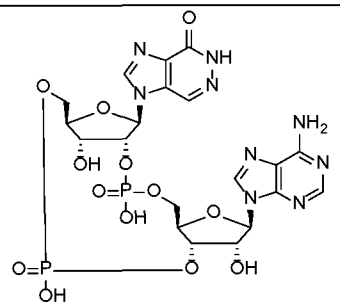
Ex.	Structure	Name	Mass [M-H]⁻
38		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-10-oxido-2-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	689
39		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16S)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	673
40		2-amino-9-[(5R,7R,8R,12aR,14S,15S,15aS,16R)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	673
41		2-amino-9-[(5R,7R,8R,12aR,14S,15S,15aS,16R)-14-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	672
42		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(2,6-diamino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	688

Ex.	Structure	Name	Mass [M-H]⁻
43		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-10,15,16-trihydroxy-2,10-dioxido-2-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	688
44		9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,9-dihydro-1H-purine-2,6-dione	674
45		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10,15-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	656
46		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS,16S)-14-(6-amino-9H-purin-9-yl)-5-fluoro-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	691
47		9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(2,6-diamino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	673

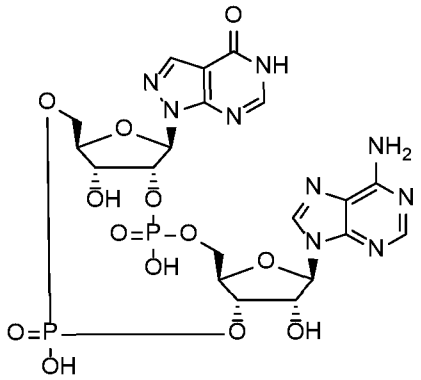
Ex.	Structure	Name	Mass [M-H]⁻
48		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	673
49		2-amino-9-[(5R,7R,8R,12aR,14S,15S,15aS,16R)-14-(7-amino-1H-pyrazolo[4,3-d]pyrimidin-3-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	673
50		9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	642
51		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(4-amino-1H-imidazo[4,5-c]pyridin-1-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	672
52		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,16S)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10,15-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	675

Ex.	Structure	Name	Mass [M-H]⁻
53		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purine-6-thione	689
54		2-amino-9-[(5R,7R,8R,12aR,14S,15S,15aS,16R)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	673
55		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	674
56		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-ethyl-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	686
57		2-amino-9-{(5R,7R,8R,12aR,14R,15R,15aS,16R)-2,10,15,16-tetrahydroxy-14-[6-(2-methoxyethyl)-9H-purin-9-yl]-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl}-1,9-dihydro-6H-purin-6-one	716

Ex.	Structure	Name	Mass [M-H]⁻
58		1-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one	657
59		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(2-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	673
60		4-amino-7-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-7H-pyrrolo[2,3-d]pyrimidine-5-carbonitrile	697
61		1-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one	656
62		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-16-methylidene-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]/pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	669

Ex.	Structure	Name	Mass [M-H]⁻
63		7-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-7-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-14-yl]-3,7-dihydro-4H-imidazo[4,5-d][1,2,3]triazin-4-one	659
64		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(7-amino-3H-imidazo[4,5-b]pyridin-3-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	672
65		1-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,5-dihydro-4H-imidazo[4,5-d]pyridazin-4-one	658

Example 66: 1-[(5*R*,7*R*,8*R*,12*aR*,14*R*,15*R*,15*aS*,16*R*)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one



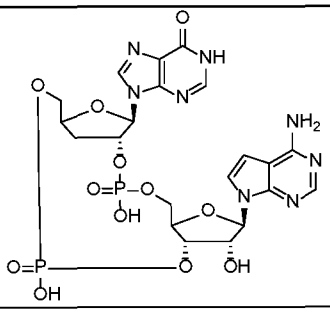
cGAS buffer consisted of 40mM Tris-HCL, pH 7.5, 100uM NaCl, 10mM $MgCl_2$. cGAS enzyme was purchased from Novoprotein (Novoprotein code: SGCAS), having been expressed in *E. coli* and purified using a HIS tag. The calculated molecular weight was 55.3kDa, and the sequence was:

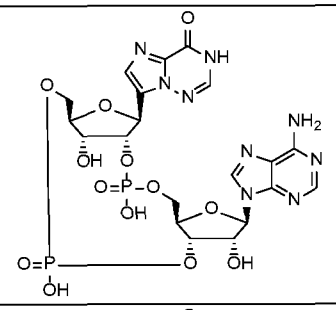
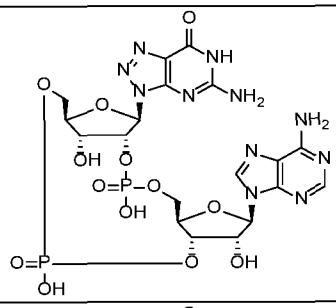
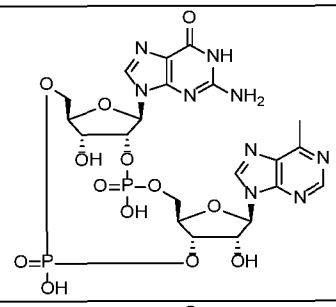
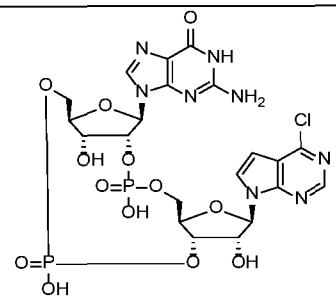
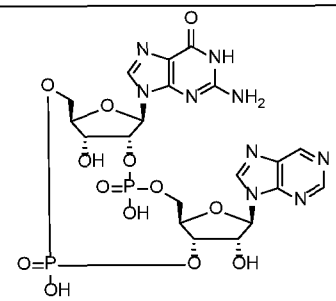
MAHHHHHHGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAF
AKRQGKEMDSLTFLYDGIEIQADQTPEDLDMEDNDIIEAHREQIGGENLYFQGGASKLR
AVLEKLKLSRDDISTAAGMVKGVVDHLLLRLKCDSAFRGVGLLNTGSYYEHVKISAPN
EFDVMFKLEVPRIQLEEYSNTRAYYFVKFKRNPKENPLSQFLEGEILSASKMLSKFRKIIK
EEINDIKDTDVIMKRKRGGSPAVTLLISEKISVDITLALESKSSWPASTQEGLRIQNWLSA
KVRKQLRLKPFYLVPKHAKEGNGFQEETWRLSFSHIEKEILNNHGKSKTCCENKEEKCC
RKDCLKLMKYLLEQLKERFKDKKHLDKFSSYHVKTAFFHVCTQNPQDSQWDRKDLGL
CFDNCVTYFLQCLRTEKLENYFIPEFNLFSSNLIDKRSKEFLTKQIEYERNNEFPVFDEF
(SEQ. ID. NO. 1)

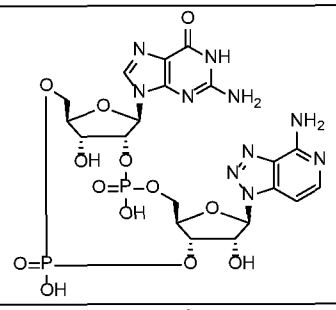
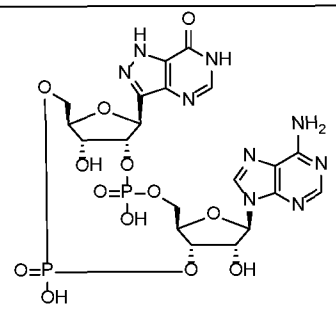
To a vial were added Herring DNA (CAS# 9007-49-2, 0.3mg/mL in cGAS buffer; 15.2mL) and cGAS enzyme (3.1mg/mL in cGAS buffer; 0.8mL), and the mixture was incubated at RT for 15min. ATP (5mM in cGAS buffer, 2.0mL, 10μmol), 7-deaza-8-aza-ITP (5mM in cGAS buffer, 2.0mL, 10μmol), and DMSO (5mL) were added, and the mixture was incubated on a Radleys Metz heater shaker set to maintain 37°C while shaking at 250rpm for 3d. The mixture was filtered, lyopholyzed, and purified by reverse phase HPLC (eluting acetonitrile/water gradient with 100mM TEAA modifier, linear gradient) to afford the title compound as the TEA salt. LCMS (ES, m/z): 658 $[M - H]^-$. ^1H NMR (600MHz, D_2O): δ 8.36 (s, 1H), 8.34 (s, 1H), 8.13 (s, 1H), 7.51 (s, 1H), 6.41 (d, J = 8.2Hz, 1H), 6.24 (s, 1H), 5.69 (m, 1H), 5.42 (m, 1H), 4.88 (d, J = 4.4Hz, 1H), 4.66 (d, J = 4.1Hz, 1H), 4.51 (m, 1H), 4.43 (m, 2H), 4.23 (m, 2H), 4.01 (m, 1H).

Examples 67 to 74 in Table 4 below were made using procedures analogous to those described above for Example 66 using the appropriate nucleoside triphosphate monomers. Where necessary, the triphosphates were formed according to methods similar to those described for Preparations 23 to 26 or by submission of the requisite 5'-OH nucleoside monomer to NuBlocks LLC (Oceanside, CA).

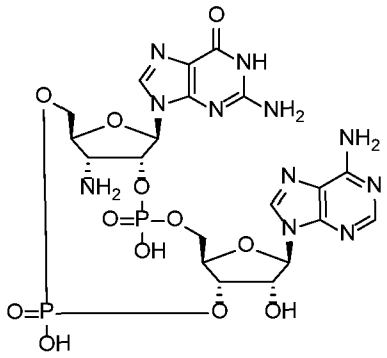
Table 4

Ex	Structure	Name	Mass $[M-H]^-$
67		9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10,15-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro [3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	641

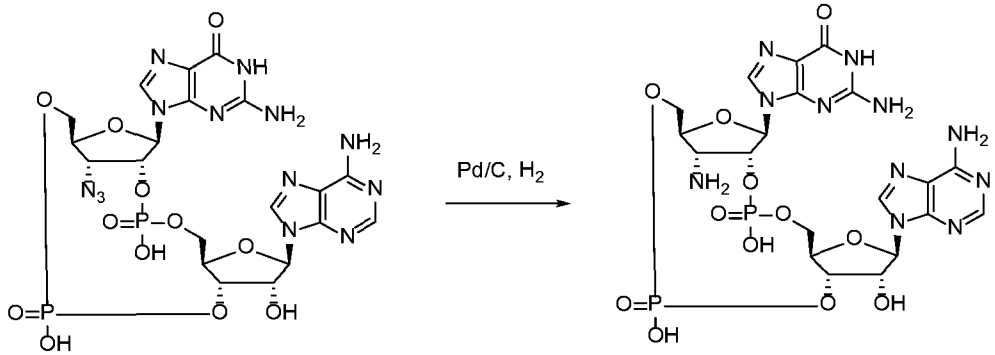
Ex	Structure	Name	Mass [M-H]⁻
68		7-[(5*R*,7*S*,8*R*,12a*R*,14*R*,15*R*,15a*S*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12*H*-5,8-methanofuro [3,2-*l*][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]imidazo[2,1-*f*][1,2,4]triazin-4(3*H*)-one	658
69		5-amino-3-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one	674
70		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-2,10,15,16-tetrahydroxy-14-(6-methyl-9H-purin-9-yl)-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	672
71		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	691
72		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-2,10,15,16-tetrahydroxy-2,10-dioxido-14-(9H-purin-9-yl)octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	658

Ex	Structure	Name	Mass [M-H]⁻
73		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(4-amino-1H-[1,2,3]triazolo[4,5-c]pyridin-1-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	673
74		3-[(5R,7S,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one	658

Example 75: 2-Amino-9-[(5S,7R,8R,12aR,14R,15R,15aS,16R)-16-amino-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one



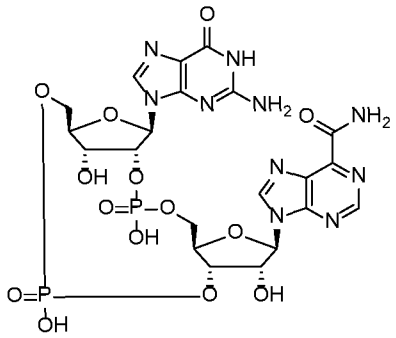
Step 1: 2-Amino-9-[(5S,7R,8R,12aR,14R,15R,15aS,16R)-16-amino-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one



To a stirred solution of 2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-azido-2,10,15-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Example 14, 4.0mg, 0.0055mmol) in absolute EtOH (1.0mL) and deionized water (1.0mL) was added palladium on carbon (1.0mg, 10wt.% loading) in one portion under Ar at RT. The reaction vessel was then flushed with hydrogen gas and attached to a hydrogen gas balloon. The reaction mixture was left to stir for 48h, filtered, and concentrated to afford the title compound. LCMS (ES, m/z): 672 [M - H]$^-$. (600MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.42 (s, 1H), 8.12 (s, 1H), 7.96 (s, 1H), 7.70 (s, 1H), 7.29 (br, 2H), 6.56 (br, 2H), 6.00 (d, J = 8.3Hz, 1H), 5.89 (d, J = 8.5Hz, 1H), 5.21 (s, 1H), 5.04 (t, J = 6.0Hz, 1H), 4.16 (s, 1H), 4.05 (dd, J = 10.5, 5.0Hz, 1H), 4.00 (s, 1H), 3.77 (d, J = 4.1Hz, 1H), 3.67 (m, 2H). ^{31}P NMR: (202MHz, DMSO-d_6): δ -0.4 (s), 2.0 (s).

Alternatively, Example 75 may be prepared from the requisite monomers, according to a method similar to that described above for Example 30.

Example 76: 9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-9*H*-purine-6-carboxamide

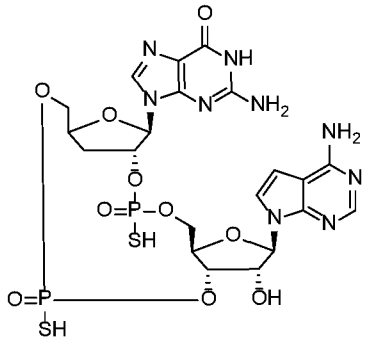


To a stirred solution of 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-14-(6-chloro-9*H*-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one, (Example 32, 16mg, 0.018mmol) in DMSO(1.7mL) was added sodium cyanide (8.0mg, 0.16mmol) in one portion under Ar at RT. The reaction mixture was heated to 80°C and left to stir at the same temperature for 3h, cooled to ambient temperature, then quenched with cold acetic acid (15uL). The mixture was filtered and lyophilyzed. The product was purified using mass-directed reverse phase HPLC with a Waters SunFire C18 OBD Prep Column, 100Å, 5µm, 19mmx150mm, [Waters Part# 186002568] using a gradient solvent system with MeCN and

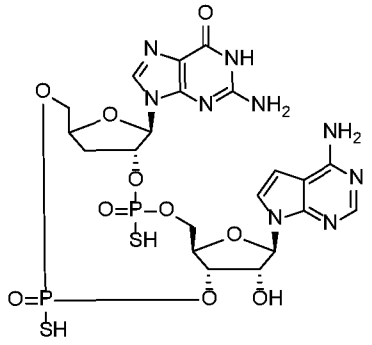
100mM aqueous triethylammonium acetate. Lyophilization of the product fractions furnished 9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-9*H*-purine-6-carbonitrile. LCMS (ES, m/z): 683 [M - H]$^-$.

To a stirred suspension of 9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-9*H*-purine-6-carbonitrile (3.0mg, 0.003mmol) in deionized water (338uL) was added Hydrido(dimethylphosphinous acid-kP)[hydrogen bis(dimethylphosphinito-kP)]platinum(II) (1.0mg, 0.002mmol). The reaction mixture was heated to 85°C and left to stir at the same temperature for 6h, cooled to ambient temperature, filtered and lyopholyzed. The product was purified using mass-directed reverse phase HPLC with a Waters SunFire C18 OBD Prep Column, 100Å, 5µm, 19mmx150mm, [Waters Part# 186002568] using a gradient solvent system with MeCN and 100mM aqueous triethylammonium acetate. Lyopholization of the product fractions furnished 9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-9*H*-purine-6-carboxamide. LCMS (ES, m/z): 701 [M - H]$^-$. ^{1}H NMR (500MHz, DMSO): δ 10.58 (s, 1H), 9.06 (s, 1H), 8.36 (s, 1H), 8.05 (s, 1H), 7.91 (s, 1H), 7.74 (s, 1H), 6.55 (br, 4 H), 6.07 (d, *J* = 7.7Hz, 1H), 5.81 (d, *J* = 6.0Hz, 1H), 5.77 (m, 1H), 5.04 (m, 1H), 4.96 (d, *J* = 4.5Hz, 1H), 4.60 (m, 1H), 4.27 (m, 1H), 4.07-4.04 (m, 2H), 3.99-3.75 (m, 2H). ^{31}P NMR: (202MHz, DMSO): δ 1.9 (s), -0.8 (s).

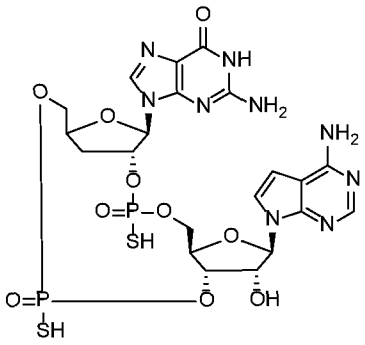
Examples 77, 78, 79, 80: 2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomers 1 – 4)



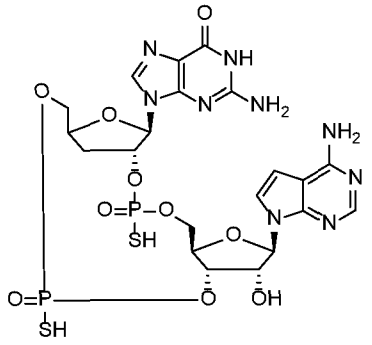
Diastereomer 1



Diastereomer 2

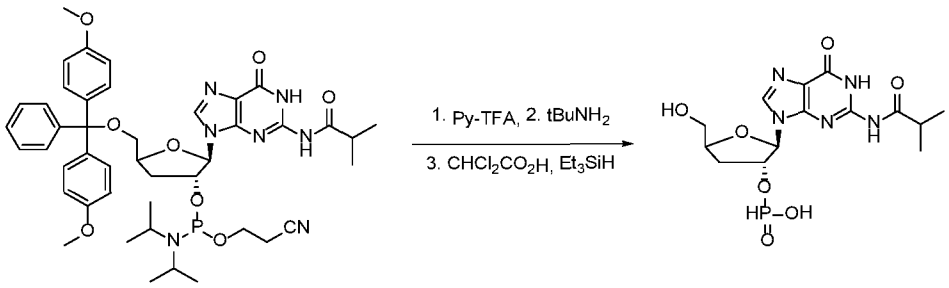


Diastereomer 3



Diastereomer 4

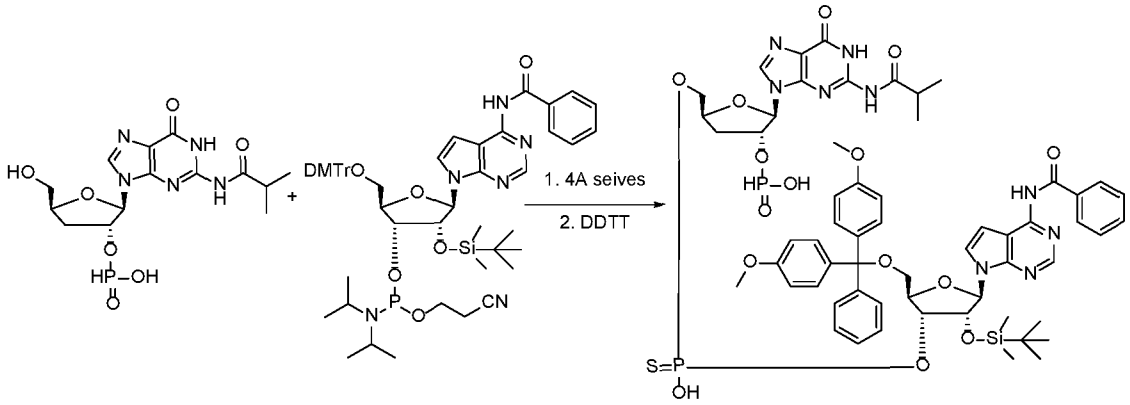
Step 1: (2R,3R)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate



To a flask was added (2R,3R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (4.00g, 4.76mmol), MeCN (23.65ml), and water (0.158ml). Pyridine trifluoroacetate (1.104g, 5.71mmol) was added, and the reaction was stirred at rt for 1h. Tert-butylamine (20.02ml, 190mmol) was then added, and stirring was continued at rt for 1h, after which time the reaction was partitioned between hexanes and acetonitrile. The acetonitrile layer was collected and concentrated under vacuum. DCM (39.9ml) and water (0.798ml) were added, followed by dichloroacetic acid (55.1ml, 33.3mmol), and the solution was stirred for 20min at rt, after which time triethylsilane (133ml, 833mmol)was added, and the reaction was stirred for a further 2h at rt. The reaction was cooled to 0°C, and pyridine (5.39mL 66.6mmol)

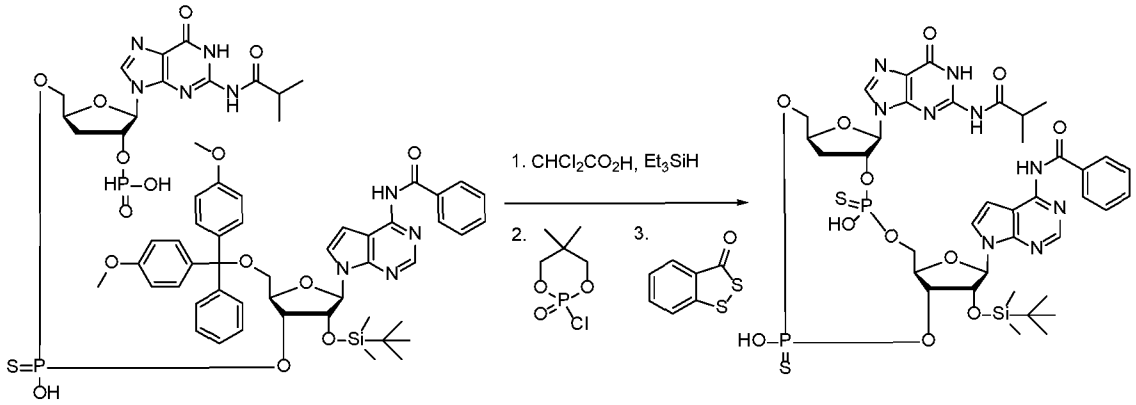
was added. Then the mixture was concentrated under reduced pressure to give the title compound, which was not purified further. LCMS (ES, m/z): 400 [M - H]⁻.

Step 2: O-((2R,3R,4R,5R)-5-(4-benzamido-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-3-yl) O-(((2S,4R,5R)-4-((hydroxyhydrophosphoryl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl) O-hydrogen phosphorothioate



To a flask was added (2R,3R,5S)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate (335mg, 0.836mmol) and MeCN (20mL), and then the solution was concentrated under reduced pressure. This process was repeated 2x, and then MeCN (8mL) was added, followed by activated 4Å sieves. The mixture was stirred for 20min at rt. (2R,3R,4R,5R)-5-(4-benzamido-7H-pyrrolo[2,3-d] pyrimidin-7-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl) oxy)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (825mg, 0.836mmol) was dissolved in MeCN (5mL). Molecular sieves (4Å) were added , and the mixture was stirred for 30min at rt, after which time this solution was transferred to the hydrogen phosphonate solution and 2x1.5mL portions of MeCN were used to complete the transfer. After stirring 30min at rt, ((dimethylamino-methylidine)amino)-3H-1,2,4-dithiazoline-3-thione (189mg, 0.919mmol) was added. After stirring 5min at rt, the mixture was concentrated under reduced pressure and purified using reverse phase HPLC with a 10-100% gradient of MeCN and 100mM aqueous triethylammonium acetate. The product-containing fractions were collected and lyophilized, during which time the cyanoethyl protecting group was cleaved, to furnish O-((2R,3R,4R,5R)-5-(4-benzamido-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((bis(4-methoxyphenyl) (phenyl)methoxy) methyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-3-yl) O-(((2S,4R,5R)-4-((hydroxyhydrophosphoryl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl) O-hydrogen phosphorothioate. LCMS (ES, m/z): 1264 [M - H]⁻.

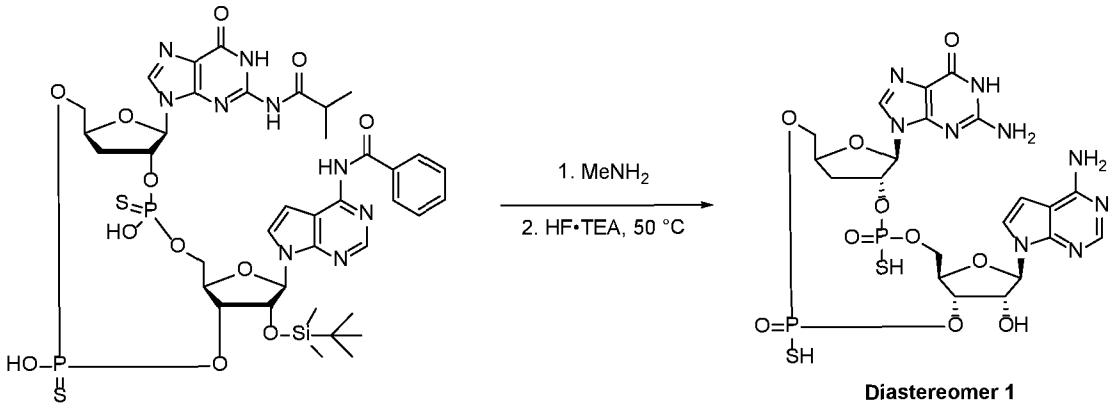
Step 3: N-{7-[(5S,7R,8R,12aR,14R,15R,15aR)-15-{[tert-butyl(dimethyl)silyl]oxy}-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl}benzamide (Diastereomers 1 – 4)



To a flask containing O-((2R,3R,4R,5R)-5-(4-benzamido-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy) tetrahydrofuran-3-yl) O-(((2S,4R,5R)-4-((hydroxyhydrophosphoryl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl) O-hydrogen phosphorothioate (581mg, 0.440mmol) was added DCM (8.81mL), water (0.079mL, 4.40mmol) and then dichloroacetic acid (8.74mL, 5.28mmol). The mixture was stirred for 15min at rt, and then triethylsilane (10.97mL, 68.7mmol) was added. The mixture was stirred at rt for 1.5h and then concentrated under reduced pressure. The mixture was dissolved in pyridine (10mL) and then concentrated under reduced pressure. This process was repeated 2x, and then the resulting sample was dissolved in pyridine (17ml) and 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinan-2-one (81mg, 0.440mmol) was added in 1 portion at rt. After stirring for 30min at rt, additional 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinan-2-one (81mg, 0.440mmol) was added. This sequence was repeated twice, and then water (238µl, 13.19mmol) and 3H-1,2-benzodithiol-3-one (111mg, 0.660mmol) were added. The mixture was stirred for 1h at rt and then partitioned between water (10mL) and 1:1 EtOAc/ether (10mL). The layers were separated, and the aqueous phase was extracted with 1:1 EtOAc/ether (3x10mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Reverse phase HPLC purification (gradient of 30-100% MeCN and 100mM aqueous triethylammonium acetate) furnished 4 diastereomers of N-{7-[(5S,7R,8R,12aR,14R,15R,15aR)-15-{[tert-butyl(dimethyl) silyl]oxy}-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-2,10-dioxido-

2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-14-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl}benzamide, all of which showed LCMS (ES, m/z): 976 [M - H]$^-$.

Step 4: 2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomers 1 – 4)



Diastereomer 1

To a flask containing N-{7-[(5S,7R,8R,12aR,14R,15R,15aR)-15-{[tert-butyl(dimethyl)silyl]oxy}-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-14-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl}benzamide (fastest eluting peak, 7.4mg, 7.57µmol) was added methylamine (33% in EtOH) (1mL, 8.03mmol), and the mixture was stirred at rt for 4h, after which time the mixture was concentrated under reduced pressure. Pyridine (1mL) was added, and the mixture was concentrated under reduced pressure. Then, pyridine (0.5ml), triethylamine (0.104ml, 0.746mmol) and triethylamine trihydrofluoride (0.030ml, 0.187mmol) were added, and the mixture was stirred at 50°C for 16h, after which time the mixture was cooled to rt and concentrated under reduced pressure. Purification by reverse phase HPLC (gradient of acetonitrile and 100mM aqueous triethylammonium acetate) furnished Example 77, 2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1). LCMS (ES, m/z): 688 [M - H]$^-$. ^{1}H NMR (600MHz, Deuterium Oxide) δ 8.06 (s, 1H), 8.03 (s, 1H), 7.41 (d, J = 3.8Hz, 1H), 6.25 (d, J = 3.7Hz, 1H), 6.17 (d, J = 2.6Hz, 1H), 5.68 (d, J = 7.5Hz, 1H), 5.42 – 5.36 (m, 2H), 5.10-5.06 (m, 1H), 4.83 – 4.81 (m, 1H), 4.51 – 4.48

(m, 1H), 4.36 – 4.33 (m, 1H), 4.28 (dt, *J* = 10.1, 4.9Hz, 1H), 4.06 – 3.94 (m, 2H), 3.03 (q, *J* = 7.3Hz, 12H), 2.44 – 2.40 (m, 2H), 1.11 (t, *J* = 7.3Hz, 18H).

The other diastereomers from Step 3 were individually processed in an analogous manner to afford three additional diastereomeric products:

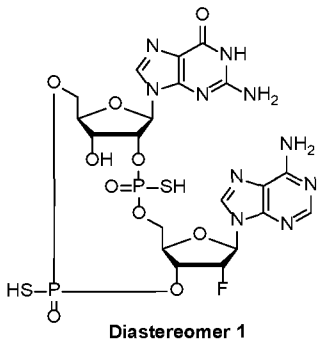
Example 78: 2-Amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2). LCMS (ES, m/z): 688 [M - H]$^-$. ^{1}H NMR (600MHz, Deuterium Oxide) δ 8.04 (s, 1H), 7.75 (s, 1H), 7.50 (d, *J* = 3.6Hz, 1H), 6.19 – 6.17 (m, 1H), 6.16 (d, *J* = 3.7Hz, 1H), 5.65 (d, *J* = 7.4Hz, 1H), 5.63 – 5.57 (m, 1H), 5.14 (td, *J* = 7.8, 4.4Hz, 1H), 4.54 (d, *J* = 4.2Hz, 1H), 4.52 – 4.46 (m, 1H), 4.33 (d, *J* = 8.9Hz, 1H), 4.27 (dd, *J* = 11.9, 3.1Hz, 1H), 4.18 – 4.15 (m, 1H), 3.96 (dd, *J* = 11.4, 3.6Hz, 2H), 3.04 (q, *J* = 7.3Hz, 12H), 2.44 – 2.36 (m, 2H), 1.11 (t, *J* = 7.3Hz, 18H).

Example 79: 2-Amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3). LCMS (ES, m/z): 688 [M - H]$^-$. ^{1}H NMR (600MHz, Deuterium Oxide) δ 8.00 (s, 1H), 7.97 (s, 1H), 7.19 (d, *J* = 3.8Hz, 1H), 6.15 (d, *J* = 3.8Hz, 2H), 5.66 (d, *J* = 7.3Hz, 1H), 5.32 (dq, *J* = 9.9, 7.2Hz, 1H), 4.95 (td, *J* = 8.6, 4.6Hz, 1H), 4.81 (d, *J* = 4.4Hz, 1H), 4.50 (d, *J* = 8.0Hz, 1H), 4.32 (d, *J* = 7.8Hz, 1H), 4.25 (dt, *J* = 12.0, 3.6Hz, 1H), 4.05 – 3.97 (m, 3H), 3.03 (q, *J* = 7.3Hz, 12H), 2.51 – 2.41 (m, 2H), 1.11 (t, *J* = 7.3Hz, 18H).

Example 80: 2-Amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4). LCMS (ES, m/z): 688 [M - H]$^-$. ^{1}H NMR (600MHz, Deuterium Oxide) δ 8.00 (s, 1H), 7.72 (s, 1H), 7.23 (d, *J* = 3.8Hz, 1H), 6.14 (s, 1H), 6.12 (d, *J* = 3.7Hz, 1H), 5.64 (d, *J* = 6.9Hz, 1H), 5.47 (dq, *J* = 14.2, 7.0Hz, 1H), 5.05 (td, *J* = 8.0, 4.5Hz, 1H), 4.52 (d, *J* = 4.4Hz, 1H), 4.49 (dt, *J* = 7.4, 2.7Hz, 1H), 4.33 – 4.27 (m, 2H), 4.19 (ddd, *J* = 11.5, 8.5, 3.0Hz, 1H), 4.00 (dd, *J* = 11.5, 3.9Hz, 1H), 3.94 (ddd, *J* = 11.6, 5.6, 2.2Hz, 1H), 3.01 (q, *J* = 7.3Hz, 12H), 2.49-2.40 (m, 2H), 1.10 (t, *J* = 7.3Hz, 18H).

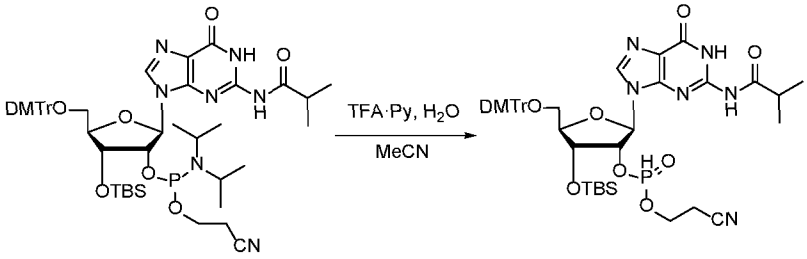
Example 81: 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-

l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)



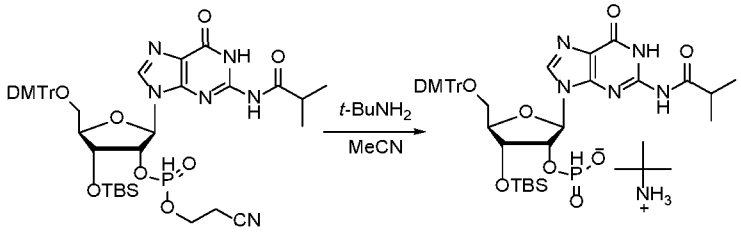
Diastereomer 1

*Step 1: (2R,3R,4R,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate*



To a stirred solution of (2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl) methoxy)methyl)-4-((*tert*-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (3g, 3.09mmol) in MeCN (15mL) at 25°C was added H_2O (0.111mL, 6.18mmol) and pyridin-1-ium 2,2,2-trifluoroacetate (0.717g, 3.71mmol). The resulting mixture was stirred at 25°C for 20min. The reaction progress was monitored by LCMS/TLC. After the phosphoramidite starting material was consumed, the reaction mixture that containing the desired product (major) was used for the next step without any after-treatment. LCMS (ES, *m/z*): 887.4 $[M + H]^+$.

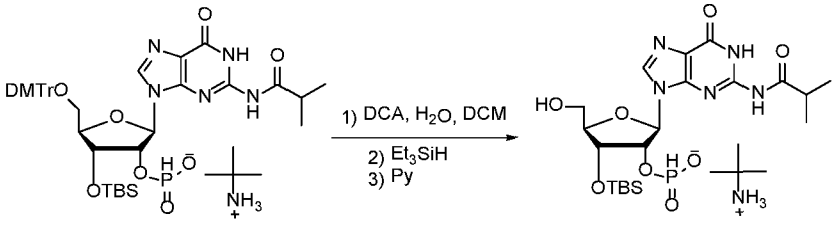
*Step 2: (2R,3R,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((*tert*-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate*



To a stirred solution of the product of Step 1 (15.0mL, 142mmol) from the previous reaction was added *tert*-butylamine in one portion, and it was stirred at 25°C for 40min. The

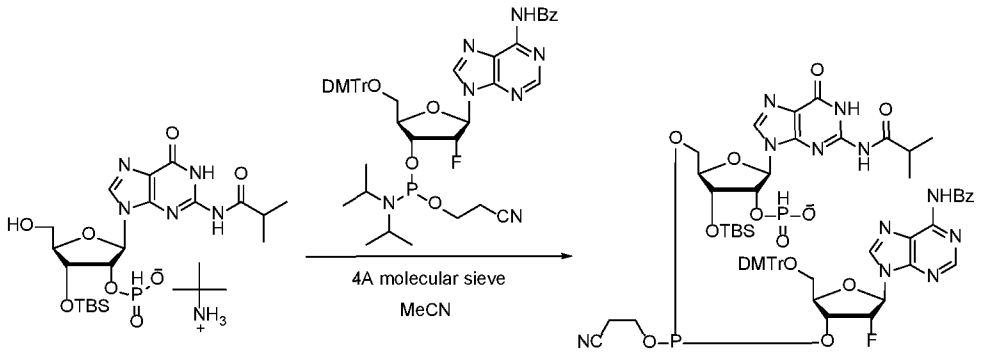
resulting solution was concentrated *in vacuo*. The residue was co-evaporated with dry MeCN (two times, 15mL each), used for the next step without purification. LCMS (ES, *m/z*): 832.3 [M - H]$^-$.

*Step 3: (2R,3R,4R,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate*



To a stirred solution of the product of Step 2 in CH_2Cl_2 (37mL) was added H_2O (0.558mL, 31.0mmol) and 6% DCA in CH_2Cl_2 (37mL, 31.5mmol) dropwise. The resulting mixture was stirred at 25°C for 40min, then Et_3SiH (60mL) was added, and the reaction mixture was stirred for 1.5h. Pyridine (4.5mL, 2eq to DCA) was added to the reaction. The resulting solution was stirred at 25°C for 5min and then concentrated *in vacuo*. The residue was triturated with MTBE/hexane (100mL, v/v, 1/1), and the supernatant was decanted. This process was repeated two more times. The final residue was concentrated at reduced pressure and was used for the next step without purification. LCMS (ES, *m/z*): 532.18 [M + H]$^+$.

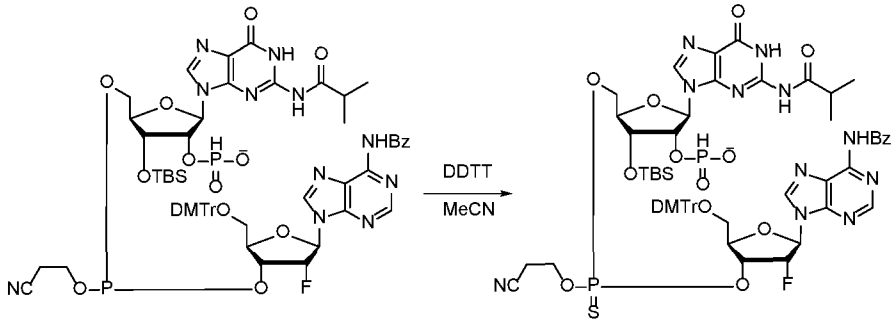
*Step 4: (2R,3R,4R,5R)-5-((((((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphanyl)oxy)methyl)-4-((*tert*-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate*



To a stirred solution of the product of Step 3 (0.704g, 0.722mmol) in MeCN (5mL) under Ar was added activated 4Å molecular sieve (200mg), and the mixture was stirred at RT over 30min. (2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl) (phenyl) methoxy)methyl)-4-fluorotetrahydrofuran-3-yl (2-cyanoethyl) diisopropyl-phosphoramidite (0.822g, 0.939mmol) was twice co-evaporated with dry MeCN (3mL). Activated 4Å molecular

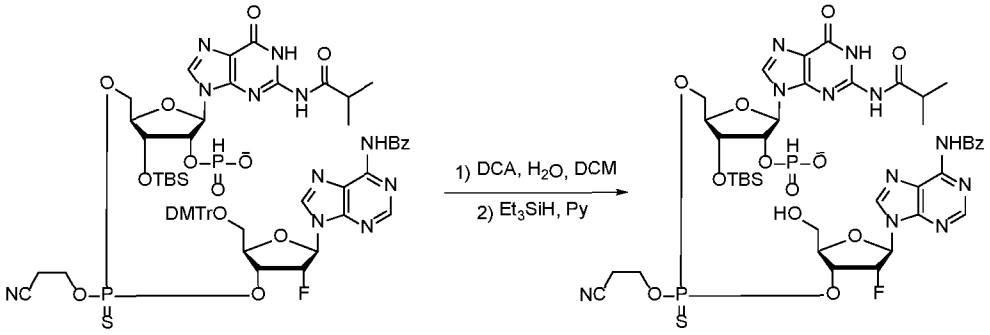
sieve (200mg) was added. After 30min, the phosphoramidite solution was transferred into the solution of the product of Step 3 by syringe. The resulting mixture was stirred at RT for 20min. The desired product was detected by TLC/LCMS, and the reaction solution was used for the next reaction without purification. LCMS (ES, *m/z*): 1306.7 $[M + H]^+$.

Step 5: (2R,3R,4R,5R)-5-((((((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



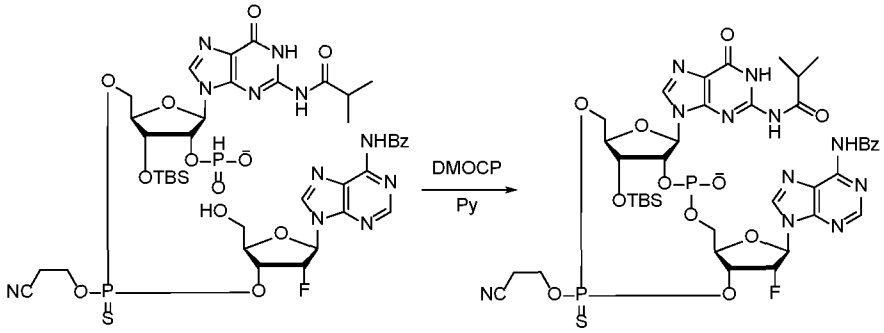
To the reaction mixture containing the product of Step 4 (~0.722mmol) under Ar was added (E)-*N,N*-dimethyl-*N'*-(3-thioxo-3H-1,2,4-dithiazol-5-yl)formimidamide (163mg, 0.794mmol) in one portion, and the mixture was stirred at RT for 30min. The reaction progress was monitored by TLC/LCMS. After the consumption of the starting phosphite, the reaction mixture was concentrated *in vacuo*, and the residue was purified by reverse phase chromatography (X-Bridge BEH130 Prep C18) eluting with 5 to 95% MeCN in H_2O (0.04% NH_4HCO_3). The product-containing fractions were combined and concentrated under reduced pressure to 2/3 volume. NaCl (10g) was added, and the aqueous mixture was extracted with EtOAc/Et_2O (v/v, 1/1, 3x80mL). The combined organic layers were dried (Na_2SO_4) and concentrated. LCMS (ES, *m/z*): 1339.5 $[M + H]^+$. ^{31}P-NMR (162MHz, CD_3OD): δ 67.83 (d, J = 43.9Hz), 2.81 (d, J = 15.7Hz).

Step 6: (2R,3R,4R,5R)-5-((((((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



To a stirred solution of the product of Step 5 (180mg, 0.128mmol) in CH_2Cl_2 (7mL) was added 2,2-dichloroacetic acid in CH_2Cl_2 (2.47mg, 1.15mmol) and H_2O (22.97mg, 1.28mmol). After stirring at RT for 20min, Et_3SiH (4.5mL) was added. After 2h, pyridine (1mL) was added, and the mixture was stirred for 10min. After removal of volatiles, the product was used for the next reaction step without purification. LCMS (ES, *m/z*): 1036.4 $[M + H]^+$.

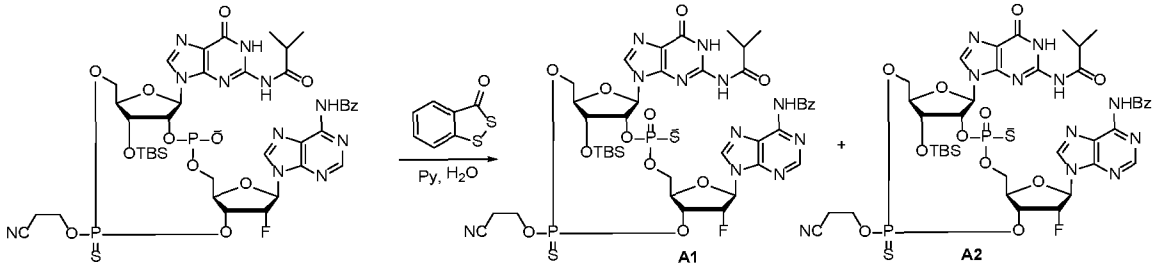
*Step 7: (5R,7R,8R,12aR,14R,15R,15aR,16R)-16-{[*tert*-butyl(dimethyl)silyl]oxy}-2-(2-cyanoethoxy)-15-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-10-olate 2-sulfide*



The product of Step 6 (570mg) was co-evaporated with dry pyridine (1mL each, three times). To the mixture in dry pyridine (4mL) at RT under Ar was added 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide (71mg, 0.384mmol) in one portion. The resulting mixture was stirred for 40min. The reaction progress was monitored by TLC/LCMS. The desired product as a mixture of diastereomers was observed, and the product was used for the next reaction step directly. LCMS (ES, *m/z*): 1018.5 $[M + H]^+$.

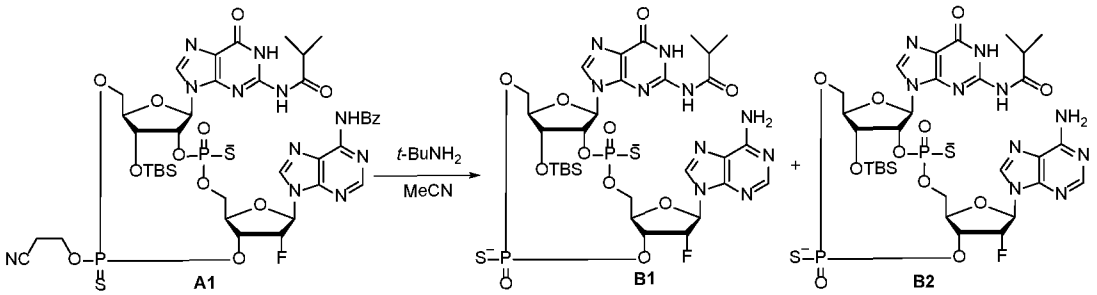
*Step 8: Diastereomeric mixtures (5R,7R,8R,12aR,14R,15R,15aR,16R)-16-{[*tert*-butyl(dimethyl)silyl]oxy}-2-(2-cyanoethoxy)-15-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-10-thiolate 10-oxide 2-sulfide (**A1**) and (5R,7R,8R,12aR,14R,15R,15aR,16R)-16-{[*tert*-butyl(dimethyl)silyl]oxy}-2-(2-*

*cyanoethoxy)-15-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-10-thiolate 10-oxide 2-sulfide (**A2**)*



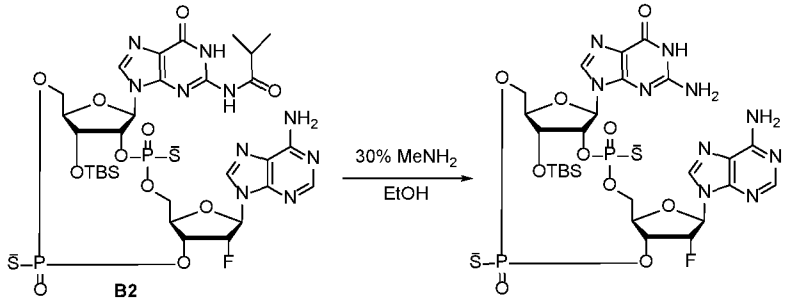
To the stirred mixture of the product of Step 7 was added H_2O (69.2mg, 3.84mmol) and 3H-benzo[c][1,2]dithiol-3-one (32.3mg, 0.192mmol). The mixture was stirred at RT for 40min. The reaction progress was monitored by TLC/LCMS. After the reaction completed, the reaction mixture was poured into aq $NaHCO_3$ (0.14g $NaHCO_3$ in 5mL H_2O) and stirred for 5min. The resulting mixture was extracted with EtOAc/ether (v/v, 1/1, 3x15mL). The combined organic layers were dried (Na_2SO_4), and purified by silica gel chromatography eluted with 0 to 15% MeOH in CH_2Cl_2 to give products: mixture of diastereomers **A1** (eluted out at 5.5% MeOH in CH_2Cl_2); mixture of diastereomers **A2** (eluted out at 9.8% MeOH in CH_2Cl_2); (2R,3R,4R,5R)-5-((((((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl) oxy)methyl)-4-((*tert*-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (eluted out at 12.6% MeOH in CH_2Cl_2). Mixture **A1**: LCMS (ES, *m/z*): 1050.30 $[M + H]^+$. ^{31}P-NMR (162MHz, CD_3OD): δ 66.34 (s), 64.63 (s). Mixture **A2**: LCMS (ES, *m/z*): 1050.30 $[M + H]^+$. ^{31}P-NMR (162MHz, CD_3OD): δ 65.94, 64.17, 62.55, 61.28.

*Step 9: Diastereomers (5R,7R,8R,12aR,14R,15R,15aR,16R)-16-{[*tert*-butyl(dimethyl)silyl]oxy}-15-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-bis(thiolate) 2,10-dioxide (**B1**) and (5R,7R,8R,12aR,14R,15R,15aR,16R)-16-{[*tert*-butyl(dimethyl)silyl]oxy}-15-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-bis(thiolate) 2,10-dioxide (**B2**)*



To a stirred suspension of crude **A1** (95mg, ~0.09mmol) from the previous step in MeCN (1mL) at RT under Ar was added *tert*-butylamine (1.5mL). After 30min, volatile components were removed *in vacuo*. The residue was purified by reverse phase prep-HPLC (X-Bridge BEH130 Prep C18) eluted with 25 to 45% MeCN in aq NH_4HCO_3 (10mM) over 8min to give compound **B2** as a single diastereomer (T_R = 5.97min). LCMS (ES, *m/z*): 891.4 $[M - H]^-$. H-NMR (300MHz, CD_3OD): δ 8.44 (s, 1H), 8.22 (s, 1H), 8.09 (s, 1H), 6.37 (d, *J* = 14.0Hz, 1H), 5.95 (d, *J* = 8.0Hz, 1H), 5.65-5.54 (m, 1H), 5.26-5.10 (m, 2H), 4.57-4.41 (m, 4H), 4.24 (s, 1H), 4.01 (d, *J* = 11.5Hz, 1H), 3.89 (d, *J* = 11.8Hz, 1H), 2.75-2.63 (m, 1H), 1.04-0.91 (m, 15H), 0.28-0.24 (m, 6H). ^{31}P-NMR (121MHz, CD_3OD): δ 57.10 (s), 53.1 (s).

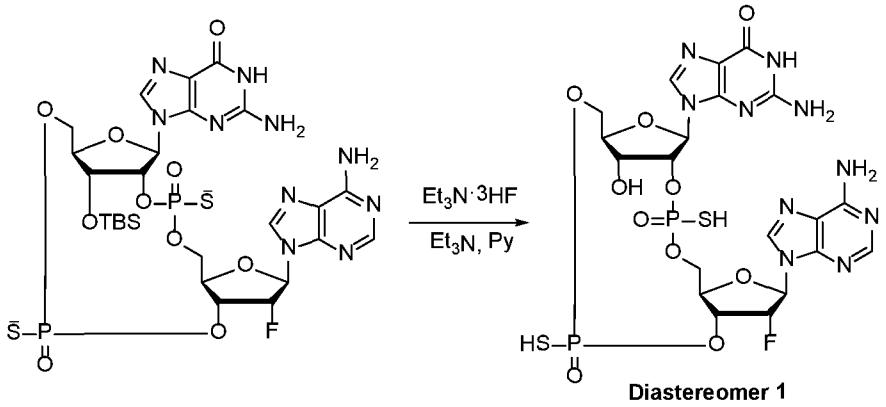
*Step 10: (5R,7R,8R,12aR,14R,15R,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-16-{[*tert*-butyl(dimethyl)silyl]oxy}-15-fluorooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-bis(thiolate) 2,10-dioxide*



To a stirred solution of compound **B2** (13mg, 0.013mmol) from the previous step was added a solution of $MeNH_2$ in EtOH (0.6mL, 30% by weight). The mixture was stirred at RT for 12h. The volatile components were removed under reduced pressure, and the residue containing product compound was used for the next reaction step without purification. LCMS (ES, *m/z*): 823.15 $[M + H]^+$.

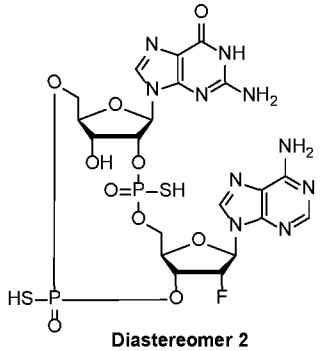
Step 11: 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-

l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)



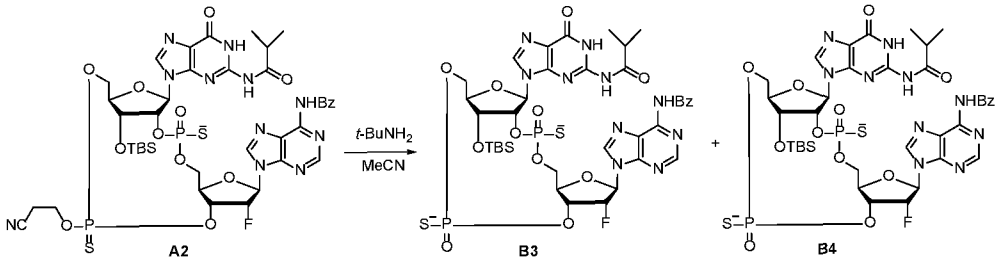
The crude product from Step 10 (25mg) was co-evaporated with pyridine/Et_3N (v/v, 3/1, 1mL each, three times) and then dissolved in pyridine (0.15mL). The mixture was charged with Ar and Et_3N (0.20mL) and triethylamine trihydrofluoride (56.4mg, 0.350mmol) were added. The resulting solution was warmed at 50°C for 6h. The reaction progress was monitored by TLC/LCMS. After completion of the reaction, the mixture was concentrated *in vacuo* and then, co-evaporated with MeCN (three times, 1mL each). The residue was purified by reverse phase prep-HPLC (X-Bridge BEH130 Prep C18) eluted with 0 to 22% MeCN in aq NH_4HCO_3 (50mM) over 15min to give the product compound (T_R = 8.3min). LCMS (ES, *m/z*): 708.95 $[M+H]^+$. ^{1}H-NMR (400MHz, D_2O): δ 8.18 (s, 1H), 8.16 (s, 1H), 7.77 (s, 1H), 6.37 (d, *J* = 14.3Hz, 1H), 5.86 (d, *J* = 8.4Hz, 1H), 5.61-5.54 (m, 1.5H), 5.43 (s, 0.5H), 5.27-5.12 (m, 2H), 4.59 (d, J = 3.6Hz, 1H), 4.47 (t, *J* = 12.9Hz, 2H), 4.36 (d, *J* = 4.8Hz, 1H), 4.04 (dd, *J* = 23.2, 12.0Hz, 2H). ^{31}P-NMR (162MHz, D_2O): δ 55.63 (s), 51.55 (s).

Example 82: 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)



Diastereomer 2

*Step 1: Diastereomers (5R,7R,8R,12aR,14R,15R,15aR,16R)-16-{[*tert*-butyl(dimethyl)silyl]oxy}-15-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-bis(thiolate) 2,10-dioxide (**B3**) and (5R,7R,8R,12aR,14R,15R,15aR,16R)-16-{[*tert*-butyl(dimethyl)silyl]oxy}-15-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-bis(thiolate) 2,10-dioxide (**B4**)*



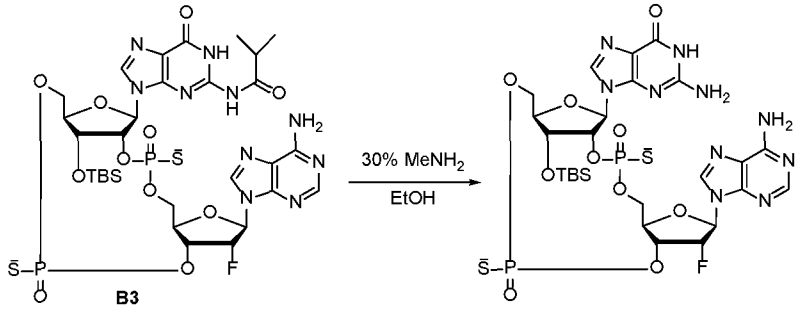
A2 B3 B4

To a stirred suspension of crude **A2** (105mg, ~0.1mmol) from Example 81, Step 8 in MeCN (1mL) under Ar was added *tert*-butylamine (1.5mL), and the mixture was stirred at RT for 30min. The volatile components were removed *in vacuo*. The residue was purified by reverse phase prep-HPLC (X-Bridge BEH130 Prep C18) eluted with 25 to 40% MeCN in aq NH_4HCO_3 (10mM) over 10min to give two diastereomeric compounds, **B3** (T_R = 6.12min, 0.025mmol) and **B4** (T_R = 7.45min, 0.021mmol).

Compound **B3**: LCMS (ES, *m/z*): 995.3 $[M - H]^-$. H-NMR (300MHz, CD_3OD): δ 8.82 (s, 1H), 8.72 (s, 1H), 8.65 (s, 1H), 8.20-8.13 (m, 2H), 7.66-7.54 (m, 3H), 6.47 (d, *J* = 14.0Hz, 1H), 6.09 (d, *J* = 8.4Hz, 1H), 5.96-5.95 (m, 0.5H), 5.81-5.78 (m, 0.5H), 5.52-5.36 (m, 2H), 4.64-4.56 (m, 2H), 4.48-4.43 (m, 1H), 4.37-4.30 (m, 1H), 4.25-4.22 (m, 1H), 4.17-4.10 (m, 1H), 3.98 (d, *J* = 11.7Hz, 1H), 2.65 (p, *J* = 6.8Hz, 1H), 1.12 (d, *J* = 6.8Hz, 3H), 0.98-0.95 (m, 12H), 0.22 (d, *J* = 8.0Hz, 6H). ^{31}P-NMR (121MHz, CD_3OD): δ 56.96 (s), 55.90 (s).

Compound **B4**: LCMS (ES, *m/z*): 995.4 $[M - H]^-$. H-NMR (300MHz, CD_3OD): δ 8.97 (s, 1H), 8.68 (s, 1H), 8.24-8.22 (m, 3H), 7.59 (ddd, *J* = 14.5, 7.9, 6.2Hz, 3H), 6.46 (d, *J* = 13.0Hz, 1H), 5.99 (d, *J* = 8.3Hz, 1H), 5.67-5.57 (m, 1H), 5.45-5.33 (m, 2H), 4.56 (dd, *J* = 13.5, 4.9Hz, 2H), 4.47-4.38 (m, 2H), 4.25 (t, *J* = 3.5Hz, 1H), 4.07 (d, *J* = 11.3Hz, 1H), 3.94 (d, *J* = 11.0Hz, 1H), 2.75 (p, *J* = 6.8Hz, 1H), 1.04 (d, *J* = 6.8Hz, 3H), 0.95 (s, 12H), 0.23 (d, *J* = 5.2Hz, 6H). ^{31}P-NMR (121MHz, CD_3OD): δ 56.81 (s), 54.76 (s).

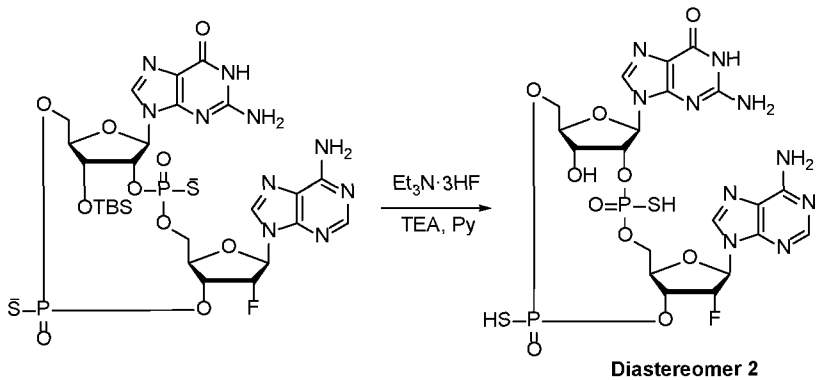
*Step 2: (5R,7R,8R,12aR,14R,15R,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-16-{[*tert*-butyl(dimethyl)silyl]oxy}-15-fluorooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-bis(thiolate) 2,10-dioxide*



B3

Compound **B3** from Step 1 (25mg, 0.025mmol) was dissolved in a solution of $MeNH_2$ in EtOH (1mL, 30% by weight), and the mixture was stirred at RT for 12h. The reaction progress was monitored by TLC/LCMS. After the reaction was complete, the volatile components were removed *in vacuo*, and the residue containing the crude product was used for the next reaction step without purification. LCMS (ES, *m/z*): 823.25 $[M + H]^+$.

Step 3: 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)

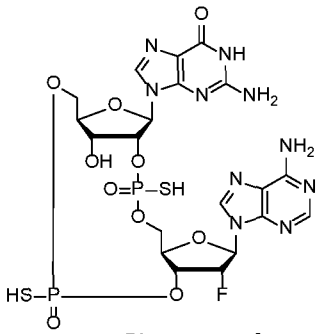


Diastereomer 2

The crude product of Step 2 (35mg) was co-evaporated with pyridine/Et_3N (v/v, 3/1, 1mL each, three times) and then re-dissolved in pyridine (0.4mL). The mixture was charged with Ar

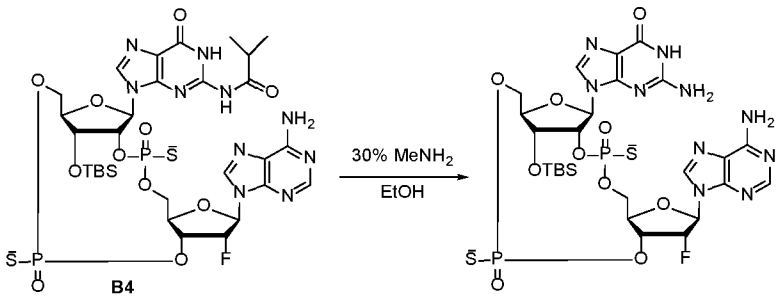
and Et_3N (0.34mL, 2.4mmol), and triethylamine trihydrofluoride (97mg, 0.6mmol) were added. The resulting solution was warmed at 50°C for 6h. Then, the mixture was concentrated at reduced pressure and then co-evaporated with MeCN (3x1mL). The residue was purified by reverse phase prep-HPLC (X-Bridge BEH130 Prep C18) eluted with 0 to 10% MeCN in aq NH_4HCO_3 (50mM) over 14min to give the product compound (T_R = 9.2min). LCMS (ES, *m/z*): 709.00 $[M + H]^+$. H-NMR (400MHz, DMF-d_7 + D_2O): δ 8.68 (s, 1H), 8.61 (s, 1H), 8.45 (s, 1H), 6.57 (d, *J* = 14.8Hz, 1H), 6.27-6.25 (m, 1.5H), 6.15-6.13 (m, 0.5H), 5.72-5.68 (m, 1H), 5.56-5.54 (m, 1H), 4.85-4.83 (m, 1H), 4.71-4.69 (m, 1H), 4.52-4.43 (m, 4H), 4.27-4.24 (m, 1H). ^{31}P-NMR (162MHz, DMF-d_7 + D_2O): δ 56.03 (s), 53.37 (s). ^{19}F-NMR (376MHz, DMF-d_7 + D_2O): δ -205.44 (s).

Example 83: 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)



Diastereomer 3

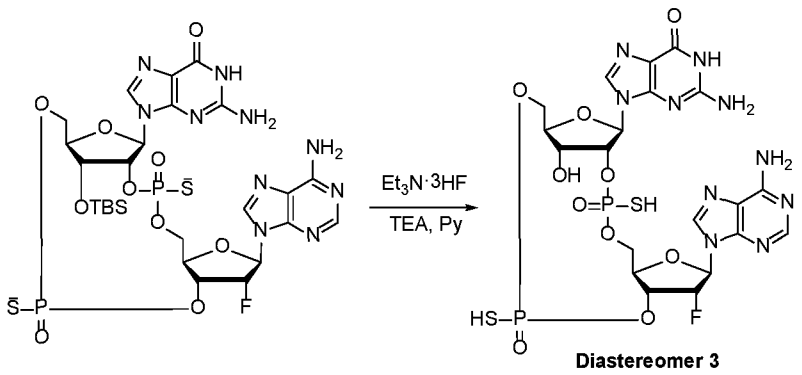
Step 1: (5R,7R,8R,12aR,14R,15R,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-16-{[tert-butyl(dimethyl)silyl]oxy}-15-fluorooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-bis(thiolate) 2,10-dioxide



B4

Compound **B4** ((5R,7R,8R,12aR,14R,15R,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-16-{[*tert*-butyl(dimethyl)silyl]oxy}-15-fluorooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclo-tetradecine-2,10-bis(thiolate) 2,10-dioxide, 21mg, 0.021mmol) from Example 82, Step 1 was dissolved in a solution of $MeNH_2$ in EtOH (1mL, 30% by weight), and the mixture was stirred at RT for 12h. The reaction progress was monitored by TLC/LCMS. After the reaction was complete, the volatile components were removed *in vacuo*, and the product was used for the next reaction step without purification. LCMS (ES, *m/z*): 823.25 $[M + H]^+$.

Step 2: 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)

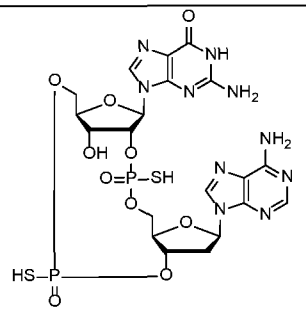
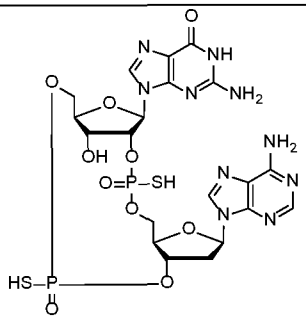
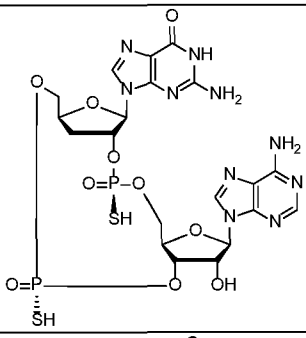
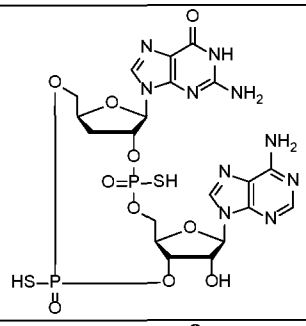
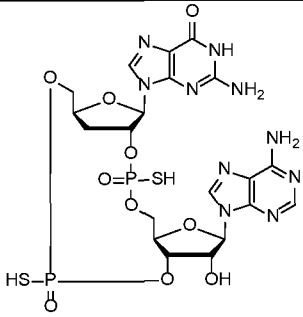


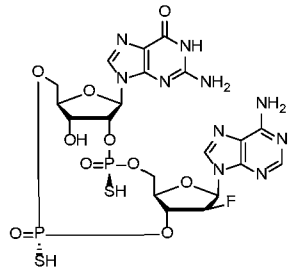
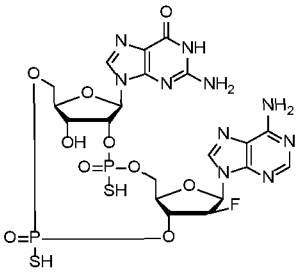
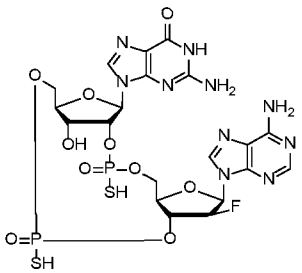
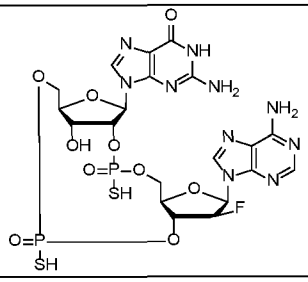
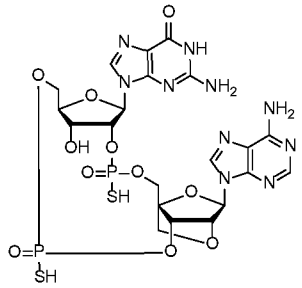
The crude product of Step 1 (31mg) was co-evaporated with pyridine/Et_3N (v/v, 3/1, 3x1mL) and then, re-dissolved in pyridine (0.4mL). The mixture was charged with Ar and Et_3N (0.28mL, 2.0mmol) and triethylamine trihydrofluoride (81mg, 0.5mmol) were added. The resulting solution was warmed at 50°C for 6h. The mixture was concentrated at reduced pressure and then co-evaporated with MeCN (3x1mL). The residue was purified by reverse phase prep-HPLC (X-Bridge BEH130 Prep C18) eluting with 0 to 10% MeCN in aq NH_4HCO_3 (50mM) over 14min to give the product compound (T_R = 10.1min). LCMS (ES, *m/z*): 709.00 $[M + H]^+$. H-NMR (400MHz, DMF-d_7 + D_2O): δ 8.73 (s, 1H), 8.28-8.20 (m, 2H), 6.55 (d, *J* = 14.8Hz, 1H), 6.25-5.85 (m, 3H), 5.62-5.56 (m, 1H), 4.76 (s, 1H), 4.62-4.60 (m, 2H), 4.49-4.41 (m, 3H), 4.18-4.15 (m, 1H). ^{31}P-NMR (162MHz, DMF-d_7 + D_2O): δ 56.09 (s), 54.75 (s). ^{19}F-NMR (376MHz, DMF-d_7 + D_2O): δ -203.33 (s).

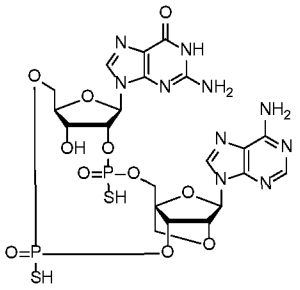
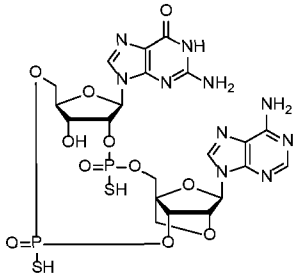
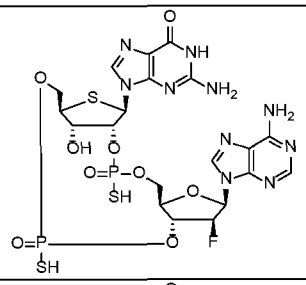
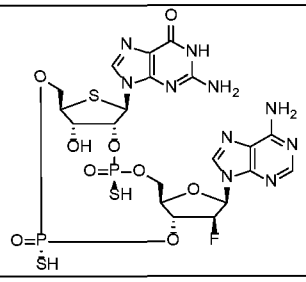
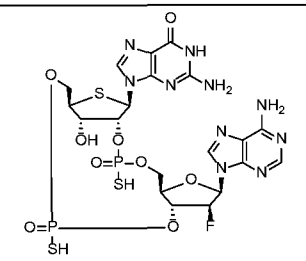
Examples 84 through 116, shown in Table 5 below, were prepared according to procedures analogous to those outlined in Examples 77 through 83 above using the appropriate

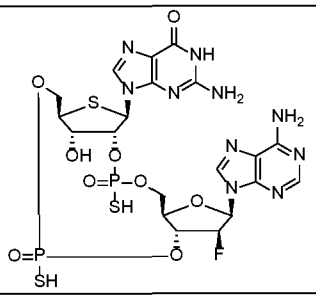
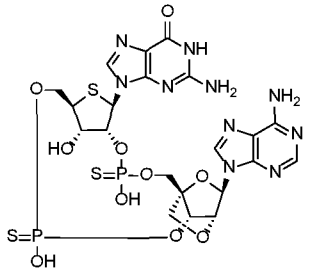
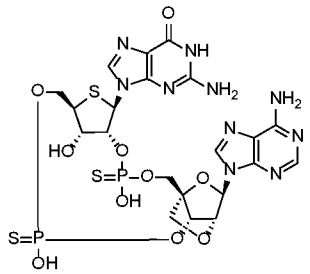
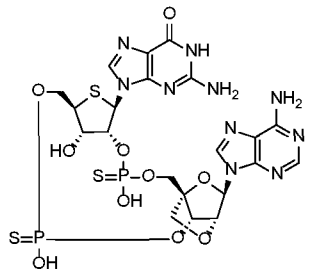
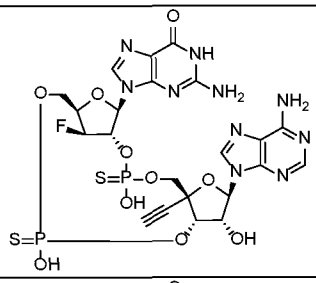
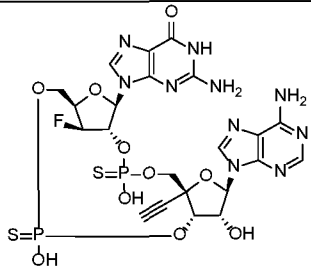
monomers, decribed as Preparations or as obtained from commercial sources, in the coupling step.

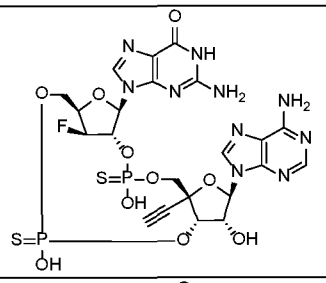
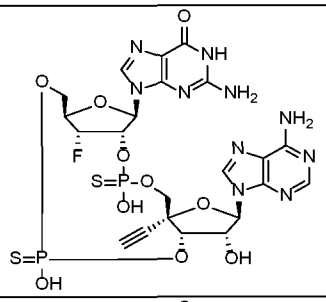
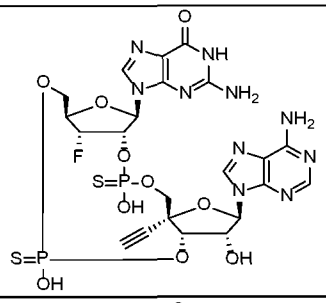
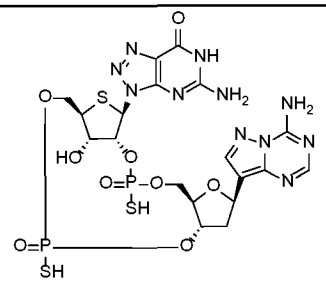
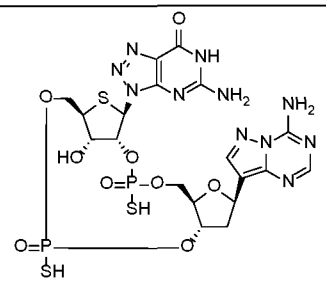
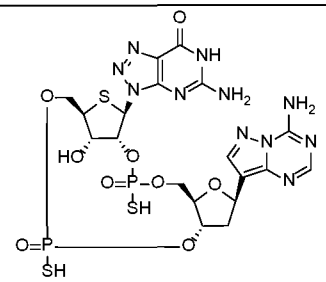
Table 5

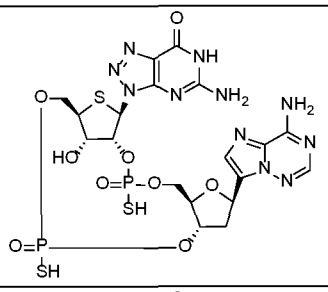
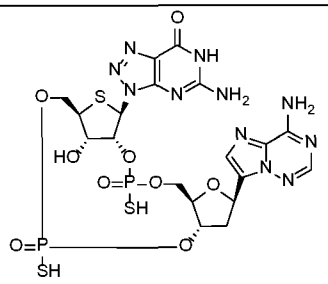
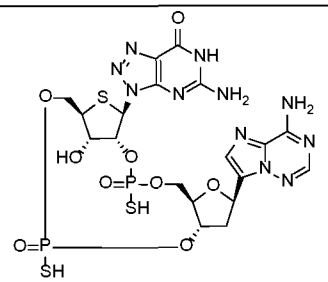
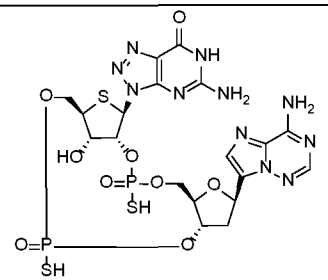
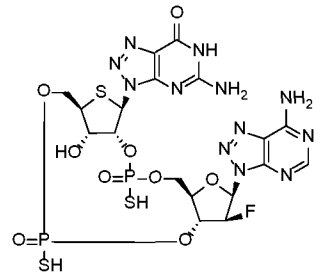
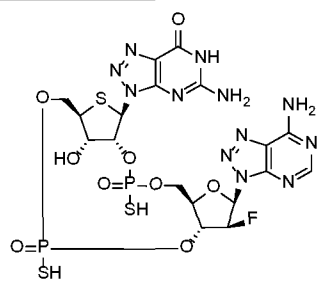
Ex.	Structure	Name	Mass $[M-H]^-$
84		2-amino-9-[(5R,7R,8R,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one(Diastereomer 1)	691 $[M+H]^+$
85		2-amino-9-[(5R,7R,8R,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	691 $[M+H]^+$
86		2-amino-9-[(2R, 5S,7R,8R,10R, 12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	689
87		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	689
88		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10,15-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	689

Ex.	Structure	Name	Mass [M-H]⁻
89		2-amino-9-[(2R, 5R,7R,8R,10,R, 12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one(Diastereomer 1)	707
90		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	707
91		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	707
92		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)	707
93		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro [3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	717

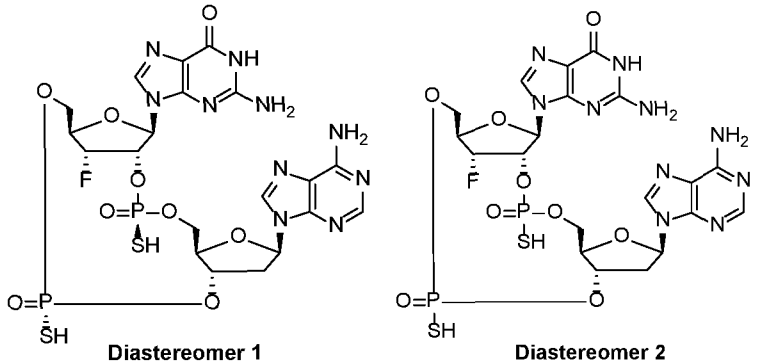
Ex.	Structure	Name	Mass $[M-H]^-$
94		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	717
95		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	719 $[M+H]^+$
96		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15-fluoro-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10] tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	723
97		2-amino-9-[(2R, 5R,7R,8R,10R, 12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15-fluoro-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10] tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	723
98		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15-fluoro-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10] tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	723

Ex.	Structure	Name	Mass [M-H]⁻
99		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15-fluoro-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10] tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)	723
100		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	733
101		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	733
102		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10] tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	733
103		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,16S)-14-(6-amino-9H-purin-9-yl)-12a-ethynyl-16-fluoro-2,10,15-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	731
104		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,16S)-14-(6-amino-9H-purin-9-yl)-12a-ethynyl-16-fluoro-2,10,15-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	731

Ex.	Structure	Name	Mass [M-H]⁻
105		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,16S)-14-(6-amino-9H-purin-9-yl)-12a-ethynyl-16-fluoro-2,10,15-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	731
106		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-12a-ethynyl-16-fluoro-2,10,15-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	731
107		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-12a-ethynyl-16-fluoro-2,10,15-trihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	731
108		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	706
109		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	706
110		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	706

Ex.	Structure	Name	Mass [M-H]⁻
111		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	706
112		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	706
113		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	706
114		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 4)	706
115		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	725
116		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-16-hydroxy-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	725

Examples 117 and 118: 2-amino-9-[(2R,5R,7R,8S,10R,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1) and 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)



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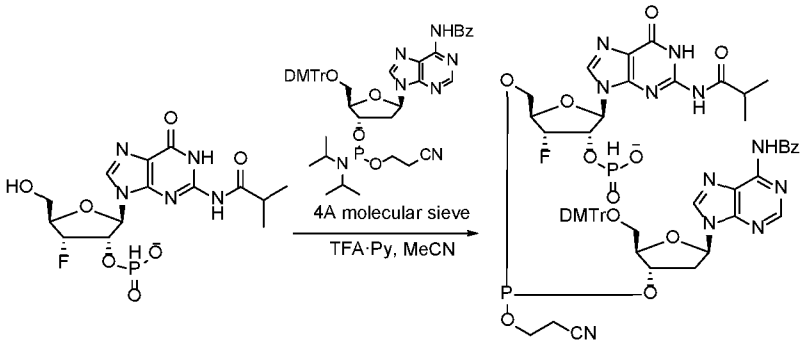
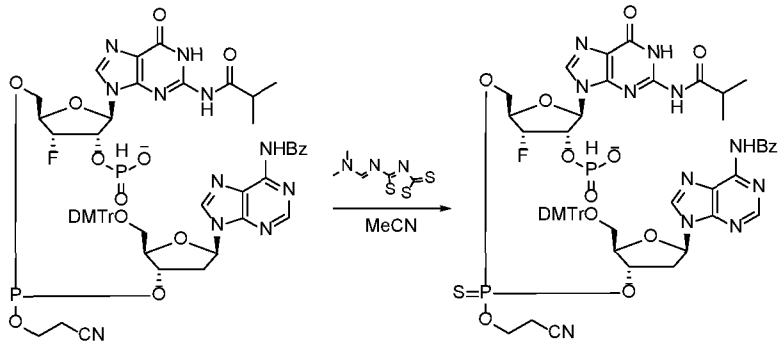
Step 1: 2R,3S,4R,5R)-5-((((((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphanyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (1058mg, 1.234mmol) was co-evaporated with dry ACN (3x5mL), re-dissolved in ACN (10mL) under Ar, and dried by adding activated 4Å molecular sieve (200mg). (2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (430mg, 1.03mmol) and pyridinium 2,2,2-trifluoroacetate (298mg, 1.54mmol) were co-evaporated with ACN (3x5mL) and then re-dissolved in ACN (10mL), and dried by adding activated 4A molecular sieve (200mg). After 30min, it was added to the previously prepared mixture

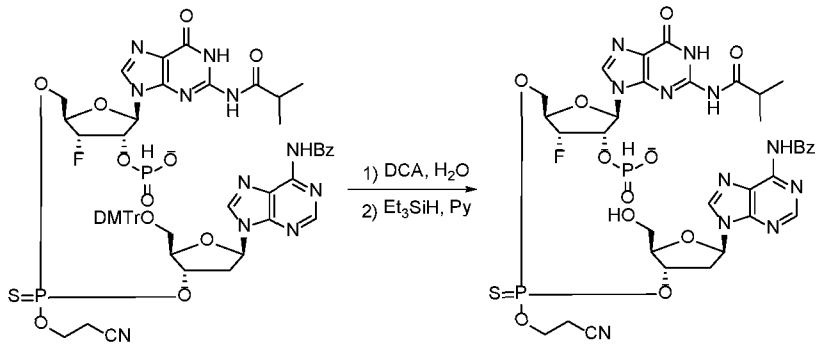
containing ((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl) methoxy)methyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite. It was stirred at rt for 30min. The reaction mixture was used for the next reaction step without purification. LCMS (ES, m/z): 1173.8 [M - H]$^-$.

Step 2: (2R,3S,4R,5R)-5-((((((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



To the reaction mixture from Step 1 was added (*E*)-*N*,*N*-dimethyl-*N*'-(3-thioxo-3*H*-1,2,4-dithiazol-5-yl)formimidamide (DDTT, 0.232g, 1.13mmol) in one portion. The mixture was stirred at rt for 1h. It was concentrated to give a crude sample containing the product, which was used for the next reaction step without purification. LCMS (ES, m/z): 1205.8 [M - H]$^-$.

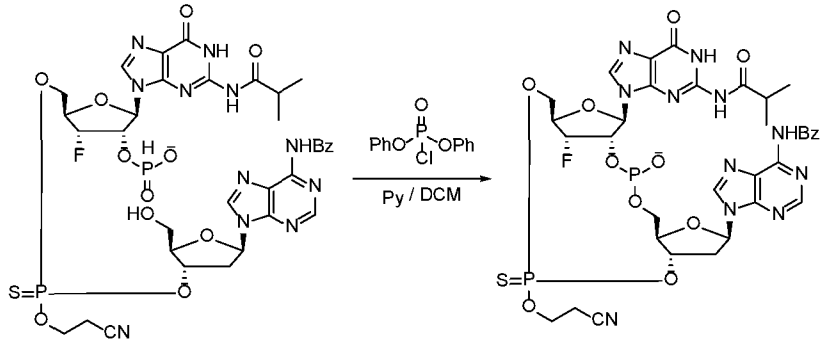
Step 3: (2R,3S,4R,5R)-5-((((((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



To a solution of the crude from Step 2 in CH_2Cl_2 (15mL) was added water (0.2mL, 10mmol) and 2,2-dichloroacetic acid in CH_2Cl_2 (0.6M, 15mL, 9mmol). After 30min, triethylsilane (28mL) was added, and it was stirred for 1.5h. Then, pyridine (1.4mL) was added. It was concentrated, and the residue was purified by reverse phase (C18) chromatography eluted with 0 to 43% ACN in aq NH_4HCO_3 (5mM) to give the product. LCMS (ES, m/z): 905.8 [M +

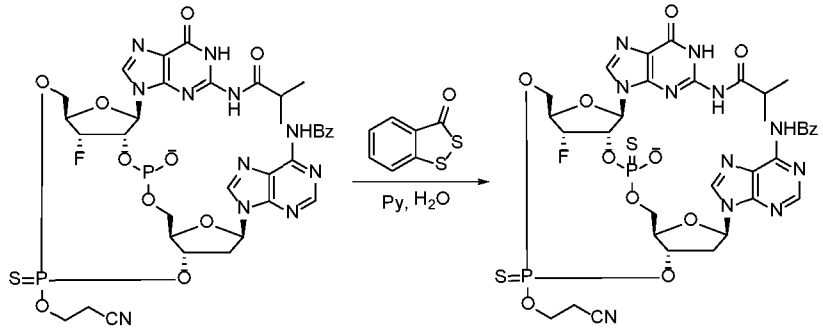
H]$^+$. ^{1}H-NMR (300MHz, CD_3OD): δ 8.71-8.44 (m, 2H), 8.21-8.03 (m, 3H), 7.80 (d, *J* = 10.4Hz, 0.5H), 7.66-7.61 (m, 1H), 7.54 (t, *J* = 7.6Hz, 2H), 6.61-6.42 (m, 1H), 6.14 (dd, *J* = 13.2, 6.0Hz, 1H), 5.68 (d, *J* = 9.9Hz, 0.5H), 5.60-5.19 (m, 3H), 4.69-4.36 (m, 3H), 4.36-4.17 (m, 3H), 3.92-3.64 (m, 2H), 3.13-2.55 (m, 5H), 1.19 (dd, *J* = 6.9, 2.1Hz, 6H). ^{19}F-NMR (282MHz, CD_3OD): δ -202.55, -202.75 (d, 1F). ^{31}P-NMR (121MHz, CD_3OD): δ 66.91, 66.69 (2s, 1P); 2.66, 2.60 (2s, 1P).

Step 4: (5R,7R,8S,12aR,14R,15aS,16R)-2-(2-cyanoethoxy)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-10-olate 2-sulfide



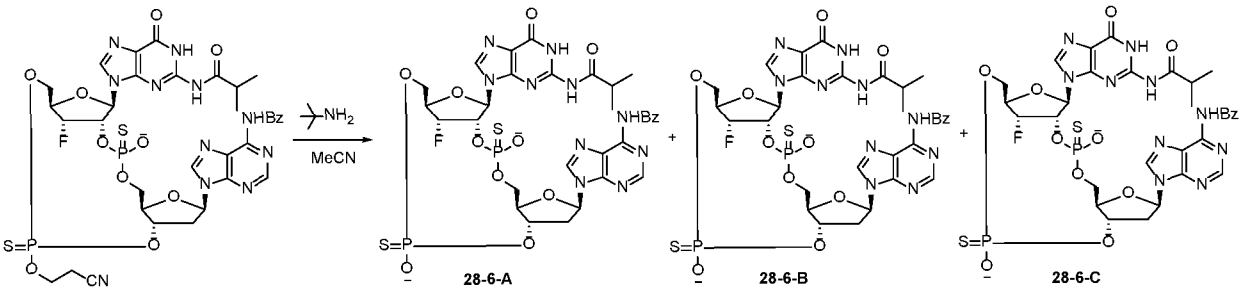
Diphenyl phosphorochloridate (2375mg, 8.84mmol) was added to pyridine (45ml) at -30°C. To the solution at -30°C was added (2R,3S,4R,5R)-5-((((((2R,3S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl) oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (400mg, 0.44mmol) in CH_2Cl_2 (45mL) dropwise over 20min. The resulting mixture was stirred at -30°C for 40min. The reaction mixture was used for the next reaction step immediately without purification. LCMS (ES, m/z): 887.8 [M + H]$^+$.

Step 5: (5R,7R,8S,12aR,14R,15aS,16R)-2-(2-cyanoethoxy)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-10-olate 2,10-disulfide



To the mixture from Step 4 at -30°C was added 3*H*-benzo[c][1,2]dithiol-3-one (112mg, 0.663mmol) and water (279μL, 15.5mmol). After stirring at rt for 1h, the mixture was poured into a solution of $Na_2S_2O_3 \cdot 5H_2O$ (280mg) in water (10mL) at 0°C. It was stirred at rt for 5min, and the mixture was concentrated under reduced pressure. The residue was purified by reverse phase (C18) chromatography eluted with 0 to 28% ACN in aq NH_4HCO_3 (5mM) to give the product. LCMS (ES, m/z): 919.8 $[M + H]^+$. ^{19}F-NMR (376MHz, CD_3OD): δ -198.51, -198.98, -200.16 (3s, 1F). $]^+$. ^{31}P-NMR (162MHz, CD_3OD): δ 65.90, 65.09, 63.64, 62.95, 57.26, 56.50 (6s, 2P).

*Step 6: Diastereomers (5R,7R,8S,12aR,14R,15aS,16R)-16-fluoro-7-{2-[(2-methylpropanoyl) amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-disulfide (**28-6-A**), (5R,7R,8S,12aR,14R,15aS,16R)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-disulfide (**28-6-B**), and (5R,7R,8S,12aR,14R,15aS,16R)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-disulfide (**28-6-C**)*



To a solution of (5R,7R,8S,12aR,14R,15aS,16R)-2-(2-cyanoethoxy)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-

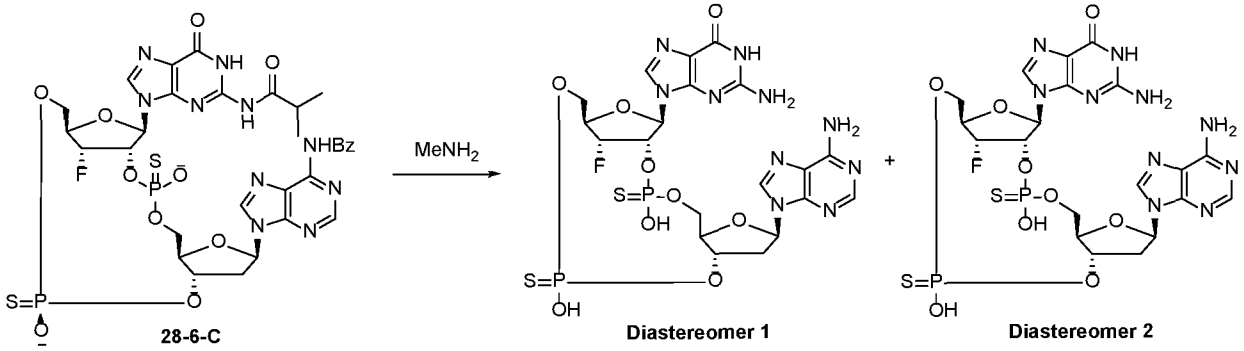
9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-10-olate 2,10-disulfide (265mg, 0.288mmol) in ACN (5mL) at rt was added *tert*-butylamine (5mL, 0.29mmol). The reaction mixture was stirred for 10min. Then, volatile components were removed under reduced pressure. The residue was purified by preparative-HPLC (T3 Prep Column, 19 mm×250 mm) eluted with 5 to 20% ACN in aq NH_4HCO_3 (50mM) over 21min.

The first fractions (T_R: 8.95 min) gave (5R,7R,8S,12aR,14R,15aS,16R)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-disulfide (**28-6-A**). LCMS (ES, m/z): 866.7 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ 8.74 (s, 1H), 8.71 (s, 1H), 8.26 (s, 1H), 8.07 (d, J = 7.5Hz, 1H), 7.65 (d, J = 7.7Hz, 1H), 7.57 (t, J = 7.5Hz, 2H), 6.60-6.34 (m, 1H), 5.94 (d, J = 8.6Hz, 1H), 5.91-5.66 (m, 1H), 5.46-5.16 (m, 2H), 4.50 (d, J = 27.0Hz, 1H), 4.27 (d, J = 9.8Hz, 1H), 4.16 (t, J = 10.1Hz, 1H), 3.98 (q, J = 11.0Hz, 1H), 3.86 (d, J = 11.9Hz, 1H), 3.72-3.69 (m, 1H), 3.10-3.06 (m, 1H), 3.00-2.82 (m, 1H), 2.74-2.70 (m, 1H), 1.06 (dd, J = 27.2, 6.8Hz, 6H). ^{31}P-NMR (162MHz, DMSO-d_6): δ 53.92 (s, 1P), 52.99 (s, 1P).

The second fractions (T_R: 10.00min) gave (5R,7R,8S,12aR,14R,15aS,16R)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl) amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-disulfide (**28-6-B**). LCMS (ES, m/z): 866.7 $[M + H]^+$.

The third fractions (T_R: 11.27-12.16min) gave (5R,7R,8S,12aR,14R,15aS,16R)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-disulfide (**28-6-C**), a mixture of two diastereomers, which was used in next step. LCMS (ES, m/z): 866.7 $[M + H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ 8.77 (s, 1H), 8.74 (s, 1H), 8.72 (s, 1H), 8.12-8.02 (m, 2H), 7.66-7.64 (m, 1H), 7.56 (t, J = 7.5Hz, 2H), 6.47-6.44 (m, 1H), 5.99 (d, J = 8.6Hz, 1H), 5.55-5.33 (m, 2H), 5.22 (d, J = 11.6Hz, 1H), 4.47 (d, J = 25.7Hz, 1H), 4.43-4.40 (m, 1H), 4.03-3.98 (m, 2H), 3.84 (d, J = 11.8Hz, 1H), 3.75-3.72 (m, 1H), 3.18-3.15 (m, 1H), 2.82-2.73 (m, 2H), 1.13 (dd, J = 6.9, 2.5Hz, 6H). ^{31}P-NMR (162MHz, DMSO): δ 53.42 (s, 1P), 52.16 (s, 1P).

Step 7: 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomers 1 and 2)



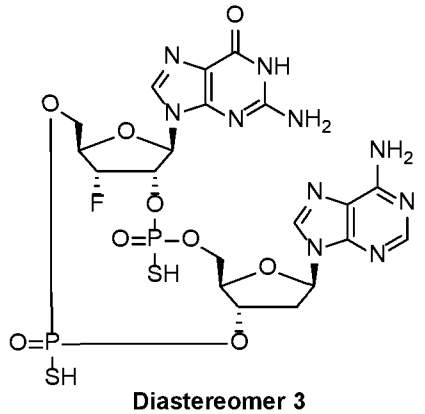
(5R,7R,8S,12aR,14R,15aS,16R)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-disulfide (**28-6-C**) (180mg, 0.208mmol) was dissolved in a solution of $MeNH_2$ in EtOH (30%, 5.0mL, 42mmol), and the resulting solution was stirred at rt for 1h. The volatile components were removed under reduced pressure to give a crude sample that was purified by Prep-HPLC (Atlantis Prep T3 OBD Column, 19mm×250mm) eluted with 5 to 19.5% ACN in aq NH_4HCO_3 (50mM) over 19min to give, after concentration:

Example 117 (T_R: 14.82min): 2-amino-9-[(2R,5R,7R,8S,10R,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1). LCMS (ES, m/z): 690.8 $[M - H]^-$. ^{1}H-NMR (400MHz, D_2O): δ 8.14 (s, 1H), 8.13 (s, 1H), 8.11 (s, 1H), 6.37 (t, J = 5.5Hz, 1H), 5.99 (d, J = 8.7Hz, 1H), 5.54 (d, J = 3.3Hz, 0.5H), 5.48-5.30 (m, 1.5H), 5.12 (dd, J = 10.2, 5.5Hz, 1H), 4.66 (d, J = 34.1Hz, 1H), 4.36 (s, 1H), 4.24-4.01 (m, 4H), 3.04 (dt, J = 14.1, 5.6Hz, 1H), 2.79 (dt, J = 13.5, 6.4Hz, 1H). ^{19}F-NMR (376MHz, D_2O): δ -198.66 (s, 1F). ^{31}P-NMR (162MHz, D_2O): δ 53.97 (s, 1P), 53.46 (s, 1P).

Example 118 (T_R: 15.93min): 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2). LCMS (ES, m/z): 690.8 $[M - H]^-$. ^{1}H-NMR (400MHz, D_2O): δ 8.16 (s, 1H), 8.11 (s, 1H), 7.83 (s, 1H), 6.34 (dd, J = 6.5, 3.0Hz, 1H), 5.95 (d, J = 8.6Hz, 1H), 5.69-5.53 (m, 1H), 5.47 (d, J = 3.4Hz, 0.5H), 5.33 (d, J = 3.4Hz, 0.5H), 5.23 (p, J = 7.3Hz, 1H), 4.64 (d, J =

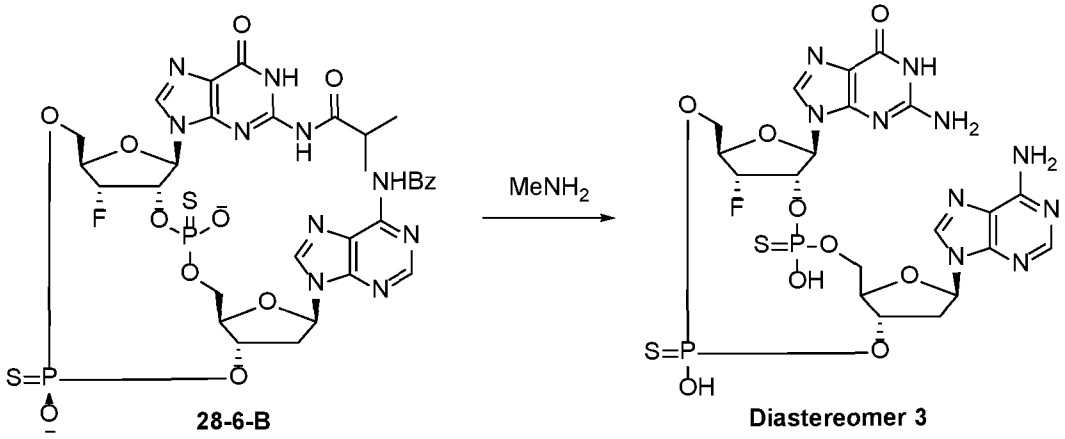
26.7Hz, 1H), 4.35 (ddd, *J* = 10.6, 6.9, 3.2Hz, 1H), 4.31-4.17 (m, 2H), 4.05-3.95 (m, 2H), 2.94-2.85 (m, 1H), 2.74 (dt, *J* = 14.0, 7.2Hz, 1H). ^{19}F-NMR (376MHz, D_2O): δ -198.74 (s, 1F). ^{31}P-NMR (162MHz, D_2O): δ 55.05 (s, 1P), 52.87 (s, 1P).

Example 119: 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)



Diastereomer 3

Step 1: 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one



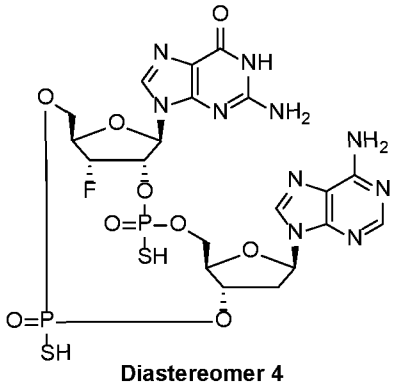
28-6-B

Diastereomer 3

(5R,7R,8S,12aR,14R,15aS,16R)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-disulfide (**28-6-B**) (45mg, 0.053mmol) was dissolved in a solution of $MeNH_2$ in EtOH (30%, 1.5mL, 11mmol), and the resulting solution was stirred at rt for 1h. The volatile components were removed under reduced pressure, and the residue was purified by Prep-HPLC (Atlantis Prep T3 OBD Column, 19mm×250mm) eluted with 18 to 19.5% ACN in aq NH_4HCO_3 (50mM)

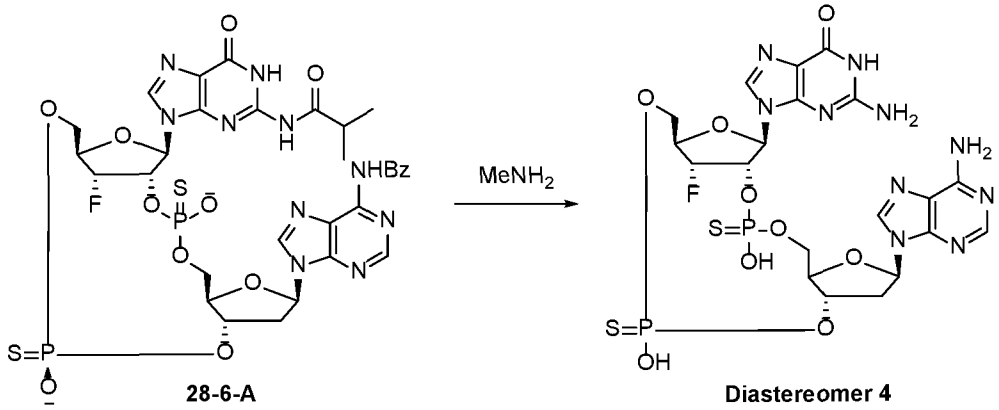
over 16min to give the product (T_R: 11.22min). LCMS (ES, m/z): 690.8 [M - H]$^-$. ^{1}H-NMR (400MHz, D_2O): δ 8.32 (s, 1H), 8.15 (s, 1H), 7.96 (s, 1H), 6.41 (t, J = 5.7Hz, 1H), 6.00 (d, J = 8.6Hz, 1H), 5.56 (dt, J = 22.9, 10.4Hz, 1H), 5.40-5.30 (m, 1.5H), 5.19 (d, J = 3.6Hz, 0.5H), 4.64 (d, J = 28.3Hz,1H), 4.40-4.27 (m, 2H), 4.27-4.17 (m, 1H), 4.02 (d, J = 11.9Hz, 1H), 3.95-3.85 (m, 1H), 2.92 (dt, J = 14.1, 5.6Hz, 1H), 2.79 (td, J = 13.8, 13.1, 6.1Hz, 1H). ^{19}F-NMR (376MHz, D_2O): δ -198.02 (s, 1F). ^{31}P-NMR (162MHz, D_2O): δ 57.89 (s, 1P), 55.05 (s, 1P).

Example 120: 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)



Diastereomer 4

Step 1: 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(6-amino-9H-purin-9-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one

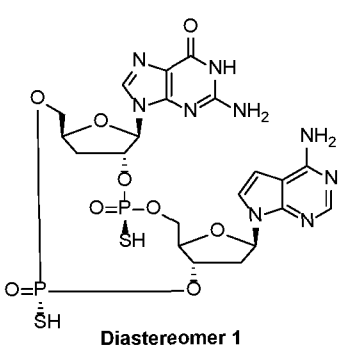


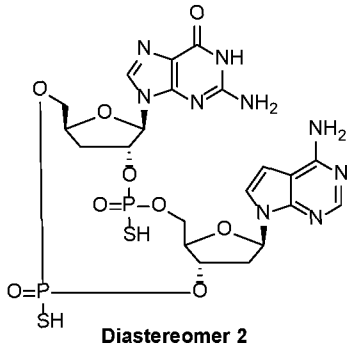
28-6-A Diastereomer 4

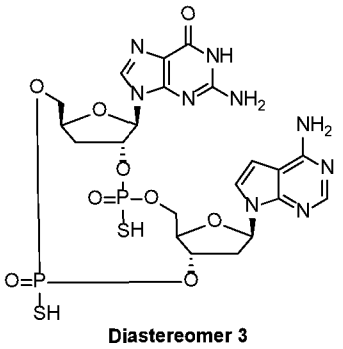
(5R,7R,8S,12aR,14R,15aS,16R)-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-diolate 2,10-disulfide (**28-6-A**) (45mg, 0.053mmol) was dissolved in a solution of $MeNH_2$ in EtOH (30%, 1.5mL, 11mmol), and the resulting solution was stirred at rt for 1h. The volatile components

were removed under reduced pressure, and the residue was purified by Prep-HPLC (Atlantis Prep T3 OBD Column, 19mm×250mm) eluted with 4 to 11% ACN in aq NH_4HCO_3 (50mM) over 17min to give the product (T_R: 11.72min). LCMS (ES, m/z): 690.8 $[M - H]^-$. ^{1}H-NMR (400MHz, D_2O): δ 8.33 (s, 1H), 8.29 (s, 1H), 8.16 (s, 1H), 6.41 (t, J = 6.5Hz, 1H), 6.05 (d, J = 8.6Hz, 1H), 5.49-5.32 (m, 1.5H), 5.23 (d, J = 3.5Hz, 0.5H), 4.79-4.73 (m, 1H), 4.69-4.59 (m, 1H), 4.40-4.32 (m, 1H), 4.23 (q, J = 8.9, 7.5Hz, 2H), 4.07 (d, J = 11.8Hz, 1H), 3.94-3.84 (m, 1H), 3.00 (dt, J = 12.7, 6.2Hz, 1H), 2.94-2.84 (m, 1H). ^{19}F-NMR (376MHz, D_2O): δ -197.92 (s, 1F). ^{31}P-NMR (162MHz, D_2O): δ 59.46 (s, 1P), 54.42 (s, 1P).

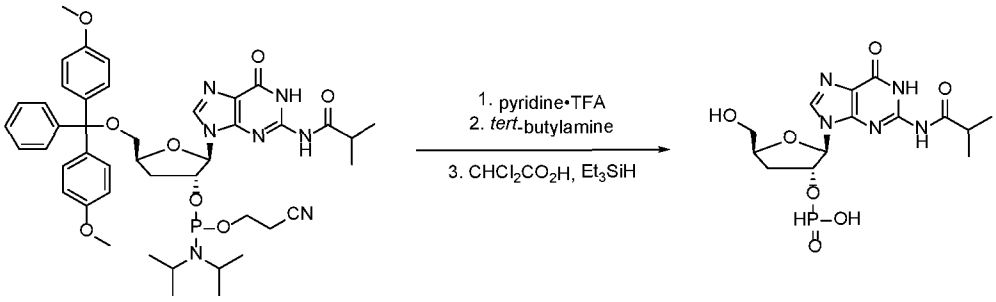
Example 121, 122, 123: 2-amino-9-[(2R,5S,7R,8R,10R,12aR,14R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1) and 2-amino-9-[(5S,7R,8R, 12aR,14R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomers 2 and 3)


Diastereomer 1


Diastereomer 2


Diastereomer 3

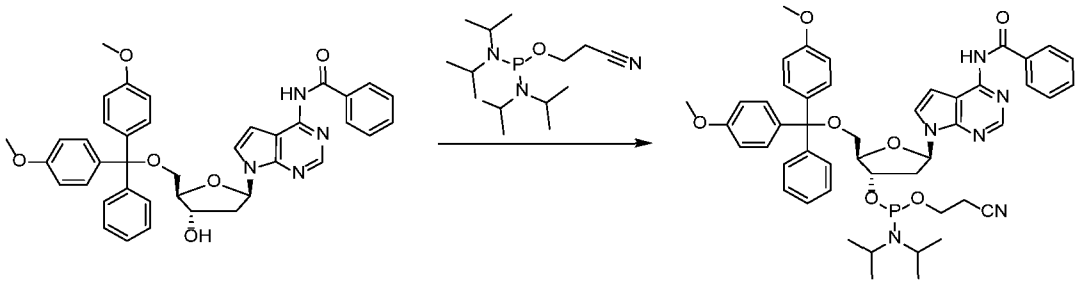
Step 1: (2R,3R,5S)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate



To a flask was added (2R,3R,5S)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl (2-cyanoethyl)

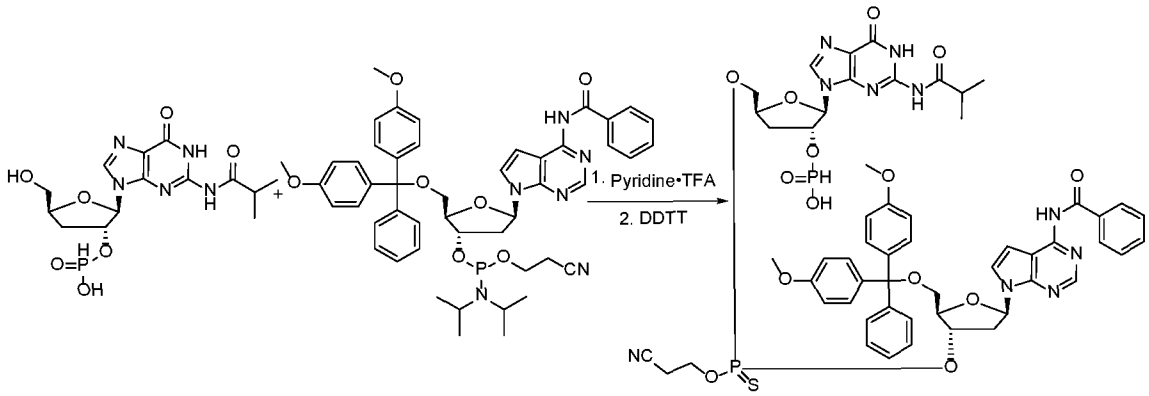
diisopropylphosphoramidite (4g, 4.76mmol), MeCN (23.65ml), and water (0.158ml). Pyridine trifluoroacetate (1.104g, 5.71mmol) was then added, and the mixture was stirred 1h at rt, after which time *tert*-butylamine (20.02ml, 190mmol) was added. After stirring 1h at rt, the mixture was concentrated under reduced pressure. The resulting mixture was dissolved in DCM (39.9ml), and then water (0.798ml) was added, followed by dichloroacetic acid (2.75ml, 33.3mmol). The solution was stirred for 20min at rt, and then triethylsilane (133ml, 833mmol) was added, and the reaction was stirred for a further 2h at rt. After cooling to 0°C, pyridine was added, and the mixture was concentrated under reduced pressure. The resulting sample was partitioned between hexanes (100mL) and water (20mL). The layers were separated, and the aqueous phase was purified by reverse phase HPLC using a gradient solvent system of 0-35% MeCN in 0.04% aq ammonium bicarbonate. The product-containing fractions were collected and lyophilized to give (2R,3R,5S)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate. LCMS (ES, m/z): 400 $[M-H]^-$.

Step 2: (2R,3S,5R)-5-(4-benzamido-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite



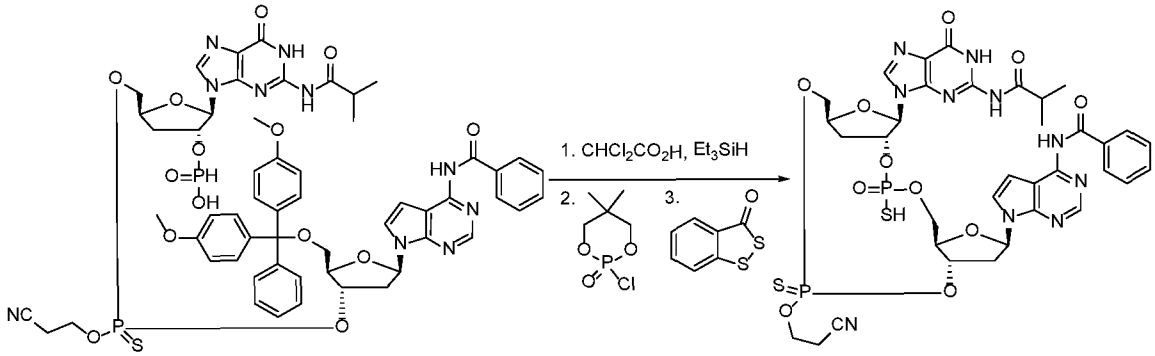
To a flask was added N-(7-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy) methyl)-4-hydroxytetrahydrofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzamide (4g, 6.09mmol), DCM (71.7ml), and 4,5-dicyanoimidazole (2.158g, 18.27mmol), and the solution was cooled to 0°C. 2-Cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite (6.77ml, 21.32mmol) was added, and the mixture was stirred for 15min at 0°C, after which time the mixture was concentrated under reduced pressure and purified by silica gel chromatography using a gradient of 30-100% EtOAc in hexanes to yield (2R,3S,5R)-5-(4-benzamido-7H-pyrrolo [2,3-d]pyrimidin-7-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl) tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite. LCMS (ES, m/z): 857 $[M+H]^+$.

Step 3: (2R,3R,5S)-5-((((((2R,3S,5R)-5-(4-benzamido-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate



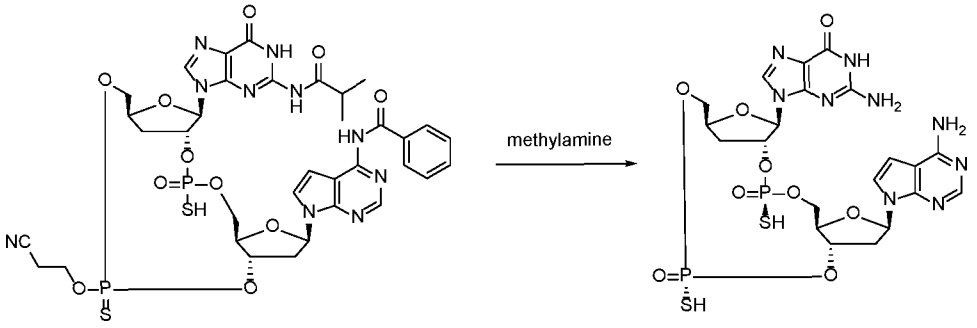
To a flask containing (2R,3R,5S)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate (1.62g, 2.81mmol) was added pyridine trifluoroacetate (0.543g, 2.81mmol), activated 4Å sieves and MeCN (10mL), and the mixture was stirred 20min at rt. To a separate flask containing (2R,3S,5R)-5-(4-benzamido-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl) tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (2.408g, 2.81mmol) was added MeCN (10mL) and activated 4Å sieves, and the mixture was stirred 20min at rt, after which time the hydrogen phosphonate solution was added, and MeCN (2x4mL) was used to complete the transfer. The mixture was stirred 1h at rt, and then ((dimethylamino-methylidene)amino)-3H-1,2,4-dithiazoline-3-thione (0.634g, 3.09mmol) was added. The resulting mixture was stirred 30min at rt and then concentrated under reduced pressure. Reverse phase HPLC purification using a gradient solvent system of 5-100% MeCN in 0.04% aqueous ammonium bicarbonate yielded (2R,3R,5S)-5-((((((2R,3S,5R)-5-(4-benzamido-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy) phosphorothioyl)oxy)methyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate. LCMS (ES, m/z): 1187 [M - H]$^-$.

Step 4: N-{7-[(5S,7R,8R,12aR,14R,15aS)-2-(2-cyanoethoxy)-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-10-oxido-10-sulfanyl-2-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl}benzamide



To a flask containing (2R,3R,5S)-5-((((((2R,3S,5R)-5-(4-benzamido-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-2-(2-isobutyramido-6-oxo-1H-purin-9(6H)-yl)tetrahydrofuran-3-yl hydrogen phosphonate (1.34g, 1.127mmol) was added DCM (22.54ml) and water (0.203ml, 11.27mmol), and then dichloroacetic acid (1.116ml, 13.52mmol) was added. The solution was stirred for 20min at rt, and then triethylsilane (28.1ml, 176mmol) was added. After stirring 3h at rt, the mixture was concentrated under reduced pressure. Pyridine (50mL) was added, and then the mixture was concentrated under reduced pressure. This process was repeated 2x, and then pyridine (37.6ml) was added, followed by 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (0.624g, 3.38mmol). The resulting mixture was stirred for 1h at rt, after which time water (610µl, 33.8mmol) was added, followed by 3H-1,2-benzodithiol-3-one (285mg, 1.692mmol). The resulting mixture was stirred 30min at rt and then concentrated under reduced pressure. HPLC purification using a gradient solvent system of MeCN in 100mM aqueous triethylammonium acetate yielded 3 separate diastereomers of N-{7-[(5S,7R,8R,12aR,14R,15aS)-2-(2-cyanoethoxy)-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-10-oxido-10-sulfanyl-2-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl}benzamide after lyopholization, all of which showed LCMS (ES, m/z): 899 $[M - H]^-$.

Step 5: 2-amino-9-[(5S,7R,8R,12aR,14R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one



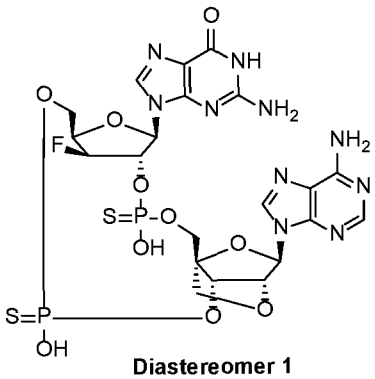
To a vial containing one diastereomer (slowest eluting) of N-{7-[(5S,7R,8R,12aR,14R,15aS)-2-(2-cyanoethoxy)-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-10-oxido-10-sulfanyl-2-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-14-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl}benzamide (122mg, 0.135mmol) was added methylamine (33% in EtOH) (2mL, 16.07mmol) and the resulting solution was stirred for 4h at rt, after which time it was concentrated under reduced pressure. Purification by reverse phase HPLC using a gradient solvent system of MeCN in 100mM aqueous triethylammonium acetate yielded Example 121, 2-amino-9-[(2R,5S,7R,8R,10R,12aR,14R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1). LCMS (ES, m/z): 672 [M - H]$^-$. ^{1}H NMR (600MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.01 (s, 1H), 7.86 (s, 1H), 7.29 (d, J = 2.7Hz, 1H), 6.97 (s, 2H), 6.60 – 6.53 (m, 3H), 6.44 (t, J = 6.9Hz, 1H), 5.73 (d, J = 2.7Hz, 1H), 4.95 (s, 1H), 4.80 (s, 1H), 4.38 – 4.27 (m, 2H), 3.97-3.91 (m, 2H), 3.90 – 3.82 (m, 1H), 3.64 (dt, J = 16.1, 9.3Hz, 1H), 2.69-2.63 (m, 1H), 2.63 (s, 12H), 2.60-2.54 (m, 3H), 2.22 – 2.13 (m, 1H), 1.01 – 0.94 (m, 18H).

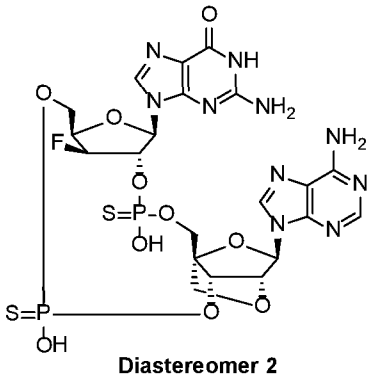
The other diastereomers from Step 4 were treated in an analogous manner to produce two additional diastereomers of 2-amino-9-[(5S,7R,8R,12aR,14R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one:

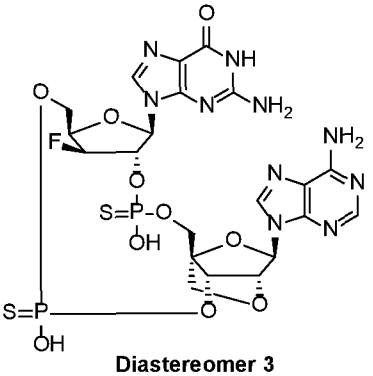
Example 122 (Diastereomer 2): LCMS (ES, m/z): 672 [M - H]$^-$. ^{1}H NMR (600MHz, DMSO-d_6) δ 10.57 (s, 1H), 9.55 (s, 2H), 8.10 (s, 1H), 7.95 (s, 1H), 7.47 (s, 1H), 7.42 (d, J = 3.2Hz, 1H), 6.66 (d, J = 3.4Hz, 1H), 6.51 (s, 2H), 6.49 – 6.44 (m, 1H), 5.73 (d, J = 4.0Hz, 1H), 5.08 – 5.00 (m, 2H), 4.31 – 4.27 (m, 1H), 4.22 (t, J = 11.3Hz, 1H), 4.07 (q, J = 10.6Hz, 1H), 4.00 – 3.95 (m, 1H), 3.76 – 3.71 (m, 1H), 3.65 (td, J = 11.6, 6.1Hz, 1H), 3.02 (d, J = 4.2Hz, 12H), 2.67 – 2.61 (m, 1H), 2.61 – 2.53 (m, 2H), 2.18 (dt, J = 11.7, 5.3Hz, 1H), 1.12 (t, J = 7.3Hz, 18H).

Example 123 (Diastereomer 3): LCMS (ES, m/z): 672 [M - H]$^-$. ^{1}H NMR (600MHz, DMSO-d_6) δ 10.53 (s, 1H), 8.01 (s, 3H), 7.28 (d, J = 3.4Hz, 1H), 6.97 (s, 2H), 6.56 (d, J = 3.5Hz, 1H), 6.49 (s, 2H), 6.44 (dd, J = 8.6, 5.8Hz, 1H), 5.70 (d, J = 3.9Hz, 1H), 5.00 – 4.92 (m, 1H), 4.90 – 4.83 (m, 1H), 4.33 – 4.27 (m, 1H), 4.12-3.96 (m, 4H), 3.81 (q, J = 12.8, 11.6Hz, 1H), 3.69 (dd, J = 12.0, 6.0Hz, 2H), 2.83 – 2.77 (m, 1H), 2.65 (d, J = 37.6Hz, 12H), 2.23 (dt, J = 12.8, 6.7Hz, 1H), 1.03 – 0.92 (m, 18H).

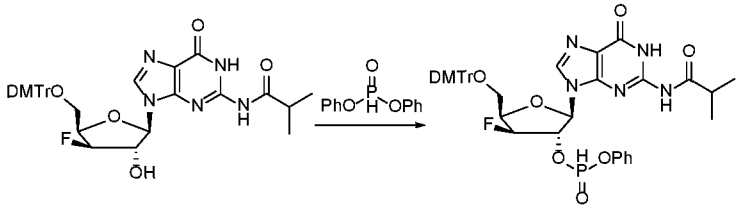
Examples 124, 125, and 126: 2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomers 1 – 3)


Diastereomer 1


Diastereomer 2

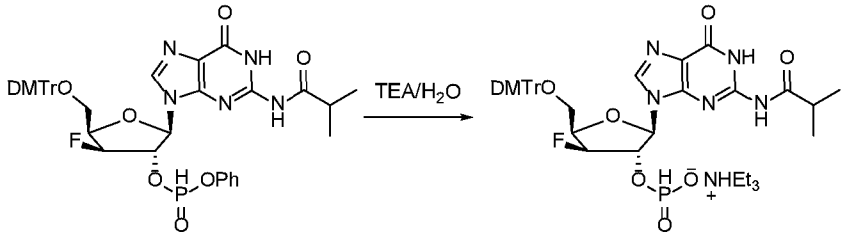

Diastereomer 3

Step 1: (2R,3S,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phenyl phosphonate



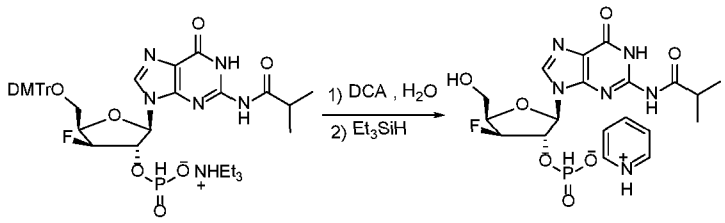
To a solution of N-(9-((2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy) methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (630mg, 0.96mmol) in pyridine (5mL) under Ar was added diphenyl phosphonate (1.07g, 4.56mmol), and the mixture was stirred at rt for 20min. It was used for the next reaction step without purification. LCMS (ES, m/z): 798.3 [M + H]$^+$.

Step 2: t (2R,3S,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



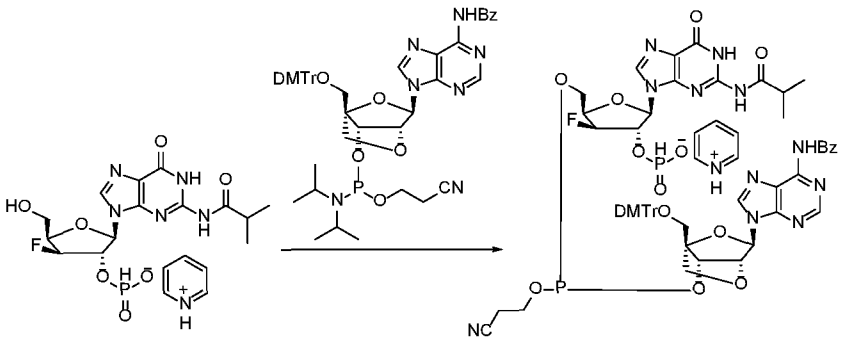
To the reaction mixture from Step 1 at 0°C was added water (1mL), triethylamine (1mL). The resulting mixture was stirred at rt for 20min. Then, it was concentrated, and the residue was partitioned between CH_2Cl_2 (50mL) and aq $NaHCO_3$ (5%, 20mL). The organic layer was washed with aq $NaHCO_3$ (5%, 20mL), dried ($Na_2S_2O_4$), concentrated and purified by silica gel column chromatography using 0-7% MeOH in CH_2Cl_2 (1% Et_3N) to give the product. LCMS (ES, m/z): 722.2 $[M + H]^+$. ^{1}H-NMR (400MHz, CD_3OD): δ 7.76 (s, 1H), 7.74 (s, 0.5H), 7.51 (d, J = 1.5Hz, 1H), 7.48 (q, J = 2.4, 1.9Hz, 1H), 7.41-7.34 (m, 4H), 7.34-7.27 (m, 2H), 7.26-7.21 (m, 1H), 6.92-6.85 (m, 4H), 6.22 (s, 1H), 6.15 (s, 0.5H), 5.37 (d, J = 2.7Hz, 0.5H), 5.28-5.19 (m, 1.5H), 4.73-4.69 (m, 0.5H), 4.66-4.62 (m, 1H), 3.80 (s, 6H), 3.65-3.55 (m, 1H), 3.53-3.44 (m, 1H), 3.12 (q, J = 7.3Hz, 8H), 2.75 (p, J = 6.8Hz, 1H), 1.33-1.22 (m, 18H). ^{31}P-NMR: (162MHz, CD_3OD): δ 2.67 (s, 1P).

Step 3: (2R,3S,4S,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



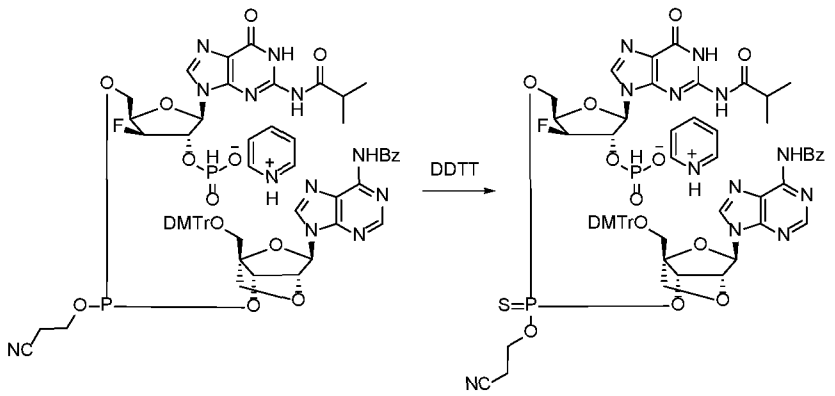
To a stirred solution of (2R,3S,4S,5R)-5-((bis(4-methoxyphenyl) (phenyl)methoxy) methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (540mg, 0.64mmol) in CH_2Cl_2 (7mL) at rt was added water (0.115g, 6.4mmol) and 2,2-dichloroacetic acid in CH_2Cl_2 (6%, 7mL, 5.76mmol). The mixture was stirred at rt for 15min, and then Et_3SiH (15mL) was added. After 40min, pyridine (0.90mL) was added, and the mixture was stirred for 5min. It was concentrated, and the residue was used for next reaction step without purification. LCMS (ES, m/z): 419.9 $[M + H]^+$.

Step 4: (2R,3S,4S,5R)-5-((((((1R,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphanyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



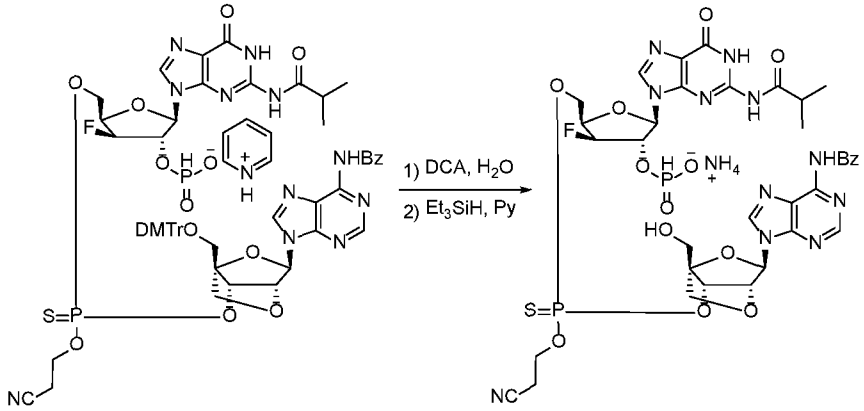
The crude from Step 3 was co-evaporated with ACN (3x5mL), re-dissolved in ACN (3mL) under Ar, and dried by adding activated 4Å molecular sieve (100mg). (1R,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl (2-cyanoethyl) diisopropylphosphoramidite (684.84mg, 0.774mmol) was co-evaporated with ACN (3x5mL), re-dissolved in ACN (3mL), and dried by adding activated 4Å molecular sieve (100mg). After 30min, it was added to the previously prepared mixture containing pyridin-1-ium (2R,3S,4S,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate. The resulting mixture was stirred at rt for 30min. Then it was used in the next reaction step without purification. LCMS (ES, m/z): 1202.1 $[M + H]^+$.

Step 5: (2R,3S,4S,5R)-5-((((((1R,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



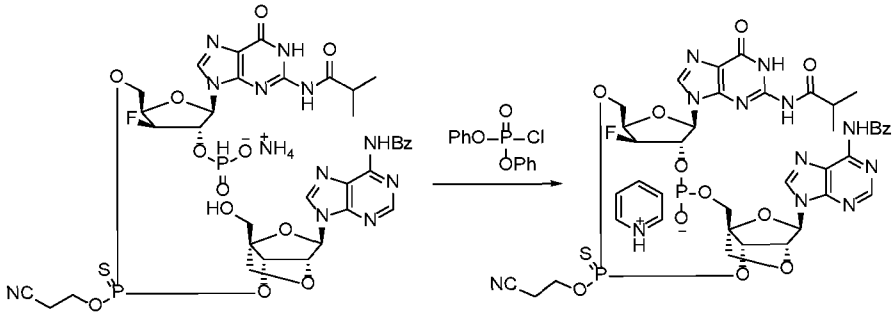
To the mixture from Step 4 was added (*E*)-*N*,*N*-dimethyl-*N*'-(3-thioxo-3*H*-1,2,4-dithiazol-5-yl)formimidamide (144.32mg, 0.704mmol), and the mixture was stirred at rt for 30min. Then, it was concentrated, and the crude was used for the next step without purification. LCMS (ES, m/z): 1234.3 $[M + H]^+$.

Step 6: (2R,3S,4S,5R)-5-((((((1S,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



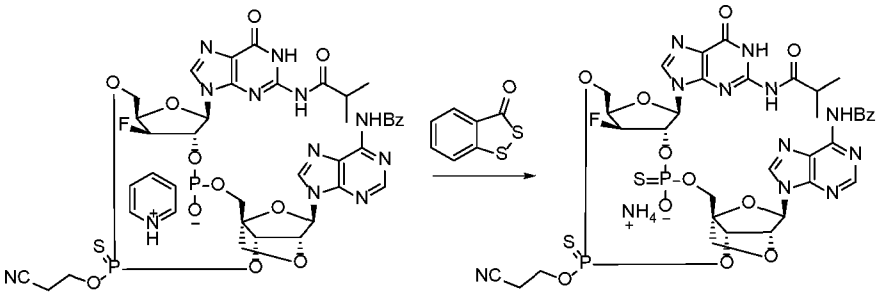
To a solution of the crude from Step 5 in CH_2Cl_2 (7mL) at rt was added water (120mg, 6.4mmol) and 2,2-dichloroacetic acid in CH_2Cl_2 (6%, 7mL, 6mmol). After 5min, triethylsilane (15mL) was added, and it was stirred for additional 2h. Then, pyridine (0.9mL) was added, and it was concentrated. The residue was purified by reverse phase (C18) chromatography eluted with 0 to 95% ACN in aq NH_4HCO_3 (0.04%) to give the product. LCMS (ES, m/z): 933.9 $[M + H]^+$. ^{1}H-NMR (400MHz, CD_3OD): δ 8.74-8.64 (m, 1H), 8.58-8.52 (m, 1H), 8.16-8.06 (m, 2H), 7.98-7.88 (m, 1H), 7.75 (d, J = 4.6Hz, 0.5H), 7.67 (t, J = 7.3Hz, 1H), 7.60-7.56 (m, 2H), 6.25-6.22 (m, 1H), 6.19-6.10 (m, 1H), 5.91 (s, 0.5H), 5.46-5.22 (m, 3H), 5.12 (d, J = 12.4Hz, 1H), 4.84-4.49 (m, 3H), 4.35 (tdd, J = 13.2, 6.1, 3.0Hz, 2H), 4.14 (d, J = 8.5Hz, 1H), 4.11-3.96 (m, 3H), 2.91 (dt, J = 16.4, 6.0Hz, 2H), 2.81-2.68 (m, 1H), 1.29-1.21 (m, 6H). ^{31}P-NMR: (162MHz, CD_3OD): δ 67.84, 66.33 (2 s, 1P); 2.65, 2.52 (2 s, 1P).

Step 7: (5R,7R,8S,12aR,14R,15R,15aS,18S)-2-(2-cyanoethoxy)-18-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}hexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-10(12H)-olate 2-sulfide



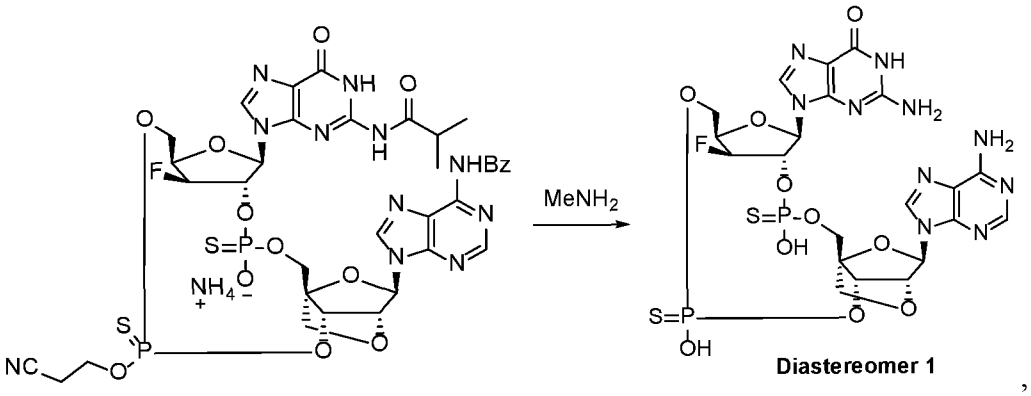
To pyridine (30mL) at -40°C under Ar was added diphenyl phosphorochloridate (1783.7mg, 6.64mmol) and then, a solution of (2R,3S,4S,5R)-5-((((((1S,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (310mg, 0.33mmol, co-evaporated with pyridine 3x5mL) in CH_2Cl_2 (30mL) over 20min. The resulting mixture was stirred at -40°C for 20min. It was used in the next step immediately without purification. LCMS (ES, m/z): 916.1 $[M + H]^+$.

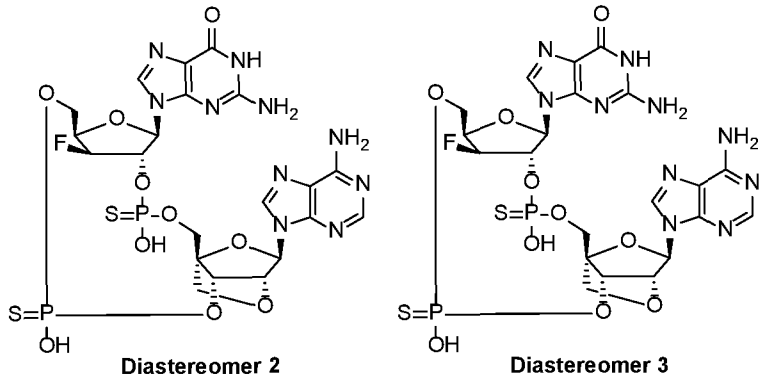
Step 8: (5R,7R,8S,12aR,14R,15R,15aS,18S)-2-(2-cyanoethoxy)-18-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}hexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-10(12H)-olate 2,10-disulfide



To the solution from Step 7 at -40°C was added 3*H*-benzo[c][1,2]dithiol-3-one (83.6mg, 0.498mmol) and water (179mg, 9.92mmol). The mixture was stirred at rt for 40min. Then, it was concentrated, and the residue was purified by reverse phase (C18) chromatography eluted with 0 to 95% ACN in aq NH_4HCO_3 (0.04%) to give the product. LCMS (ES, m/z): 947.8 $[M + H]^+$. ^{1}H-NMR (400MHz, CD_3OD): δ 8.79-8.58 (m, 2H), 8.18-8.06 (m, 2H), 7.92 (d, *J* = 13.5Hz, 1H), 7.69-7.66 (m, 1H), 7.63-7.54 (m, 2H), 6.40- 6.14 (m, 2H), 6.03-5.56 (m, 1.5H), 5.39-5.10 (m, 2.5H), 4.93-4.85 (m, 2H), 4.84-4.43 (m, 3H), 4.43-3.98 (m, 3H), 2.95 (t, *J* = 5.9Hz, 1H), 2.84-2.66 (m, 2H), 1.30-1.19 (m, 6H). ^{31}P-NMR (162MHz, CD_3OD): δ 66.60-64.98 (m, 1P), 56.95-55.65 (m, 1P).

Step 9: 2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomers 1 – 3)





(5R,7R,8S,12aR,14R,15R,15aS,18S)-2-(2-cyanoethoxy)-18-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}hexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-10(12H)-olate 2,10-disulfide (260mg, 0.27mmol) was dissolved in a solution of $MeNH_2$ in EtOH (30%, 20mL), and the resulting solution was stirred at rt for 3h. Then, it was concentrated, and the residue was purified by Prep-HPLC (Atlantis Prep RP C18 OBD Column, 19mm×250mm) eluted with 0 to 14% ACN in aq NH_4HCO_3 (50mM) over 25min to afford thee diastereomers of 2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one:

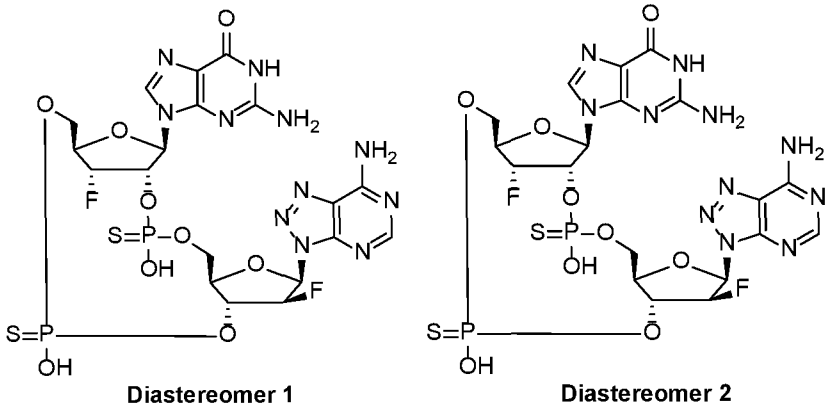
Example 124 (T_R: 22.52min): LCMS (ES, m/z): 719.0 $[M - H]^-$. ^{1}H-NMR: (400MHz, D_2O + DCl): δ 8.71 (s, 1H), 8.26 (s, 1H), 8.09 (s, 1H), 5.73 (s, 1H), 5.65 (s, 1H), 5.22-5.10 (m, 1H), 4.74-4.72 (m, 2H), 4.45 (d, J = 4.1Hz, 1H), 4.21-4.11 (m, 1H), 4.05-3.94 (m, 2H), 3.83-3.56 (m, 2H), 3.46 (s, 2H). ^{31}P-NMR: (162MHz, D_2O + DCl): δ 60.35 (s, 1P), 56.87 (s, 1P).

Example 125 (T_R: 15.75min): LCMS (ES, m/z): 719.0 $[M - H]^-$. ^{1}H-NMR: (400MHz, D_2O): δ 8.31 (s, 1H), 8.14 (s, 1H), 7.74 (s, 1H), 6.11 (s, 1H), 6.06 (s, 1H), 5.61-5.49 (m, 1H),

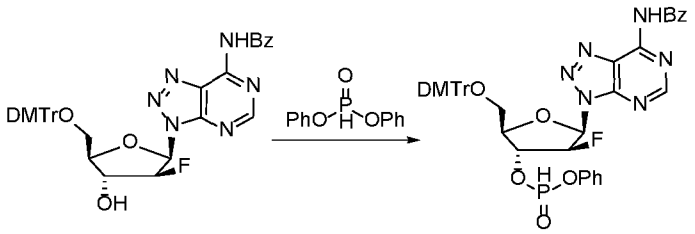
5.35 (s, 1H), 5.10 (d, J = 9.8Hz, 1H), 4.71-4.55 (m, 1H), 4.51-4.20 (m, 3H), 4.18-3.95 (m, 4H). ^{31}P-NMR: (162MHz, D_2O): δ 54.87-51.81 (m, 2P).

Example 126 (T_R: 13.17min): LCMS (ES, m/z): 718.8 $[M - H]^-$. ^{1}H-NMR: (300MHz, D_2O + DCl): δ 8.89 (s, 1H), 8.46 (s, 1H), 8.29 (s, 1H), 5.95 (s, 1H), 5.88 (s, 1H), 5.30-5.14 (m, 1H), 4.98 (t, J = 5.2Hz, 1H), 4.89 (s, 1H), 4.60 (s, 1H), 4.35-4.14 (m, 2H), 4.07 (d, J = 11.4Hz, 1H), 4.01-3.85 (m, 2H), 3.68-3.62 (m, 2H). ^{31}P-NMR: (121MHz, D_2O + DCl): δ 60.15 (s, 1P), 56.60 (s, 1P).

Examples 127 and 128: 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer1), and 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)



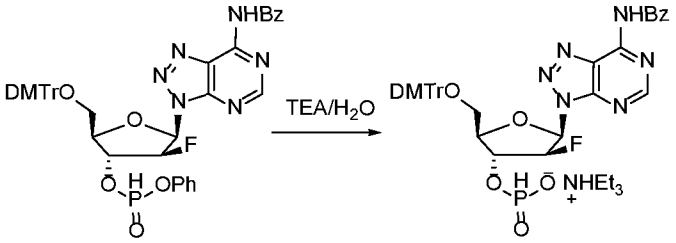
Step 1: (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl phenyl phosphonate



To a stirred solution of N-(3-((2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy) methyl)-3-fluoro-4-hydroxytetrahydrofuran-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-

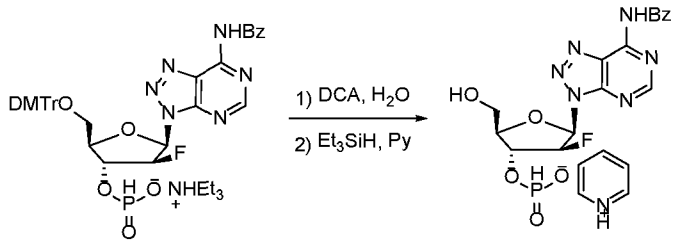
yl)benzamide (770mg, 1.138mmol) in pyridine (5ml) at 0°C under Ar was added diphenyl phosphonate (1.33g, 5.69mmol) over 2min. The resulting mixture was stirred at rt for 20min. It was used for the next reaction step directly without purification.

Step 2: (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl phosphonate



To the reaction mixture from Step 1 at 0 °C was added Et_3N (0.56mL) in water (0.56mL) over 5min. The resulting mixture was stirred at rt for 30min. It was concentrated, and the residue was partitioned between CH_2Cl_2 (60mL) and aq $NaHCO_3$ (5%, 24mL). The organic layer was washed with aq $NaHCO_3$ (5%, 2x24mL), dried (Na_2SO_4), concentrated, and purified by chromatography on silica gel using 0-10% MeOH in CH_2Cl_2 (0.5% Et_3N) to give the product. LCMS (ES, m/z): 741.2 $[M + H]^+$. ^{1}H-NMR (400MHz, CD_3OD): δ 8.79 (s, 1H), 8.21-8.11 (m, 2H), 7.71 (t, J = 7.4Hz, 1H), 7.67-7.58 (m, 2.5H), 7.46-7.37 (m, 2H), 7.30-7.23 (m, 4H), 7.18-7.13 (m, 2H), 7.03 (dd, J = 6.4, 2.8Hz, 1H), 6.76-6.73 (m, 2H), 6.69-6.65 (m, 2H), 6.04 (s, 0.5H), 5.85 (t, J = 6.4Hz, 0.5H), 5.72 (t, J = 6.4Hz, 0.5H), 5.60 (td, J = 16.9, 6.9Hz, 1H), 4.44-4.40 (m, 1H), 3.77-3.68 (m, 8H), 3.57 (dd, J = 10.6, 2.9Hz, 1H), 2.91 (q, J = 7.3Hz, 18H), 1.20 (t, J = 7.3Hz, 27H). ^{31}P-NMR: (162MHz, CD_3OD): δ 2.48 (s, 1P).

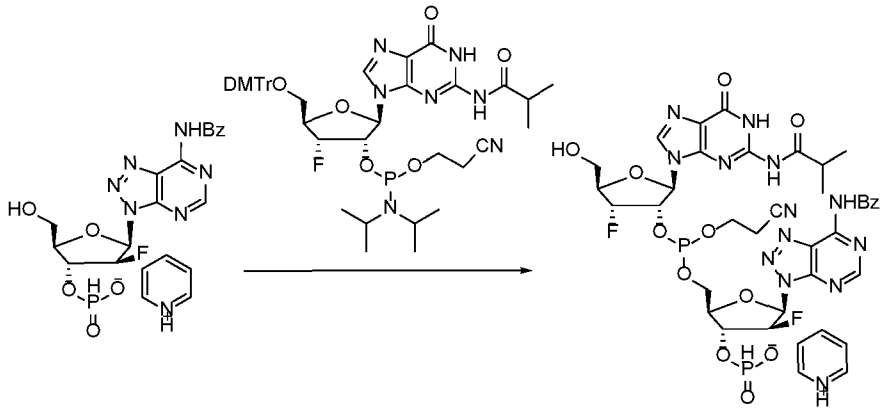
Step 3: (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl phosphonate



To a solution of (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl phosphonate (610mg, 0.65mmol) in CH_2Cl_2 (9.8mL) was added water (0.12g, 6.5mmol) and dichloroacetic acid in CH_2Cl_2 (0.6M, 9.8mL, 5.9mmol). After 30 in, triethylsilane (20mL) was added and stirring was continued for additional 2h. Then, pyridine (5mL) was added, and it was

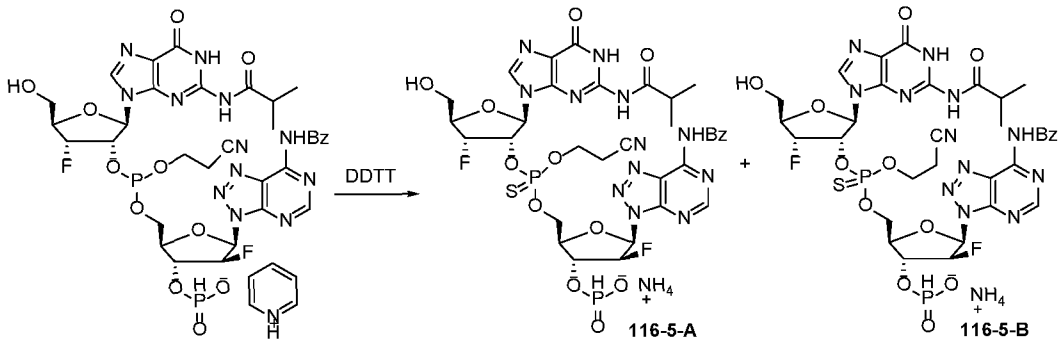
concentrated. The crude was used for the next reaction step without purification. LCMS (ES, m/z): 439.1 $[M + H]^+$.

Step 4: (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((((2-cyanoethoxy)(((2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)phosphanyl)oxy)methyl)-4-fluorotetrahydrofuran-3-yl phosphonate



The crude product of Step 3 was co-evaporated with dry ACN (3x3mL), re-dissolved in ACN (3mL), and dried by adding activated 4Å molecular sieve (200mg). (2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (0.824g, 0.960mmol) was co-evaporated with dry ACN (3x3mL), re-dissolved in ACN (5mL), and dried by adding activated 4Å molecular sieve (200mg). After 30min, it was added to the previously prepared mixture containing pyridin-1-ium (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl phosphonate. It was stirred at rt for 30min, and the reaction mixture was used in the next reaction step directly without purification. LCMS (ES, m/z): 893.2 $[M + H]^+$.

*Step 5: Diastereomers (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((((2-cyanoethoxy)(((2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)phosphorothioyl)oxy)methyl)-4-fluorotetrahydrofuran-3-yl phosphonate (**116-5-A**) and (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((((2-cyanoethoxy)(((2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)phosphorothioyl)oxy)methyl)-4-fluorotetrahydrofuran-3-yl phosphonate (**116-5-B**)*



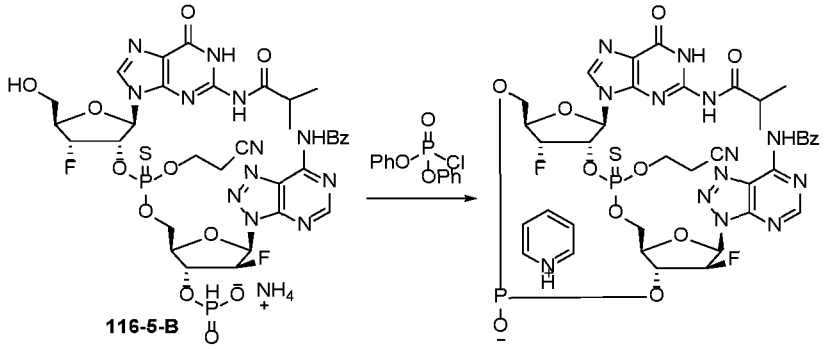
To the reaction mixture from Step 4 at rt, was added (E)-N,N-dimethyl-*N'*-(3-thioxo-3*H*-1,2,4-dithiazol-5-yl)formimidamide (181mg, 0.880mmol), and the mixture was stirred for 1h. Then, it was concentrated, and the residue was purified by reverse phase (AQ-C18) chromatography eluted with 0 to 28% ACN in aq NH_4HCO_3 (5mM) over 88min.

The first fractions with desired mass (T_R = 50min) gave (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((((2-cyanoethoxy)(((2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)phosphorothioyl)oxy)methyl)-4-fluorotetrahydrofuran-3-yl phosphonate (**116-5-A**). LCMS (ES, m/z): 924.9 $[M + H]^+$. ^{1}H-NMR: (400MHz, CD_3OD): δ 8.90 (s, 1H), 8.31 (s, 1H), 8.10 (d, *J* = 7.8Hz, 2H), 7.75 (s, 0.5H), 7.69 (t, *J* = 7.4Hz, 1H), 7.57 (t, *J* = 7.7Hz, 2H), 7.02 (dd, *J* = 6.5, 2.6Hz, 1H), 6.27-6.13 (m, 1.5H), 5.79-5.51 (m, 3H), 5.47-5.21 (m, 12H), 4.58 (dd, *J* = 15.4, 7.6Hz, 2H), 4.50-4.25 (m, 3H), 4.05 (ddd, *J* = 24.7, 13.2, 6.1Hz, 2H), 3.90-3.76 (m, 4H), 2.85-2.56 (m, 3H), 1.34 (d, *J* = 6.5Hz, 3H), 1.25-1.19 (m, 3H). ^{31}P-NMR (162MHz, CD_3OD): δ 67.56 (s, 1P), 3.09 (s, 1P).

The second fractions with desired mass (T_R = 55min) gave (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((((2-cyanoethoxy)(((2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)phosphorothioyl)oxy)methyl)-4-fluorotetrahydrofuran-3-yl phosphonate (**116-5-B**). LCMS (ES, m/z): 925.1 $[M + H]^+$. ^{1}H-NMR: (400MHz, CD_3OD): δ 8.87 (s, 1H), 8.33 (s, 1H), 8.16 (d, *J* = 7.8Hz, 2H), 7.75 (s, 0.5H), 7.70 (t, *J* = 7.4Hz, 1H), 7.61 (t, *J* = 7.7Hz, 2H), 6.91 (dd, *J* = 6.2, 3.4Hz, 1H), 6.23 (d, *J* = 7.1Hz, 1H), 6.17 (s, 0.5H), 5.74-5.54 (m, 3H), 5.46 (d, *J* = 4.6Hz, 0.5H), 5.33 (d, *J* = 4.6Hz, 0.5H), 4.65-4.24 (m, 4H), 4.15 (dt, *J* = 13.0, 5.8Hz, 2H), 3.95-3.75 (m, 2H), 2.90-2.67 (m, 3H), 1.36-1.31 (m, 3H), 1.19 (d, *J* = 6.8Hz, 3H). ^{31}P-NMR: (162MHz, CD_3OD): δ 67.29 (s, 1P), 3.07 (s, 1P).

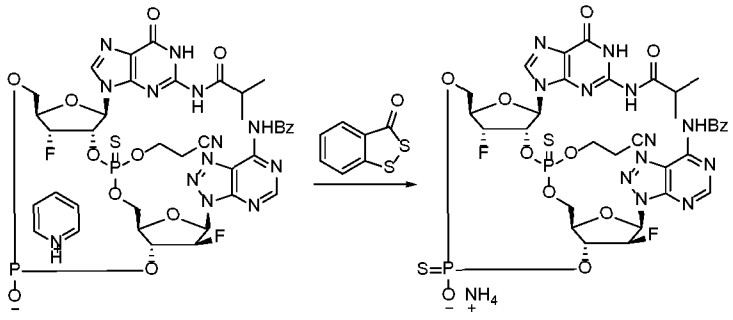
Step 6: (5R,7R,8S,12aR,14R,15S,15aR,16R)-10-(2-cyanoethoxy)-15,16-difluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{7-[(phenylcarbonyl)amino]-3H-

[1,2,3]triazolo[4,5-d]pyrimidin-3-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-2-olate 10-sulfide



To pyridine (10mL) at -40°C under Ar was added diphenyl phosphorochloridate (628mg, 2.34mmol), and then, a solution of (2R,3R,4S,5R)-5-(7-benzamido-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2-((((2-cyanoethoxy)(((2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)phosphorothioyl)-oxy)methyl)-4-fluorotetrahydrofuran-3-yl phosphonate (**116-5-B**) (110mg, 0.105mmol, co-evaporated with pyridine 3x5mL) in CH_2Cl_2 (10mL) over 20min. It was stirred at -40°C for 20min. The reaction mixture was used in the next step immediately without purification.

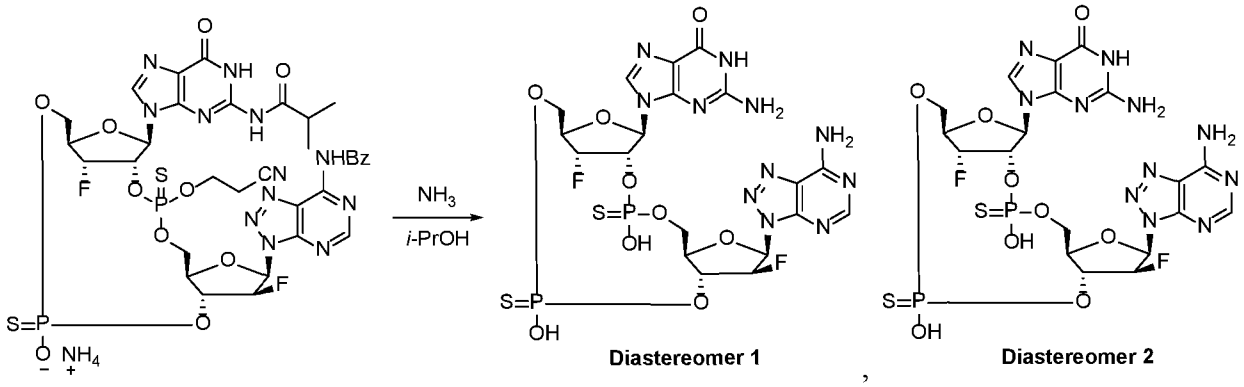
Step 7: (5R,7R,8S,12aR,14R,15S,15aR,16R)-10-(2-cyanoethoxy)-15,16-difluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{7-[(phenylcarbonyl)amino]-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-2-olate 2,10-disulfide



To the mixture from Step 6 at -20°C was added *3H*-benzo[c][1,2]dithiol-3-one (27.8mg, 0.165mmol) and water (42mg, 2.3mmol). The resulting mixture was stirred at rt for 30min. It was concentrated, and the residue was purified by reverse phase (AQ-C18) chromatography eluted with 0 to 28% ACN in aq NH_4HCO_3 (5mM) to give the product. LCMS (ES, m/z): 938.9 $[M + H]^+$. ^{1}H-NMR: (400MHz, CD_3OD): δ 8.94-8.71 (m, 2H), 8.23-8.06 (m, 2H), 7.75-7.67 (m, 1H), 7.64 (t, *J* = 7.5Hz, 2H), 7.11-6.97 (m, 1H), 6.32-6.17 (m, 2H), 6.02-5.73 (m, 2H), 5.68-5.38 (m, 1H), 4.73 (d, *J* = 24.3Hz, 1H), 4.68-4.42 (m, 4H), 4.39-4.19 (m, 2H), 4.06-3.66 (m, 2H),

2.99-2.62 (m, 2H), 2.61-2.37 (m, 1H), 1.20 (d, J = 6.8Hz, 3H), 1.12 (d, J = 6.7Hz, 3H). ^{31}P-NMR: (162MHz, CD_3OD): δ 64.05, 63.79 (2 s, 1P); 56.67, 56.27 (2 s, 1P).

Step 8: 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomers 1 and 2)



To pyridinium (5R,7R,8S,12aR,14R,15S,15aR,16R)-10-(2-cyanoethoxy)-15,16-difluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{7-[(phenylcarbonyl) amino]-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-2-olate 2,10-disulfide in a steel tank (150mL) at -60°C was added ammonia in isopropanol (50mL) at -60°C. The reactor was tightly sealed and then, heated at 50°C for 16h. Then, the volatile components were removed under reduced pressure, and the residue was purified by prep-HPLC (Atlantis Prep T3 OBD Column, 19×250 mm) eluted with 0 to 5% ACN in aq NH_4HCO_3 (50mM) over 25min.

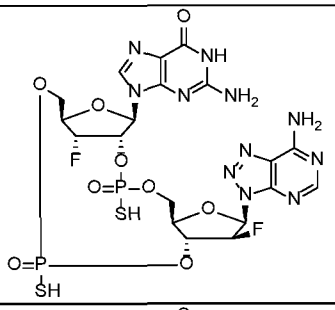
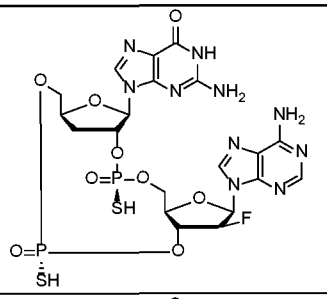
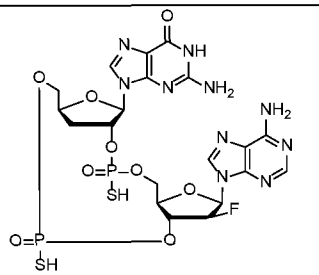
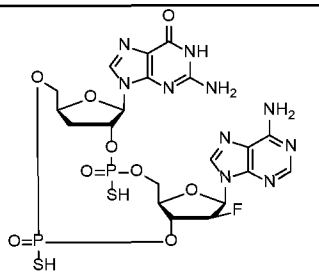
Example 127 (T_R = 17.32min): 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphospha-cyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1). LCMS (ES, m/z): 709.8 [M - H]$^-$. ^{1}H-NMR: (400MHz, D_2O): δ 8.32 (s, 1H), 8.27 (s, 1H), 6.78 (dd, J = 11.1, 4.9Hz, 1H), 6.07 (d, J = 8.6Hz, 1H), 5.81 (t, J = 4.6Hz, 0.5H), 5.68 (t, J = 4.7Hz, 0.5H), 5.63 (dq, J = 9.8, 5.0Hz, 1H), 5.58 (d, J = 3.5Hz, 0.5H), 5.45 (d, J = 3.4Hz, 0.5H), 5.43-5.26 (m, 1H), 4.65-4.63 (m, 1H), 4.56 (q, J = 5.6Hz, 1H), 4.31-4.23 (m, 3H), 4.12-4.05 (m, 1H). ^{31}P-NMR: (162MHz, D_2O): δ 55.76 (s, 1P), 54.26 (s, 1P).

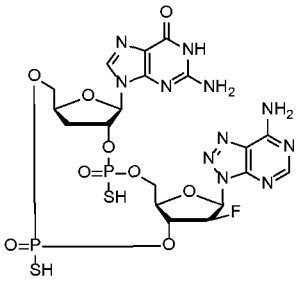
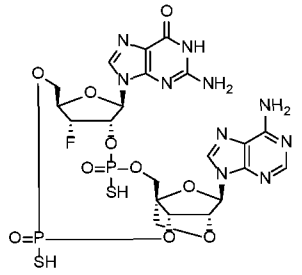
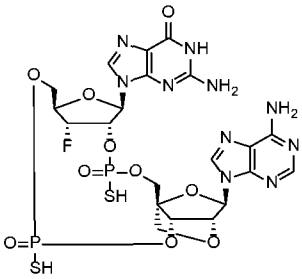
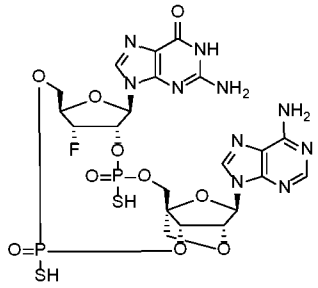
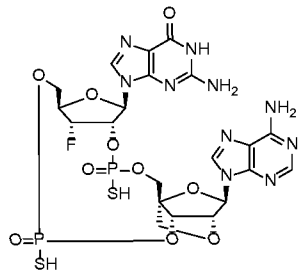
Example 128 (T_R = 21.10min): 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphospha-

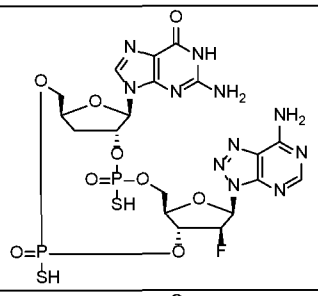
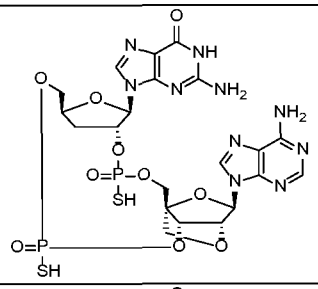
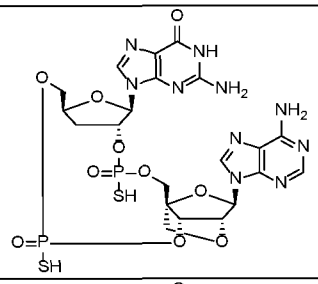
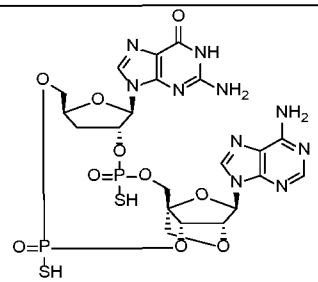
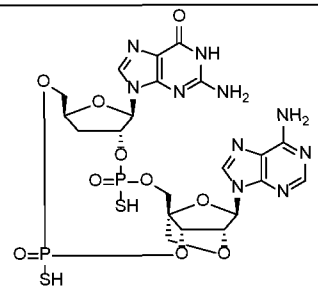
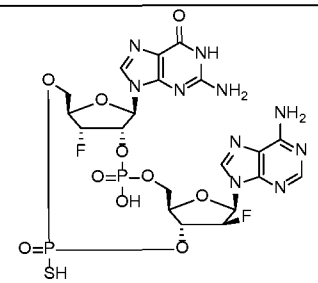
cyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2). LCMS (ES, m/z): 709.8 [M - H]$^-$. ^{1}H-NMR: (400MHz, D_2O): δ 8.26 (s, 1H), 8.01 (s, 1H), 6.79 (dd, J = 9.4, 5.3Hz, 1H), 6.05 (d, J = 8.6Hz, 1H), 5.85 (t, J = 5.0Hz, 0.5H), 5.72 (t, J = 5.0Hz, 0.5H), 5.65-5.35 (m, 3H), 4.69-4.62 (m, 1H), 4.48 (d, J = 5.5Hz, 1H), 4.42 (t, J = 11.1Hz, 1H), 4.18 (t, J = 6.2Hz, 2H), 4.09-4.06 (m, 1H). ^{31}P-NMR (162MHz, D_2O): δ 54.74 (s, 1P), 53.84 (s, 1P).

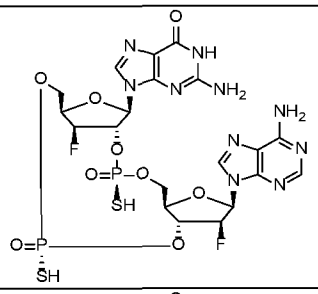
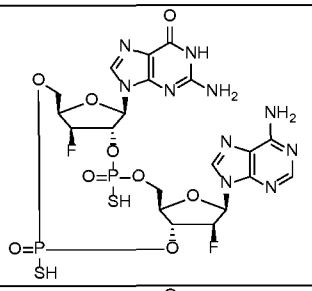
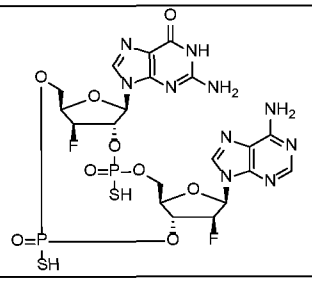
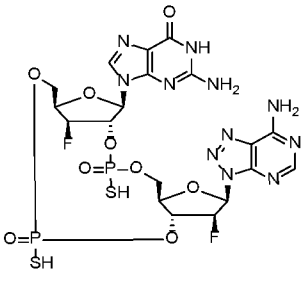
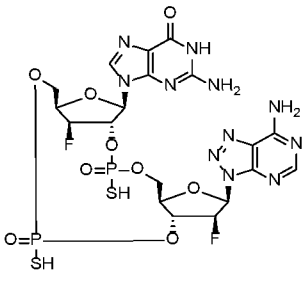
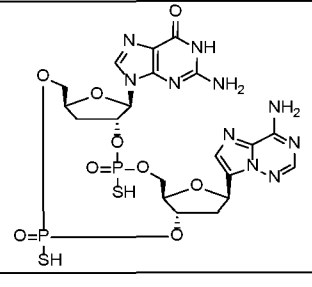
Examples 129 through 243, as shown in Table 6 below, were or may be prepared according to procedures analogous to those outlined in Examples 116 through 128 above using the appropriate monomers, decribed as Preparations or as obtained from commercial sources, in the coupling step.

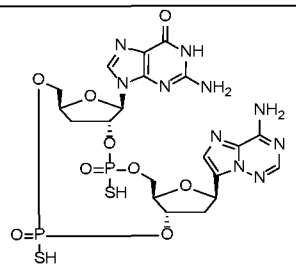
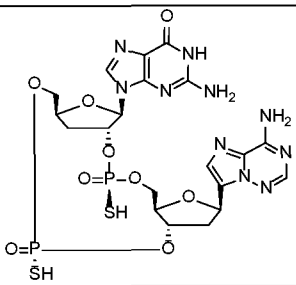
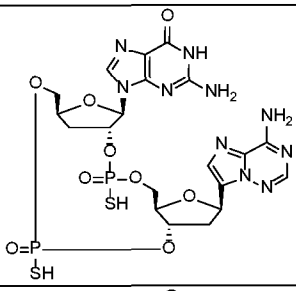
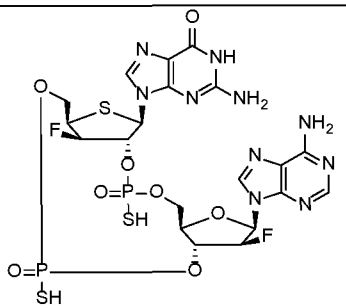
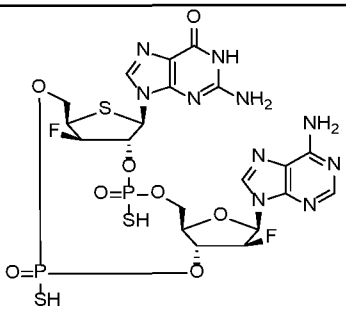
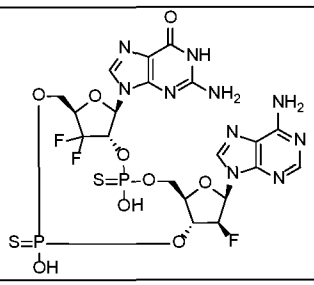
Table 6

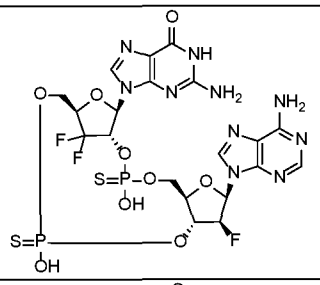
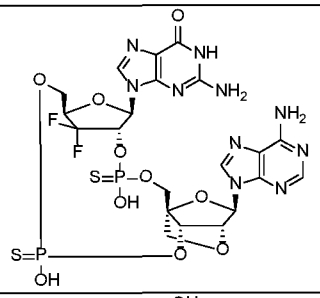
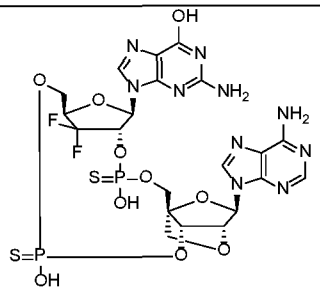
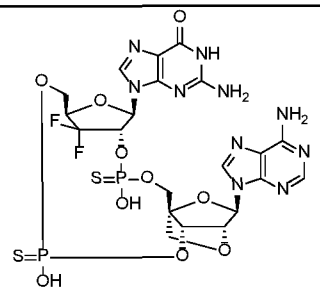
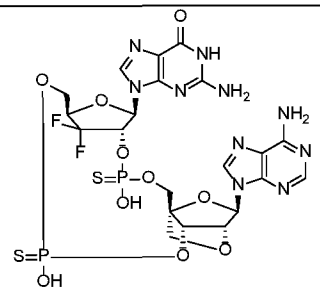
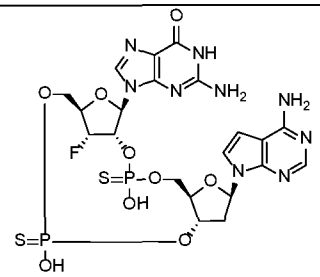
Ex.	Structure	Name	Mass [M-H]$^-$
129		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	710
130		2-amino-9-[(2R,5S,7R,8R,10R,12aR,14R,15S,15aR)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	691
131		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	691
132		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-oe (Diastereomer 3)	691

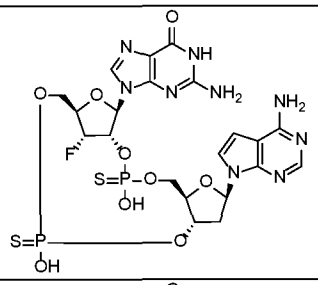
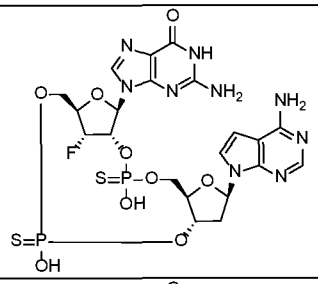
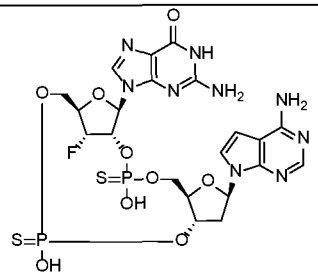
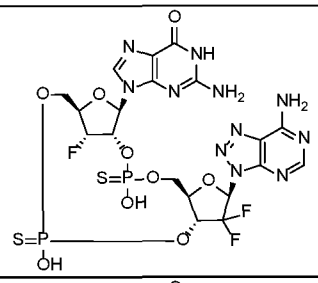
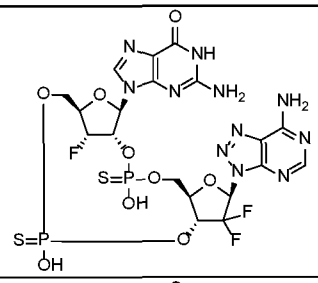
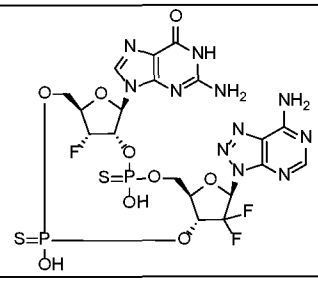
Ex.	Structure	Name	Mass [M-H]⁻
133		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	694
134		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	719
135		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	719
136		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	719
137		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)	719

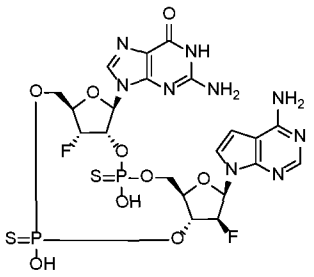
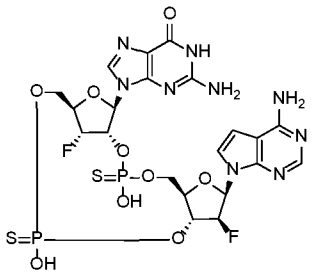
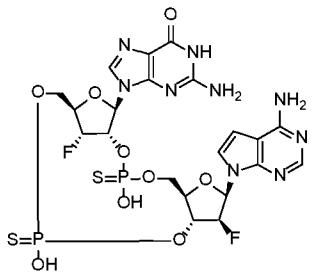
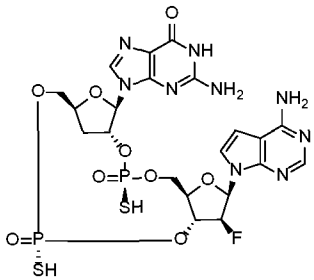
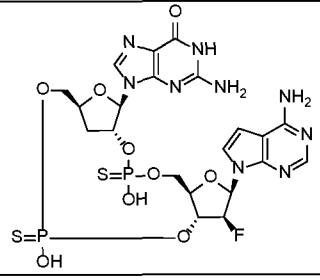
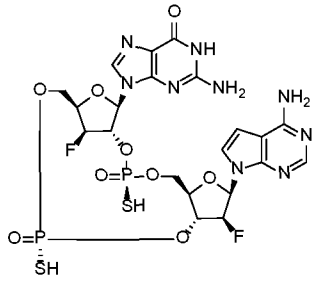
Ex.	Structure	Name	Mass [M-H]⁻
138		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	692
139		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	701
140		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	701
141		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diasteroemer 3)	701
142		2-amino-9-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)	701
143		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-10-oxido-2-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	694

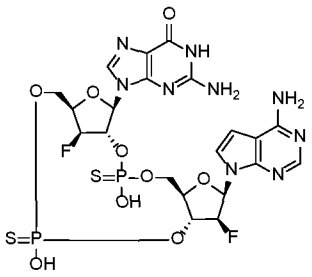
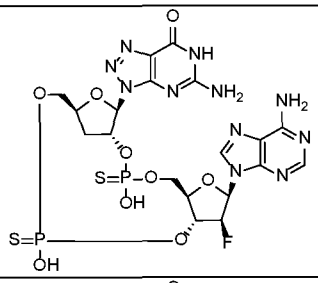
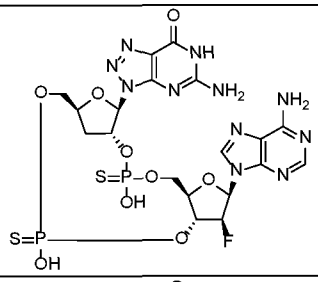
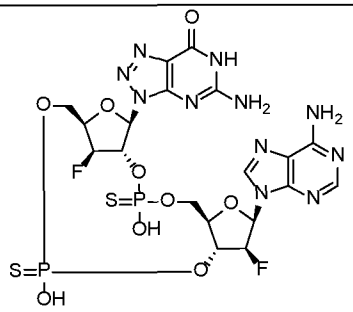
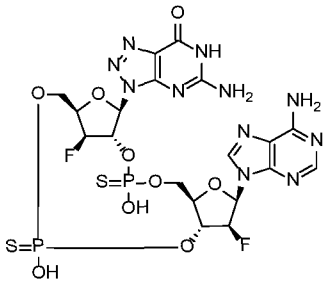
Ex.	Structure	Name	Mass [M-H]⁻
144		2-amino-9-[(2R,5R,7R,8S,10R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	709
145		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	709
146		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	709
147		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	710
148		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	710
149		2-amino-9-[(5S,7R,8R,12aR,14R,15aS)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	673

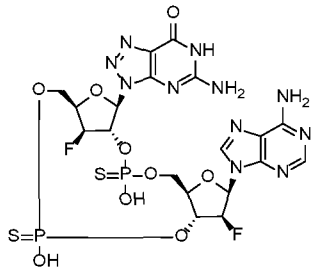
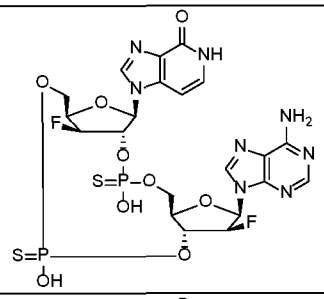
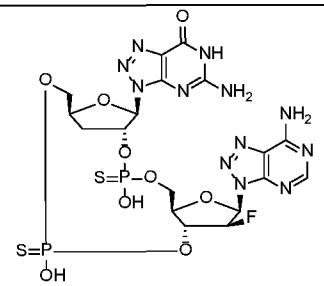
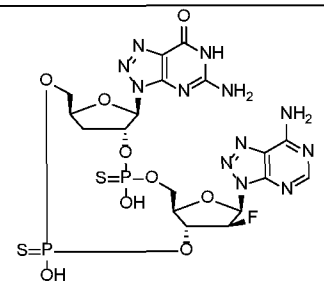
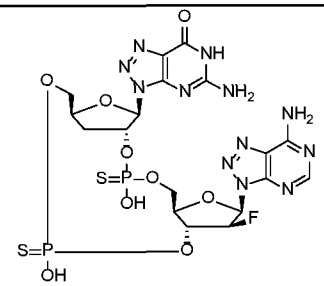
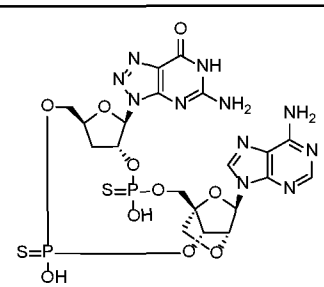
Ex.	Structure	Name	Mass [M-H]⁻
150		2-amino-9-[(5S,7R,8R,12aR,14R,15aS)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	673
151		2-amino-9-[(2R, 5S,7R,8R,10R, 12aR,14R,15aS)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	673
152		2-amino-9-[(5S,7R,8R,12aR,14R,15aS)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)	673
153		(5R,7R,8R,12aR,14R,15S,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-15,16-difluorooctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecine-2,10-bis(thiolate) 2,10-dioxide (Diastereomer 1)	725
154		(5R,7R,8R,12aR,14R,15S,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-15,16-difluorooctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecine-2,10-bis(thiolate) 2,10-dioxide (Diastereomer 2)	725
155		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR)-14-(6-amino-9H-purin-9-yl)-15,16,16-trifluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	727

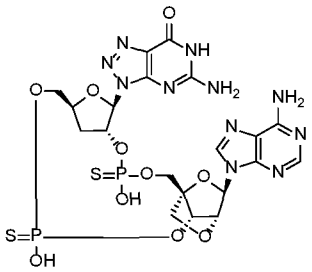
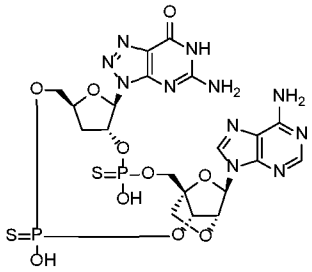
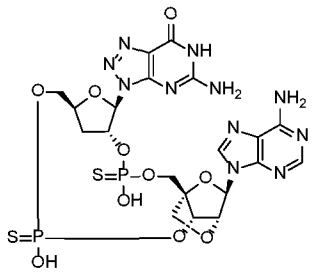
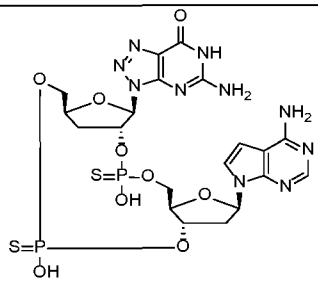
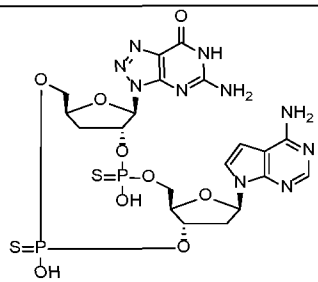
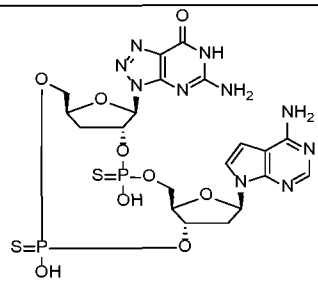
Ex.	Structure	Name	Mass [M-H]⁻
156		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR)-14-(6-amino-9H-purin-9-yl)-15,16,16-trifluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	727
157		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-18,18-difluoro-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	737
158		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-18,18-difluoro-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	737
159		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-18,18-difluoro-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	737
160		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-18,18-difluoro-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)	737
161		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	690

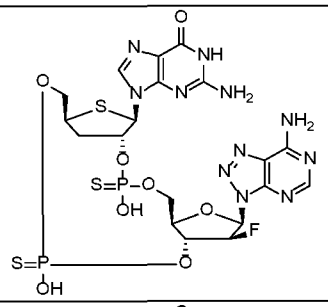
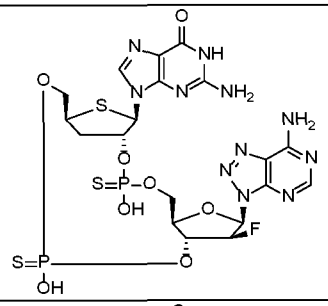
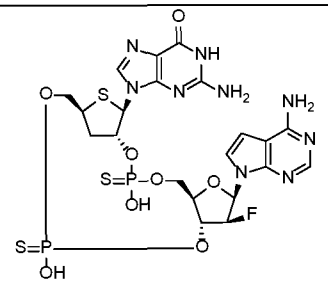
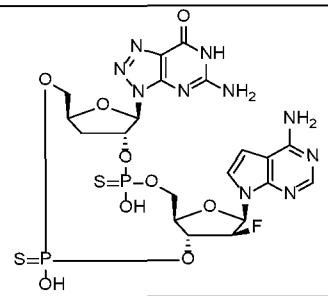
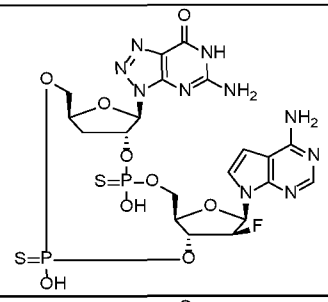
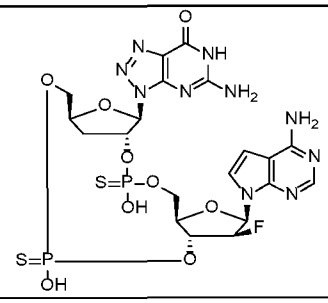
Ex.	Structure	Name	Mass [M-H]⁻
162		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	690
163		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	690
164		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)	690
165		2-amino-9-[(5R,7R,8S,12aR,14R,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,15,16-trifluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	728
166		2-amino-9-[(5R,7R,8S,12aR,14R,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,15,16-trifluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	728
167		2-amino-9-[(5R,7R,8S,12aR,14R,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,15,16-trifluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	728

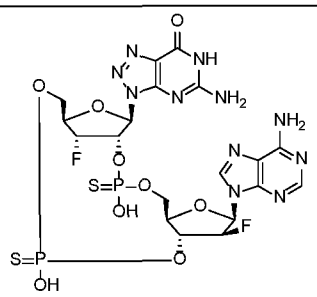
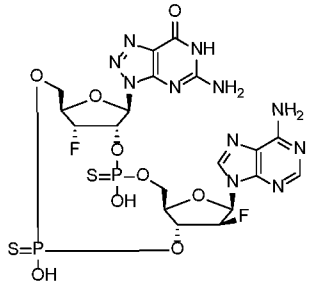
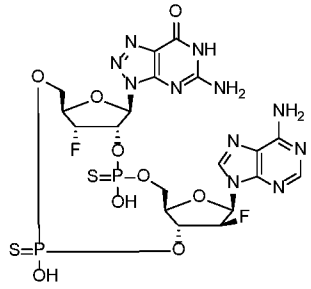
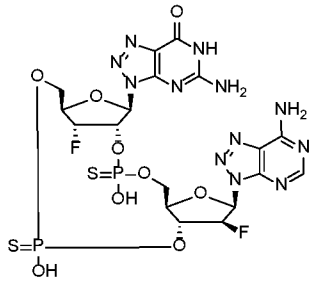
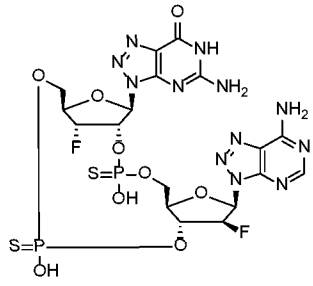
Ex.	Structure	Name	Mass [M-H]⁻
168		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	708
169		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	708
170		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	708
171		2-amino-9-[(2R, 5S,7R,8R,10R, 12aR,14R,15S,15aR)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	690
172		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	690
173		2-amino-9-[(2R, 5R,7R,8S,10R, 12aR,14R,15S,15aR,16S)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	708

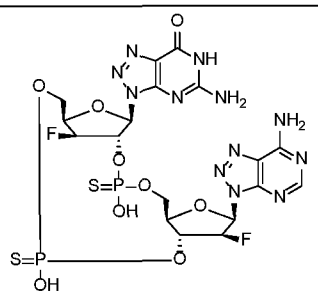
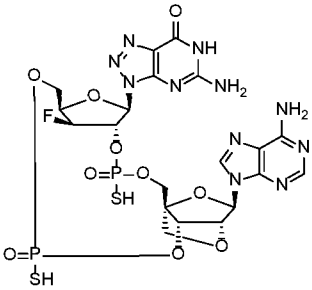
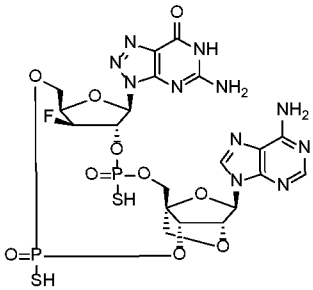
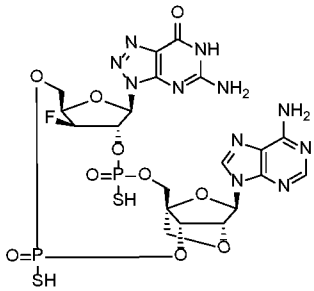
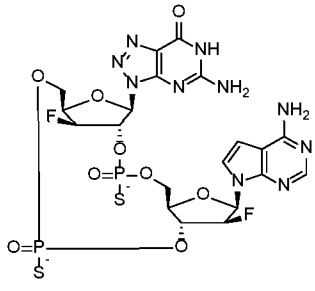
Ex.	Structure	Name	Mass [M-H]⁻
174		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	708
175		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	692
176		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	692
177		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	710
178		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	710

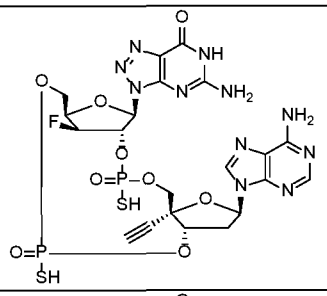
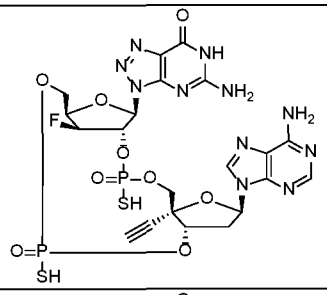
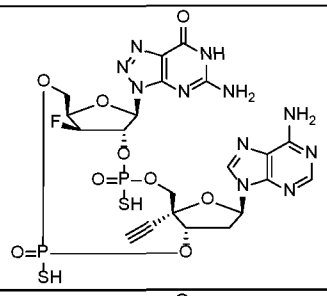
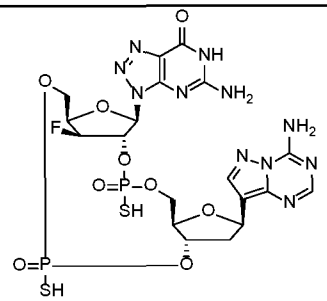
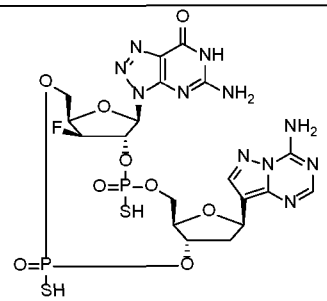
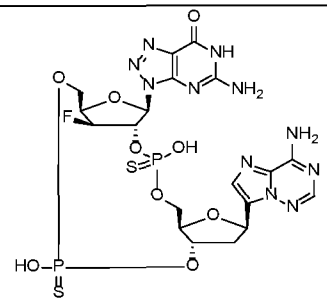
Ex.	Structure	Name	Mass [M-H]⁻
179		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	710
180		1-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one	693
181		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	693
182		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	693
183		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	693
184		5-amino-3-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	702

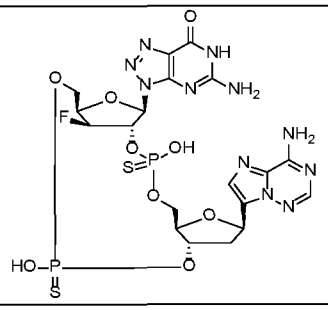
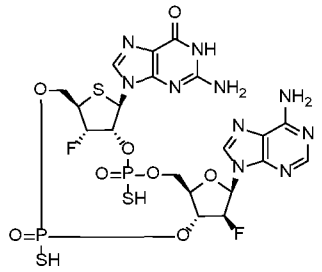
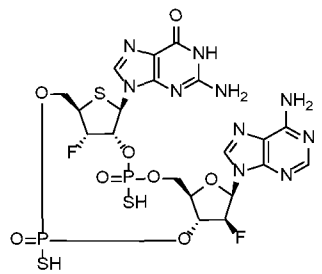
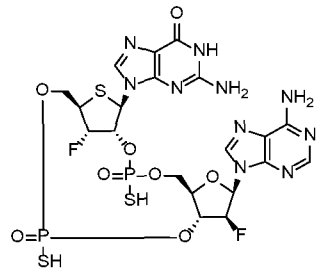
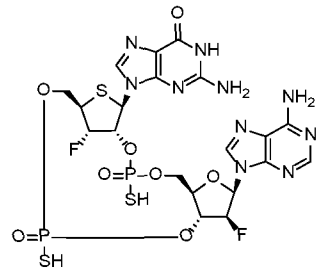
Ex.	Structure	Name	Mass [M-H]⁻
185		5-amino-3-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	702
186		5-amino-3-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	702
187		5-amino-3-[(5S,7R,8R,12aR,14R,15R,15aS)-14-(6-amino-9H-purin-9-yl)-2,10-dihydroxy-2,10-disulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 4)	702
188		5-amino-3-[(5S,7R,8R,12aR,14R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	673
189		5-amino-3-[(5S,7R,8R,12aR,14R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	673
190		5-amino-3-[(5S,7R,8R,12aR,14R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	673

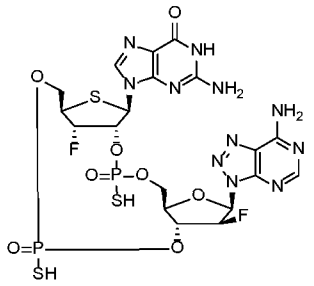
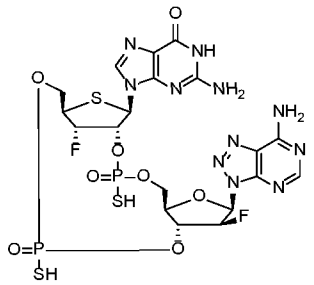
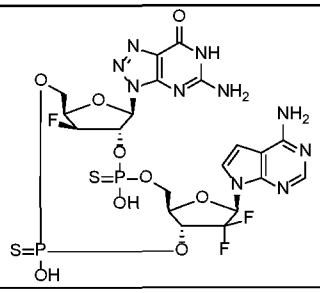
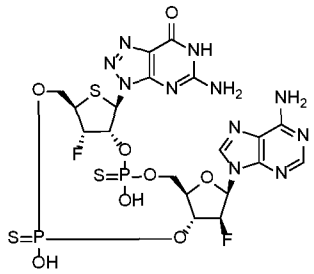
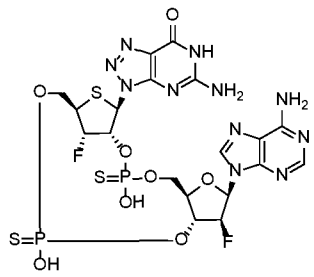
Ex.	Structure	Name	Mass [M-H]⁻
191		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	708
192		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	708
193		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	706
194		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	691
195		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	691
196		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	691

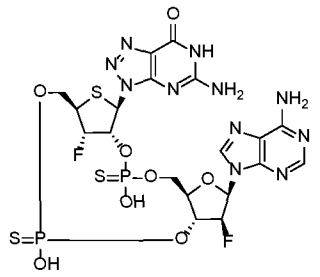
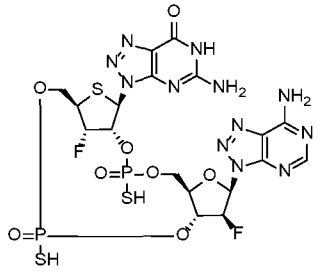
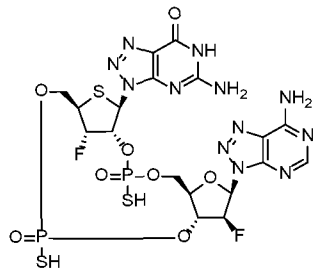
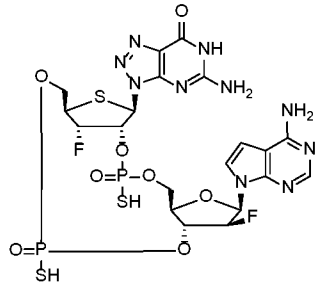
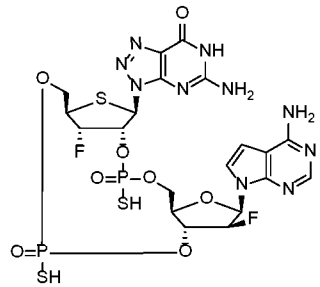
Ex.	Structure	Name	Mass [M-H]⁻
197		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	710
198		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	710
199		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	710
200		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	711
201		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	711

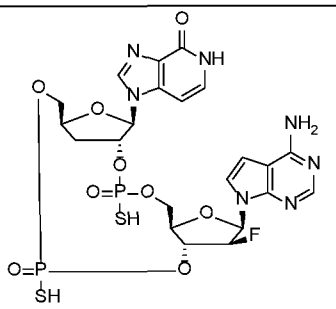
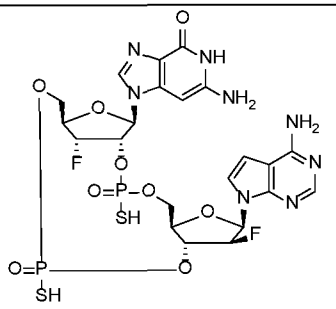
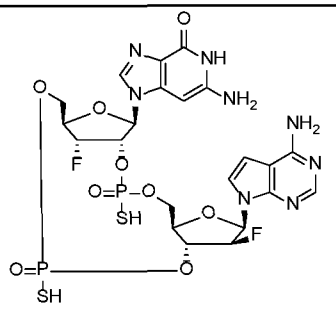
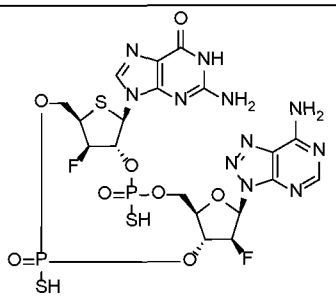
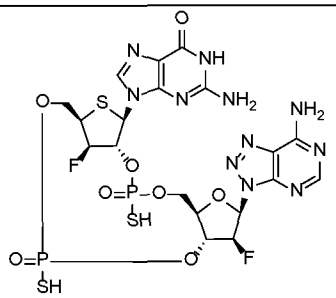
Ex.	Structure	Name	Mass [M-H]⁻
202		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one	711
203		5-amino-3-[(5R,7R,8S,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	720
204		5-amino-3-[(5R,7R,8S,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	720
205		5-amino-3-[(5R,7R,8S,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	720
206		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one	709

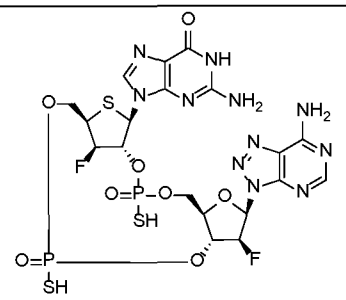
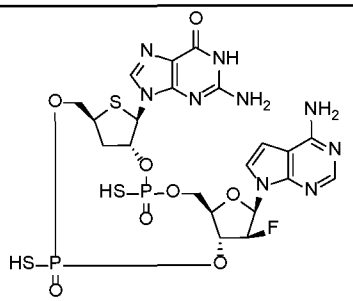
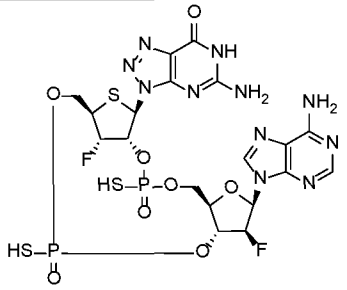
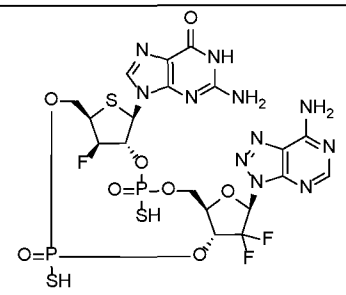
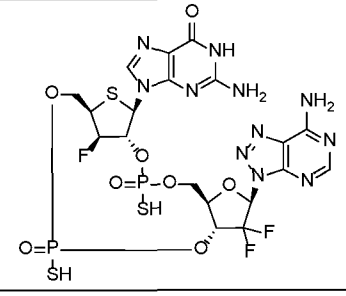
Ex.	Structure	Name	Mass [M-H]⁻
207		5-amino-3-[(5R,7R,8S,112aR,14R,15aS,16S)-14-(6-amino-9H-purin-9-yl)-12a-ethynyl-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	716
208		5-amino-3-[(5R,7R,8S,112aR,14R,15aS,16S)-14-(6-amino-9H-purin-9-yl)-12a-ethynyl-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	716
209		5-amino-3-[(5R,7R,8S,112aR,14R,15aS,16S)-14-(6-amino-9H-purin-9-yl)-12a-ethynyl-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	716
210		5-amino-3-[(5R,7R,8S,12aR,14R,15aS,16S)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	692
211		5-amino-3-[(5R,7R,8S,12aR,14R,15aS,16S)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	692
212		5-amino-3-[(5R,7R,8S,12aR,14R,15aS,16S)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	692

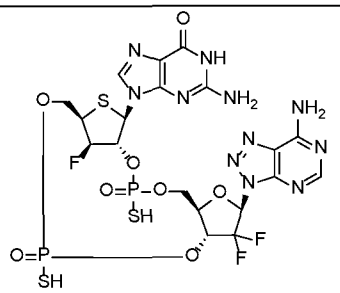
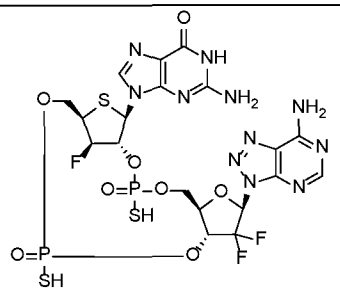
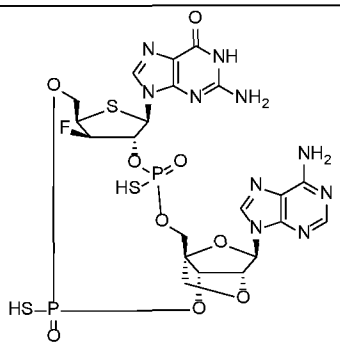
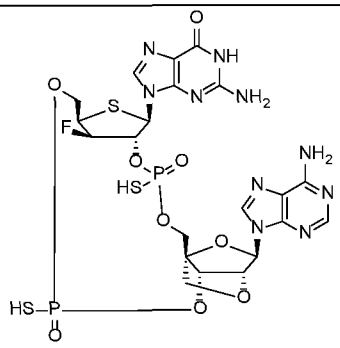
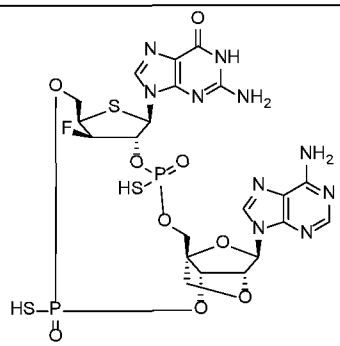
Ex.	Structure	Name	Mass [M-H]⁻
213		5-amino-3-[(5R,7R,8S,12aR,14R,15aS,16S)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	692
214		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	725
215		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	725
216		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	725
217		2-amino-9-[(2R,5R,7R,8R,10R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)	725

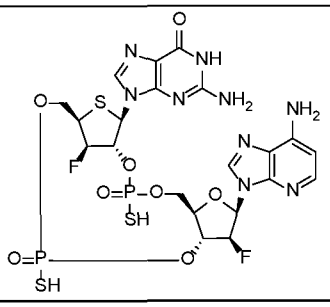
Ex.	Structure	Name	Mass [M-H]⁻
218		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	726
219		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	726
220		5-amino-3-[(5R,7R,8S,12aR,14R,15aR,16S)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,15,16-trifluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one	727
221		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	726
222		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	726

Ex.	Structure	Name	Mass [M-H]⁻
223		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	726
224		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	727
225		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	727
226		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	725
227		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	725

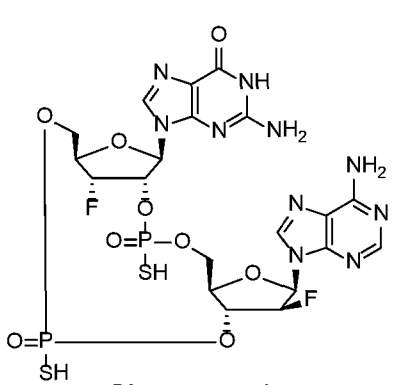
Ex.	Structure	Name	Mass [M-H]⁻
228		1-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one	674
229		6-amino-1-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one (Diastereomer 1)	707
230		6-amino-1-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one (Diastereomer 2)	707
231		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	726
232		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	726

Ex.	Structure	Name	Mass [M-H]⁻
233		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	726
234		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	706
235		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one	726
236		2-amino-9-[(5R,7R,8R,12aR,14R,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,15,16-trifluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	744
237		2-amino-9-[(5R,7R,8R,12aR,14R,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,15,16-trifluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	744

Ex.	Structure	Name	Mass [M-H]⁻
238		2-amino-9-[(5R,7R,8R,12aR,14R,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,15,16-trifluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	744
239		2-amino-9-[(5R,7R,8R,12aR,14R,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,15,16-trifluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)	744
240		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	735
241		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	735
242		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dioxido-2,10-disulfanylhexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	735

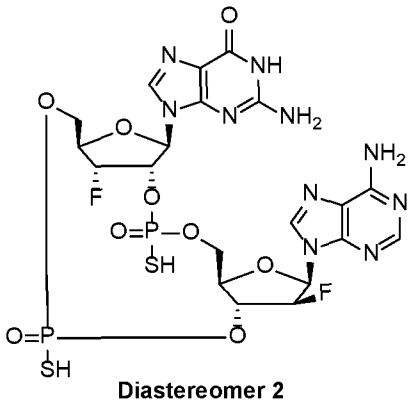
Ex.	Structure	Name	Mass [M-H]⁻
243		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-imidazo[4,5-b]pyridin-3-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	724

Examples 244, 245, 246, and 247: 2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomers 1 – 3) and 2-amino-9-[(2R,5*R*,7*R*,8*S*,10R,12a*R*,14*R*,15*S*,15a*R*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 4)



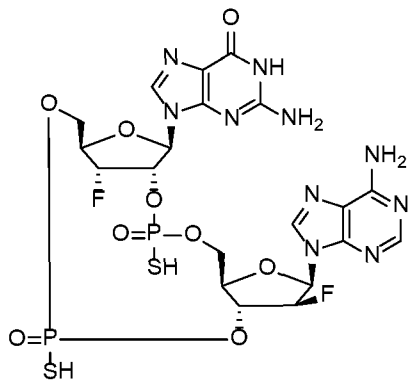
Diastereomer 1

,



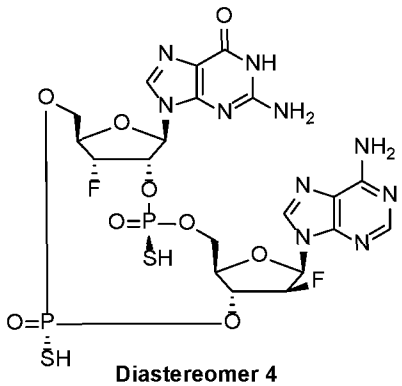
Diastereomer 2

,



Diastereomer 3

,

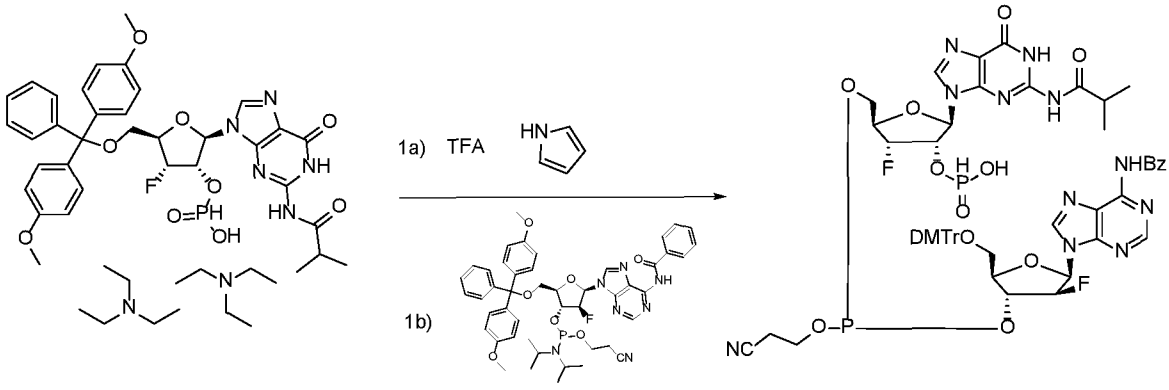


Diastereomer 4

,

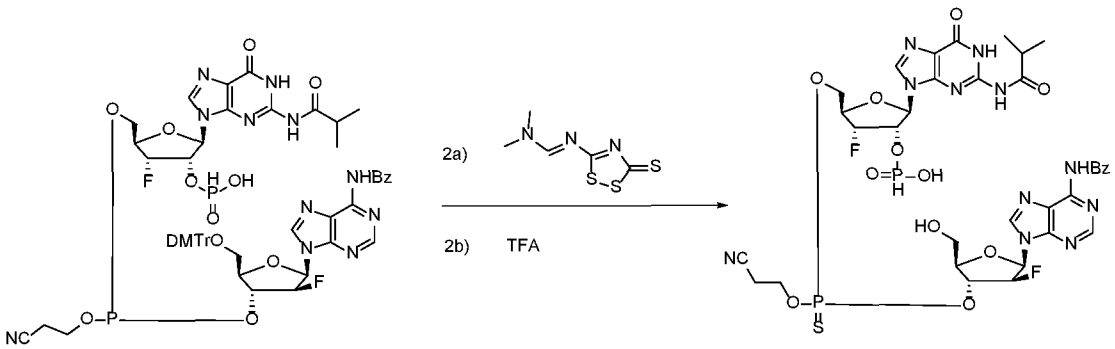
Step 1: (2R,3S,4R,5R)-5-((((((2R,3R,4S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl)oxy)(2-

cyanoethoxy)phosphanyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate



Pyrrole (0.087mL, 1.2mmol) was added to a solution of (2R,3S,4R,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate triethylamine salt (1:2) (0.34g, 0.41mmol) in acetonitrile (3.0mL) under an argon atmosphere at 0°C. After 5min, TFA (0.096mL, 0.14mmol) was added, and the reaction mixture was stirred at 0°C for 30min. Pyridine (0.13mL, 1.7mmol) was added drop wise at 0°C. The reaction mixture was then stirred for 10min at 0°C. At that time, a mixture of (2R,3R,4S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (0.48g, 0.55mmol) in acetonitrile (3.0mL) was added drop wise over 5min to the reaction mixture under an argon atmosphere at 0°C. The reaction mixture was stirred at 0°C for 20min and immediately used in the next step without further manipulation.

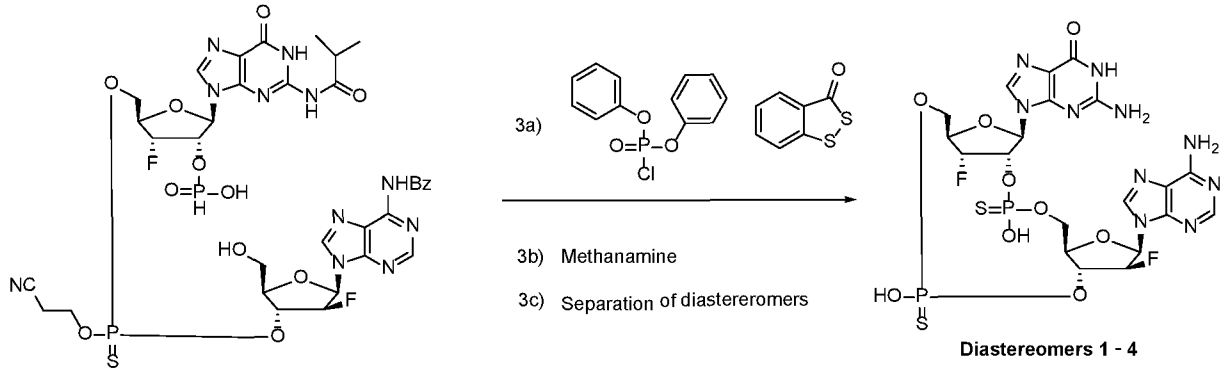
Step 2: (2R,3S,4R,5R)-5-((((((2R,3R,4S,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate



To the crude reaction mixture from Step 1 was added (*E*)-N,N-dimethyl-N'-(3-thioxo-3H-1,2,4-dithiazol-5-yl)formimidamide (0.10g, 0.50mmol) under an argon atmosphere at 0°C. The

reaction mixture was stirred for 45 minutes at 0°C. At that time, 1-propanol (0.31mL, 4.13mmol) was added to the reaction mixture under an argon atmosphere at 0°C. The reaction mixture was then allowed to warm to ambient temperature and stirred for 10min. TFA (0.32mL, 4.1mmol) was added to the reaction mixture, and the reaction mixture was stirred for 30min at ambient temperature. Pyridine (0.37mL, 4.6mmol) was added at ambient temperature, and the reaction mixture was stirred for 10min. The reaction mixture was concentrated under reduced pressure to approximately one-half volume. The mixture was then diluted with isopropyl acetate (20mL) and stirred for 30min at ambient temperature. The resulting suspension was filtered. The collected solids were dried overnight under high vacuum to afford (2R,3S,4R,5R)-5-((((((2R,3R,4S,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate. LCMS (ES, m/z): 922 [M - H]$^-$.

Step 3: 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one



(2R,3S,4R,5R)-5-((((((2R,3R,4S,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate (0.30g, 0.33mmol) was azeotroped with dry pyridine (2x10mL) and then dried under high vacuum for 1h. In a separate flask, diphenyl phosphorochloridate (0.34mL, 1.6mmol) was added to a mixture of acetonitrile (15mL) and pyridine (1.0mL). The resulting solution was then cooled to -20°C. To this mixture was added drop wise over a period of 5min a mixture of (2R,3S,4R,5R)-5-((((((2R,3R,4S,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)-methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl hydrogen

phosphonate (0.30g, 0.33mmol) in pyridine (4.0mL) at -20°C. The reaction mixture was then stirred at -20°C for 15min post-addition. 3H-benzo[c][1,2]dithiol-3-one (0.066g, 0.39mmol) and water (0.12mL, 6.5mmol) were then added to the reaction mixture at -20°C. The reaction mixture was allowed to gradually warm to ambient temperature. The reaction mixture was stirred for 30min at ambient temperature. The reaction mixture was then concentrated under reduced pressure to approximately one quarter volume. The reaction mixture was cooled to 0°C, and methanamine (33% in ethanol) (2.63mL, 24mmol) was added drop wise. After the addition was complete, the reaction mixture was allowed to warm to ambient temperature. The reaction mixture was stirred at ambient temperature for 18h. The reaction mixture was concentrated under reduced pressure to afford the crude product residue. The crude product residue was azeotroped (3x30mL ethanol) to afford the crude product. This material was dissolved in water (5mL) and acetonitrile (1mL). The resulting mixture was purified by mass-directed reverse phase HPLC (Waters Sunfire 19x250 mm, UV 215/254 nm, fraction trigger by SIM negative MS monitoring mass 709; mobile phase = 100mM triethylammonium acetate in water/acetonitrile gradient, 2-30% acetonitrile over 40 min) to afford the 4 diastereomers of 2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one.

Example 244: 2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 1): T_R = 17.7 min. LCMS (ES, m/z): 709 $[M - H]^-$.

Example 245: 2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 2): T_R = 21.9 min. LCMS (ES, m/z): 709 $[M - H]^-$. 1H NMR (500MHz, DMSO-d_6) δ 8.32 (s, 1H), 8.21 – 8.09 (m, 2H), 7.46 – 7.29 (m, 2H), 6.59 – 6.43 (m, 2H), 6.40 – 6.29 (m, 1H), 5.88 (d, J = 8.8Hz, 1H), 5.49 – 5.19 (m, 4H), 4.45 – 4.32 (m, 2H), 4.10 – 3.93 (m, 2H), 3.94 – 3.82 (m, 1H), 3.80 – 3.68 (m, 1H).

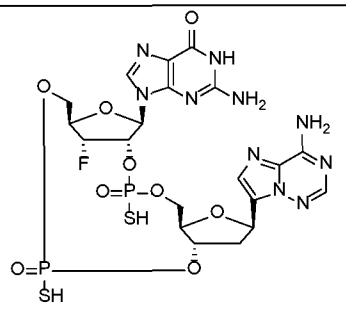
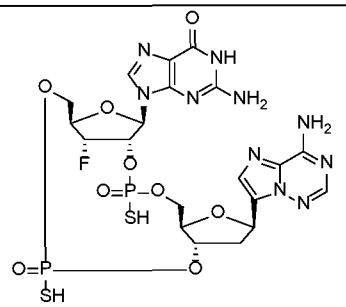
Example 246: 2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 3): T_R = 23.8 min. LCMS (ES, m/z): 709 $[M - H]^-$. 1H NMR (500MHz, DMSO-

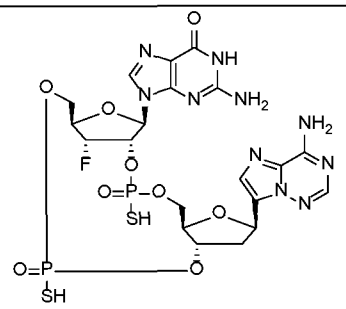
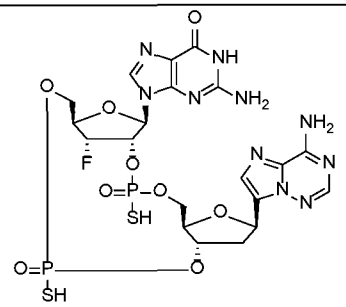
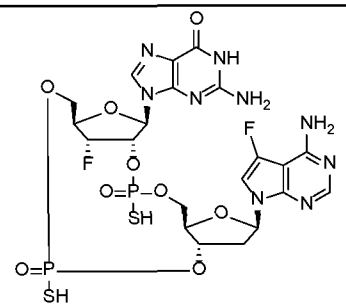
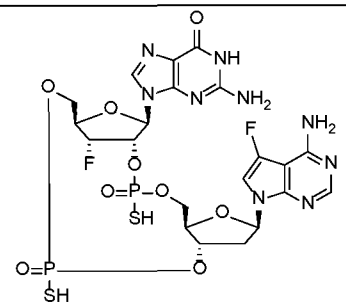
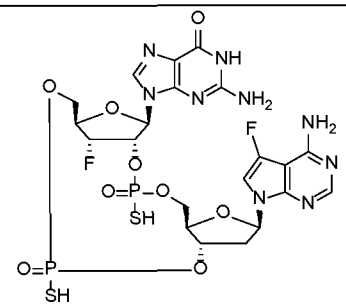
d$_6$) δ 8.18 – 8.08 (m, 3H), 7.41 – 7.33 (m, 2H), 6.59 – 6.47 (m, 2H), 6.37 – 6.27 (m, 1H), 5.84 (d, J = 8.7Hz, 1H), 5.52 – 5.26 (m, 2H), 5.21 – 5.11 (m, 1H), 4.46 – 4.35 (m, 2H), 4.19 – 4.02 (m, 2H), 3.83 – 3.65 (m, 2H).

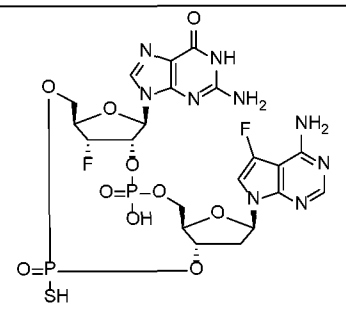
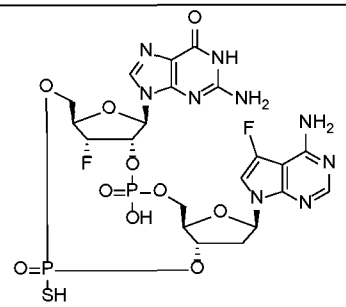
Example 247: 2-amino-9-[(2R,5*R*,7*R*,8*S*,10R,12a*R*,14*R*,15*S*,15a*R*,16*R*)-14-(6-amino-9*H*-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 4): T_R = 26.4 min. LCMS (ES, m/z): 709 [M - H]$^-$. ^{1}H NMR (500MHz, DMSO-*d*$_6$) δ 8.19 – 8.07 (m, 3H), 7.41 – 7.32 (m, 2H), 6.70 – 6.50 (m, 2H), 6.40 – 6.29 (m, 1H), 5.85 (d, J = 8.7Hz, 1H), 5.33 – 5.25 (m, 2H), 5.23 – 5.12 (m, 1H), 4.48 – 4.35 (m, 1H), 4.33 – 4.24 (m, 1H), 4.09 – 3.93 (m, 2H), 3.92 – 3.81 (m, 1H), 3.83 – 3.70 (m, 1H).

Examples 248 through 256, as shown in Table 7 below, were prepared according to procedures analogous to those outlined in Examples 244 through 247 above using the appropriate monomers, decribed as Preparations or as obtained from commercial sources, in the coupling step.

Table 7

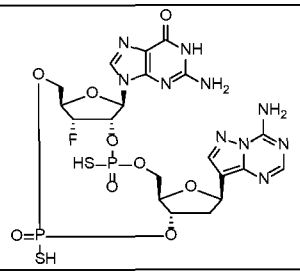
Ex.	Structure	Name	Mass [M-H]$^-$
248		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	691
249		2-amino-9-[(5R,7R,8S, 12aR,14R,15aS,16R)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	691

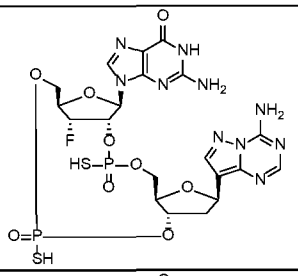
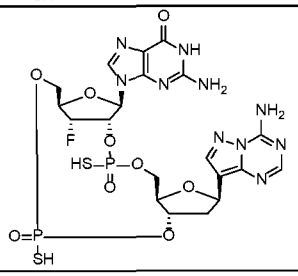
Ex.	Structure	Name	Mass [M-H]⁻
250		2-amino-9-[(5R,7R,8S, 12aR,14R,15aS,16R)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	691
251		2-amino-9-[(5R,7R,8S, 12aR,14R,15aS,16R)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 4)	691
252		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	708
253		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	708
254		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	708

Ex.	Structure	Name	Mass [M-H]⁻
255		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-16-fluoro-10-hydroxy-2,10-dioxido-2-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	692
256		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-16-fluoro-10-hydroxy-2,10-dioxido-2-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	692

Examples 257 through 259 in Table 8 were made according to procedures analogous to those described above for Examples 244 through 247 using the appropriate monomeric nucleosides, decribed as Preparations or as obtained from commercial sources, with the following additional representative treatment as a final step: A sample (0.12mmol) was dissolved in water (6mL), and the resulting mixture was applied to ion exchange resin in a column (DOWEX 50WX2 hydrogen form, 100-200 mesh, 1.5g, pre-washed with 10mL water, and then packed in column before compound loading occurred). After loading mixture had completely absorbed to column, the column was then washed with additional water (10mL). The eluent was lyophilized to afford product.

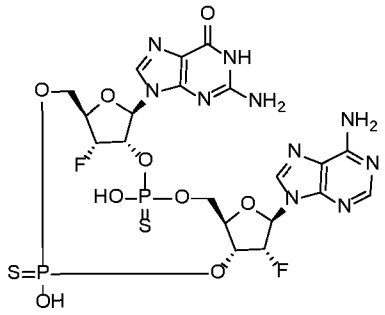
Table 8

Ex.	Structure	Name	Mass [M-H]-
257		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	691

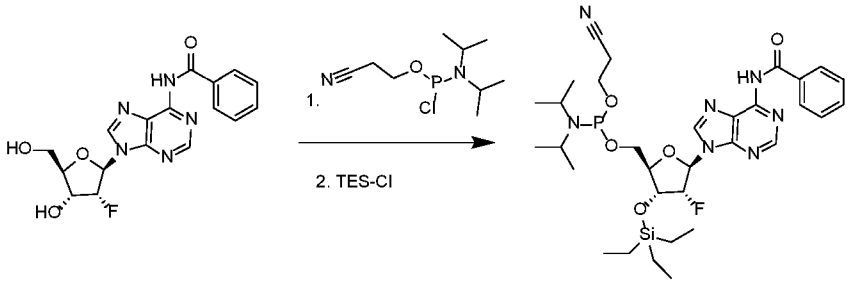
Ex.	Structure	Name	Mass [M-H]-
258		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	691
259		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	691

Alternatively, Examples 117-142, 144-152, 172, 174, and 244-247, above were made using procedures similar to those described for Examples 81 through 83 above.

Example 260: 2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one



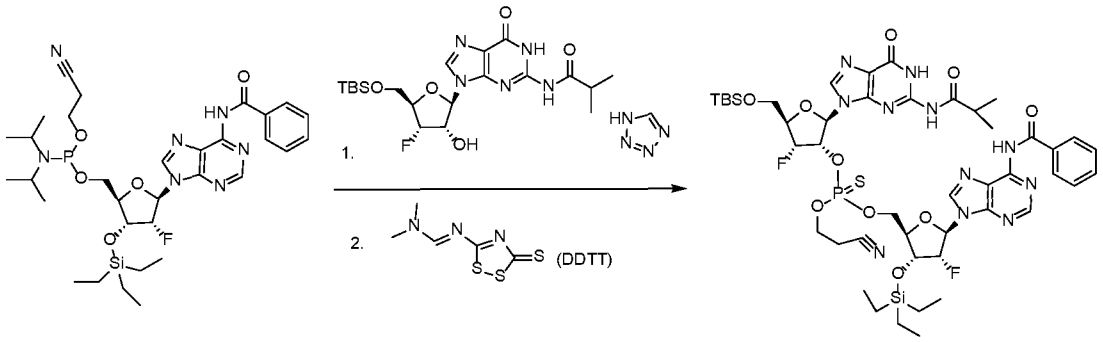
Step 1: ((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-3-((triethylsilyl)oxy)tetrahydrofuran-2-yl)methyl (2-cyanoethyl) diisopropylphosphoramidite



To a solution of N-(9-((2R,3R,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-9H-purin-6-yl)benzamide (780mg, 2.089mmol) in DMF (8mL) and

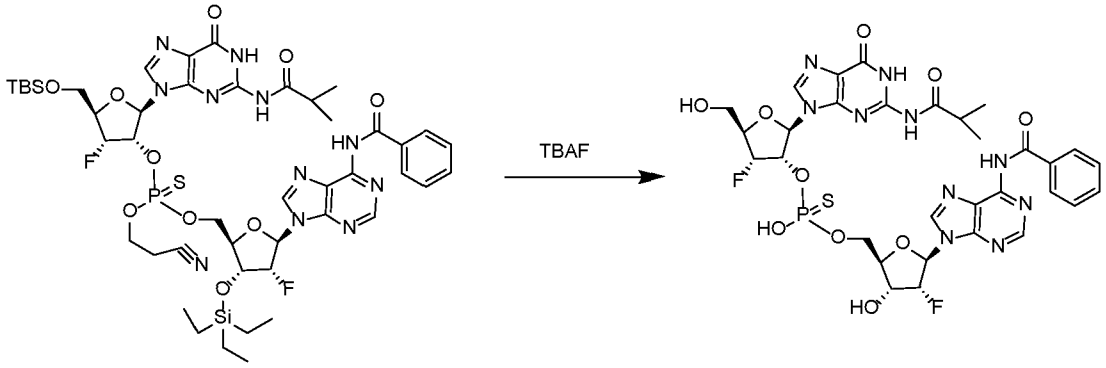
DIPEA (1.116mL, 6.39mmol) at 0°C was added 200mg actived molecular sieve 4Å and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (572mg, 2.343mmol) in 1ml dry CH_3CN. The resulting mixture was stirred at 0°C for 5h; chlorotriethylsilane (401mg, 2.66mmol) was added dropwise. The resulting mixture was stirred at rt overnight. The reaction carried directly to next step. LCMS (ES, m/z): 686 $[M - H]^-$

Step 2: O-(((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-3-((triethylsilyl)oxy) tetrahydrofuran-2-yl)methyl) O-((2R,3S,4R,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl) O-(2-cyanoethyl) phosphorothioate



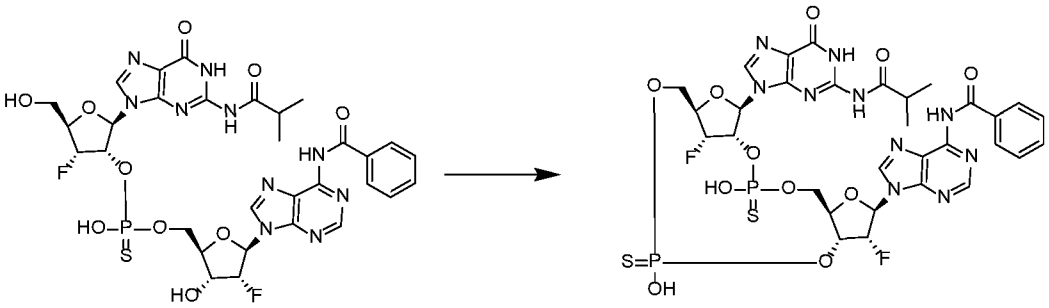
To a solution of the product of step 1was added N-(9-((2R,3S,4S,5R)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (500mg, 1.065mmol) and 1H-tetrazole (895mg, 12.78mmol). The mixture was stirred at RT for 2h, and DDTT (568g, 2.77mmol) was added. The mixture was stirred for 1h, then partitioned between ethyl acetate and H_2O, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel , eluting with 0-6% MeOH/DCM to give O-(((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-3-((triethylsilyl)oxy)tetrahydrofuran-2-yl)methyl) O-((2R,3S,4R,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl) O-(2-cyanoethyl) phosphorothioate. LCMS (ES, m/z): 1088 $[M + H]^+$

Step 3: O-(((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methyl) O-((2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl) O-hydrogen phosphorothioate



To a solution of the mixture (520mg) from step 3 in THF (5mL) was added TBAF (1.0M in THF) (1.140mL, 1.140mmol). The resulting mixture was stirred at RT for 2h and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel , eluting with 0-8% MeOH/DCM to give O-(((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methyl) O-((2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1H-purin-9(6H)-yl)tetrahydrofuran-3-yl) O-hydrogen phosphorothioate. LCMS (ES, m/z): 807 $[M + H]^+$

Step 4: N-{9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-15,16-difluoro-2,10-dihydroxy-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-9H-purin-6-yl}benzamide

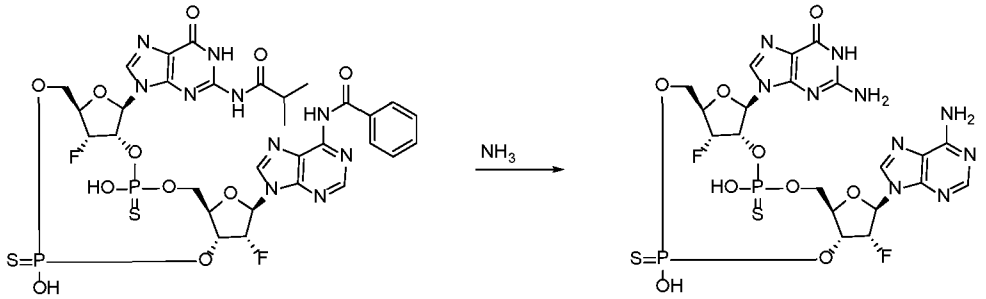


O-(((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methyl) O-((2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1H-purin-9(6H)-yl)tetrahydrofuran-3-yl) O-hydrogen phosphorothioate (250mg, 0.310mmol) and diisopropylammonium tetrazolide (80mg, 0.465mmol) were azeotrope with dry CH_3CN (3x10ml) and dried under high vacum for 30min.

The above mixture was dissolved in DMF (1mL) and acetonitrile (7mL) and added 200mg active molecular sieve 4Å and a solution of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite (128mg, 0.403mmol) in 1ml dry CH_3CN. The resulting mixture was stirred at rt for 30min, followed by addition of 1H-tetrazole (109mg, 1.550mmol).

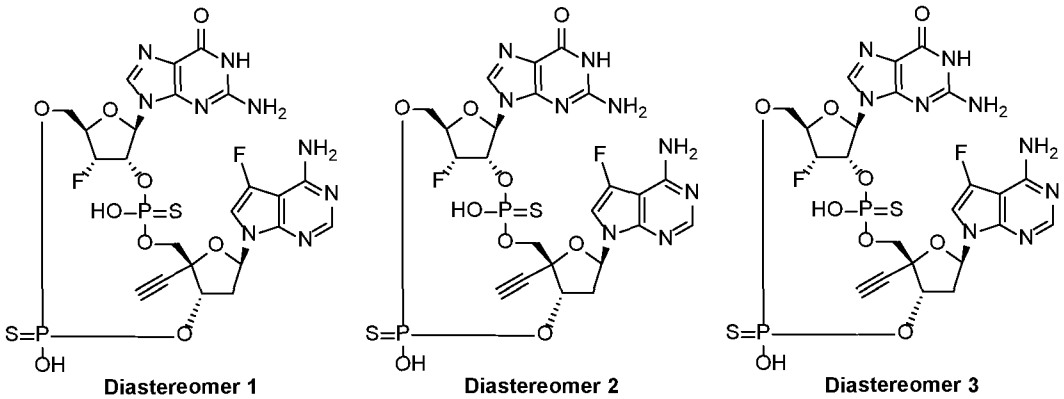
The reaction stayed at rt for 1h and added DDTT (95mg, 0.465mmol). The stirring continued for 1h and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with 0-10% MeOH / DCM with 1% ET3N to give the desired product. LCMS (ES, m/z): 883 $[M - H]^-$

Step 5: 2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one

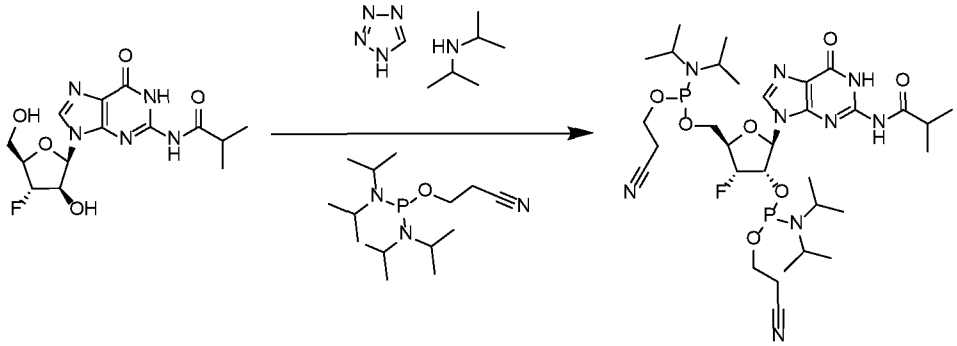


N-{9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-15,16-difluoro-2,10-dihydroxy-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-9H-purin-6-yl}benzamide (120mg, 0.136mmol) and ammonia (7.0M in MeOH) (2ml, 14.00mmol) were sealed in a microwave tube. The reaction mixture was heated to 50°C and stirred for 4h. The reaction mixture was concentrated, and purified using mass-directed reverse phase HPLC (X-Bridge BEH 150 Prep C18) using a gradient solvent system with MeCN and 100mM aqueous triethylammonium acetate to give desired product. Lyopholization of the product fractions furnished 2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]-pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one. LCMS (ES, m/z): 709 $[M - H]^-$. ^1H NMR (H_2O-d2, 500MHz): δ_H 8.23 (1H, s), 8.17 (1H, s), 7.93 (1H, s), 6.43 (1H, d, J = 15.0Hz), 6.00 (1H, d, J = 8.7Hz), 5.75 (1H, m), 5.58 (2H, m), 5.15 (1H, m), 4.65 (2H, m), 4.54 (1H, m), 4.15-4.30 (3H, m). ^{31}P NMR: (H_2O-d_2, 202MHz): δ 52.1, 52.2.

Examples 261, 262, 263: 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-12a-ethynyl-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomers 1 – 3)

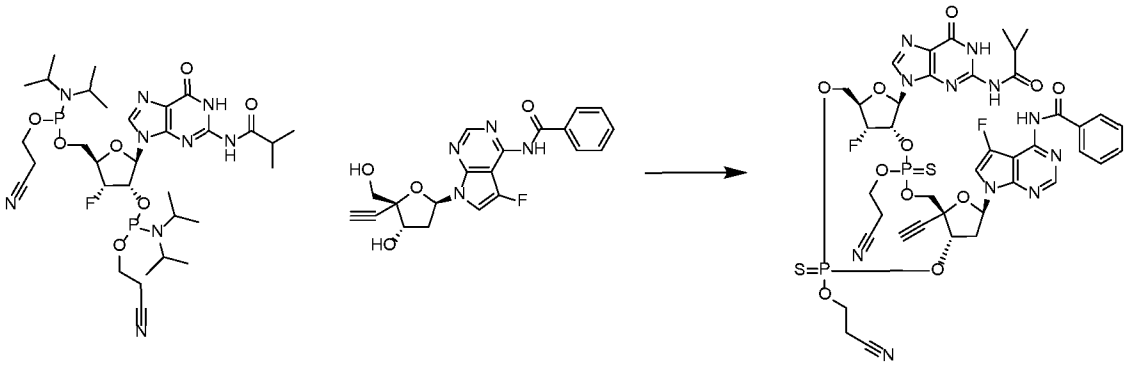


Step 1: ((2R,3R,4S,5R)-4-(((2-cyanoethoxy)(diisopropylamino)phosphanyl)oxy)-3-fluoro-5-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl (2-cyanoethyl) diisopropylphosphoramidite



To a solution of N-(9-((2R,3R,4S,5R)-4-fluoro-3-hydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)isobutyramide (190mg, 0.535mmol) and 3-((bis(diisopropylamino)phosphino)oxy)propanenitrile (95%) (373mg, 1.176mmol) in DMF (5ml) was added diisopropylammonium tetrazolide (137mg, 0.802mmol). The mixture was stirred at RT for 2h, then added 1H-tetrazole (15.77mg, 0.225mmol) and continued for 2h. 300mg actived molecular sieve 4A was added and contuned stirring at RT for 2h. The reaction carried directly to next step. LCMS (ES, m/z): 754 $[M - H]^-$

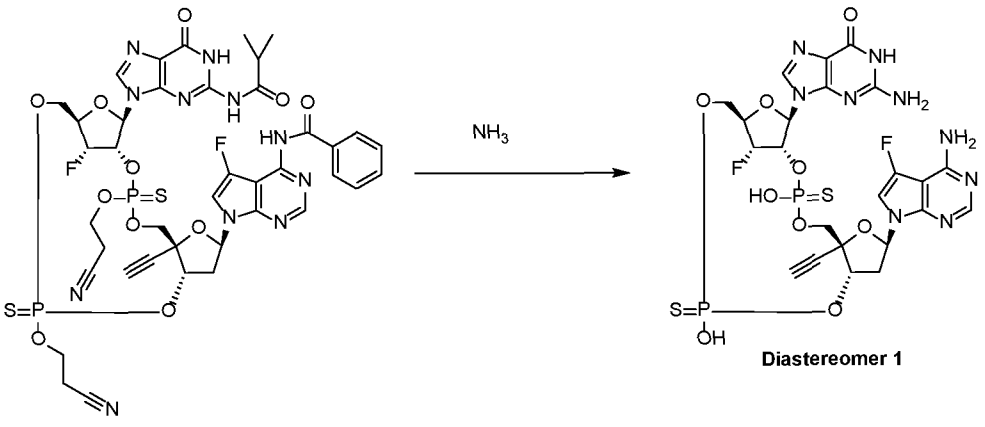
Step 2: N-{7-[(5R,7R,8S,12aR,14R,15aS,16R)-2,10-bis(2-cyanoethoxy)-12a-ethynyl-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-4-yl}benzamide



To a solution of N-(7-((2R,4S,5R)-5-ethynyl-4-hydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzamide (180mg, 0.454mmol) and Et_3N (0.114mL, 0.817mmol) in DMF (4mL) at -78°C was added a solution of TMS-Cl (0.070mL, 0.545mmol) in CH_2Cl_2 (1mL) dropwise. The mixture was stirred at -78°C for 20min and warmed to 0°C within 1h, then added 200mg actived molecular sieve 4Å and continued stirring at RT for 2h. The mixture was cooled to 0°C and transfered to a stirred solution of the product of Step 1 (394mg, 0.454mmol) (precooled to -0°C), followed by addition of 1H-tetrazole (191mg, 2.72mmol). The mixture was gradually warmed to RT and stirred at RT for 2h. The filtration removed the solids. After washed with 3ml DMF, the combined filtrate was added additional 1H-tetrazole (191mg, 2.72mmol) and stirred at RT overnight.

After completion of reaction, the reaction mixture was diluted with ethyl acetate and washed successively with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4 and purified by a silica gel chromatography, eluting with 0-8% MeOH/DCM to give two desired fractions with the same molecular weight. LCMS (ES, m/z): 1014 $[M + H]^+$

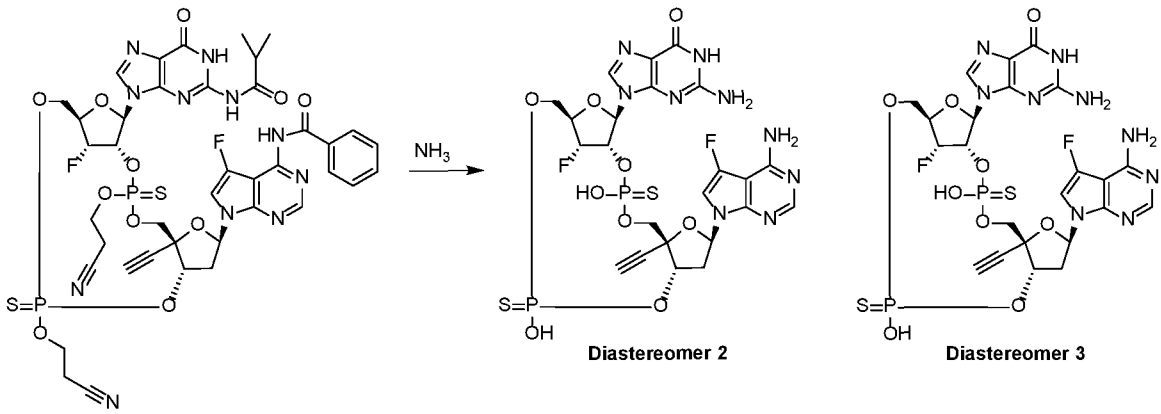
Step 3: 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-12a-ethynyl-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one



N-{7-[(5R,7R,8S,12aR,14R,15aS,16R)-2,10-bis(2-cyanoethoxy)-12a-ethynyl-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-4-yl}benzamide (faster fraction from step 2) (30mg, 0.030mmol) and 7.0M ammonia in MeOH (2ml, 14.00mmol) were sealed in a microwave tube. The mixture was heated to 50°C and stirred for 8h. The reaction mixture was concentrated, and the product was purified using mass-directed reverse phase HPLC (X-Bridge BEH 150 Prep C18) using a gradient solvent system with MeCN and 100mM aqueous triethylammonium acetate. Lyopholization of the product fractions furnished 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-12a-ethynyl-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one as the bis-triethylamine salt.

Example 261 (Diastereomer 1): LCMS (ES, m/z): 732 [M - H]$^-$. ^{1}H NMR (H_2O-d_2, 500MHz): δ_H 8.32 (1H, s), 8.08 (1H, s), 7.05 (1H, s), 6.70 (1H, t, J = 6.1Hz), 6.08 (1H, d, J = 8.7Hz), 5.71 (1H, d, J = 53.2Hz), 5.47-5.40 (1H, m), 5.14-5.10 (1H, m), 4.65 (2H, m), 4.28 (1H, t, J = 8.7Hz), 4.14-4.02 (3H, m), 2.85 (2H, d, *J* = 6.4Hz). ^{31}P NMR: (H_2O-d_2, 202MHz): δ 53.7, 54.0.

Step 4: 2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-14-(4-amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-12a-ethynyl-16-fluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one



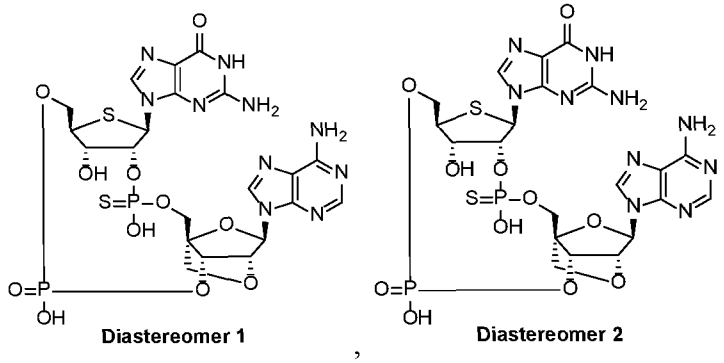
N-{7-[(5R,7R,8S,12aR,14R,15aS,16R)-2,10-bis(2-cyanoethoxy)-12a-ethynyl-16-fluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-5-fluoro-

7H-pyrrolo[2,3-d]pyrimidin-4-yl}benzamide (slow fraction from Step 2, 65mg, 0.064mmol) and 7.0M ammonia in MeOH (2ml, 14.00mmol) were sealed in a microwave tube and heated to 50°C and stirred for 8h. The reaction mixture was concentrated and purified using mass-directed reverse phase HPLC (X-Bridge BEH 150 Prep C18) using a gradient solvent system with MeCN and 100mM aqueous triethylammonium acetate to give two additional diastereomers after lyopholization of the product fractions.

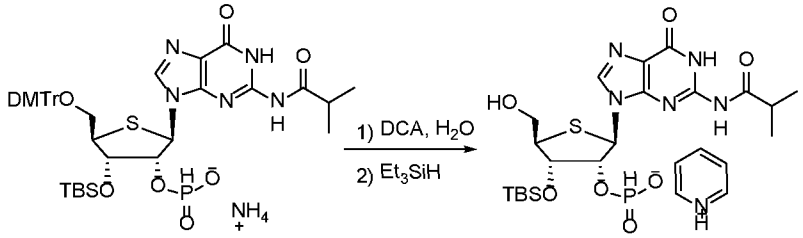
Example 262 (Diastereomer 2): LCMS (ES, m/z): 732 $[M - H]^-$. ^1H NMR (H_2O-d_2, 500MHz): δ_H 8.09 (2H, d, J = 3.0Hz), 7.19 (1H, s), 6.74 (1H, s), 6.08 (1H, d, J = 8.3Hz), 5.63 (1H, s), 5.53 (1H, s), 5.35-5.33 (1H, m), 4.65 (2H, m), 4.34 (1H, d, J = 8.8Hz), 4.28 (1H, t, J = 10.3Hz), 4.11 (1H, d, J = 12.1Hz), 3.89 (1H, dd, J = 10.6, 4.0Hz), 2.85 (2H, d, J = 6.4Hz). ^{31}P NMR: (H_2O-d_2, 202MHz): δ 54.7, 59.8.

Example 263 (Diastereomer 3): LCMS (ES, m/z): 732 $[M - H]^-$. ^1H NMR (H_2O-d_2, 500MHz): δ_H 8.08 (1H, s), 7.94 (1H, s), 7.04 (1H, s), 6.68 (1H, s), 6.04 (1H, d, J = 8.6Hz), 5.64 (1H, s), 5.54 (1H, d, J = 3.3Hz), 5.24 (1H, d, J = 7.7Hz), 4.65 (2H, m), 4.40 (1H, d, J = 8.2Hz), 4.18 (1H, dd, J = 11.0, 5.5Hz), 4.09-4.00 (2H, m), 2.93-2.89 (1H, m), 2.82 (1H, d, J = 7.1Hz). ^{31}P NMR: (H_2O-d_2, 202MHz): δ 53.1, 54.9.

Examples 264 and 265: 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2-oxido-10-sulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1) and 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2-oxido-10-sulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)

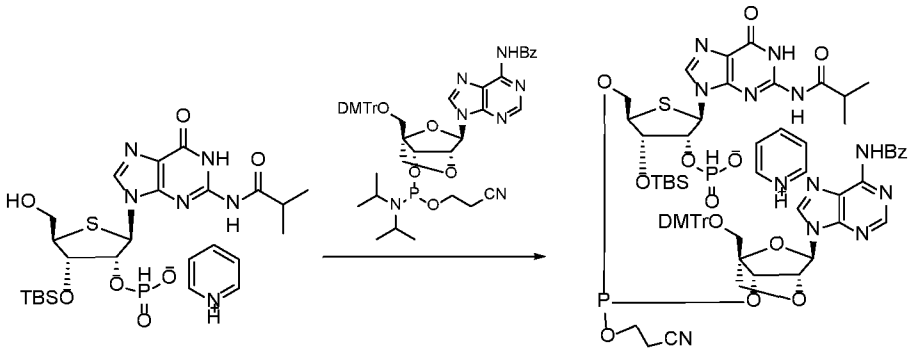


Step 1: (2R,3R,4S,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl phosphonate



To a solution of (2R,3R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl phosphonate (750mg, 0.865mmol) in CH_2Cl_2 (9mL) was added water (156mg, 8.65mmol) and 2,2-dichloroacetic acid in CH_2Cl_2 (0.6M, 11mL, 6.6mmol). The mixture was stirred at rt for 15min, and then, Et_3SiH (4.00mL) was added. After 1h, pyridine (1232mg, 15.57mmol) was added, and it was concentrated to give a crude sample that was used for next reaction step without purification. LCMS (ES, m/z): 548.1 $[M + H]^+$.

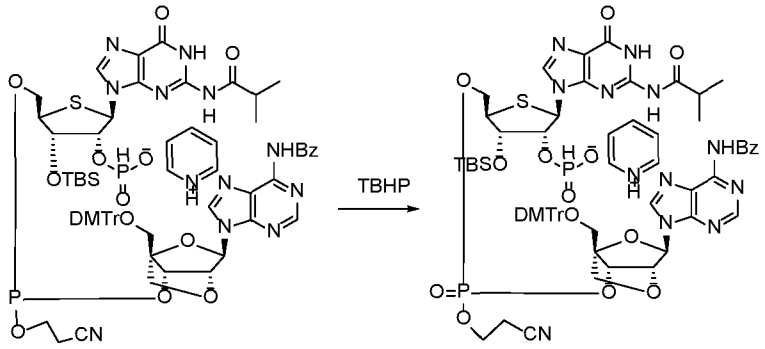
Step 2: (2R,3R,4S,5R)-5-((((((1R,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphanyl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl phosphonate



The crude sample from Step 1 was co-evaporated with ACN (3x2mL), re-dissolved in ACN (3mL), and dried by adding activated 4Å molecular sieve (150mg). (1R,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl (2-cyanoethyl) diisopropylphosphoramidite (0.843g, 0.952mmol) was also co-evaporated with ACN (3x1mL), re-dissolved in ACN (3mL), and dried by adding activated 4 Å molecular sieve (150mg). After 30min, it was added to the previously prepared mixture containing pyridin-1-ium (2R,3R,4S,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl

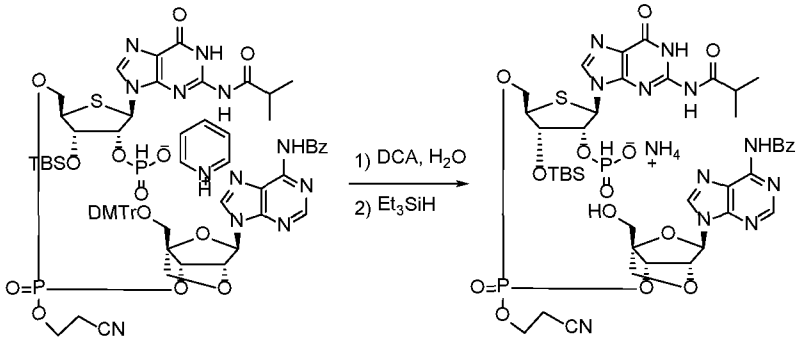
phosphonate. The reaction mixture was used in the next reaction step without purification. LCMS (ES, m/z): 1332.1 $[M + H]^+$.

Step 3: (2R,3R,4S,5R)-5-((((((1R,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl phosphonate



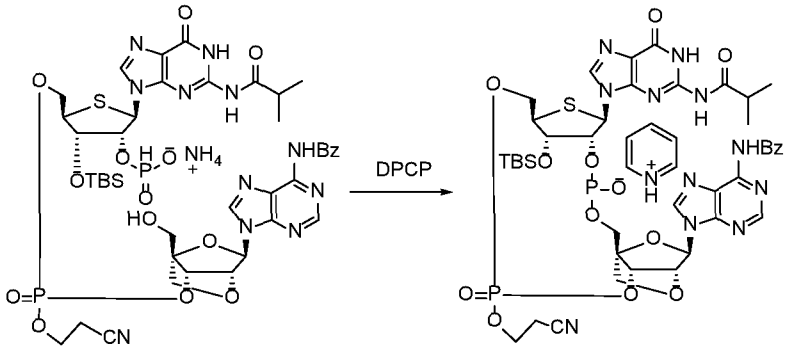
To a reaction mixture from Step 2 at rt was added 2-hydroperoxy-2-methylpropane (0.234g, 2.60mmol), and it was stirred for 40min. It was concentrated to give a crude product, which was used for the next step without purification. LCMS (ES, m/z): 1347.1 $[M + H]^+$.

Step 4: (2R,3R,4S,5R)-5-((((((1S,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl phosphonate



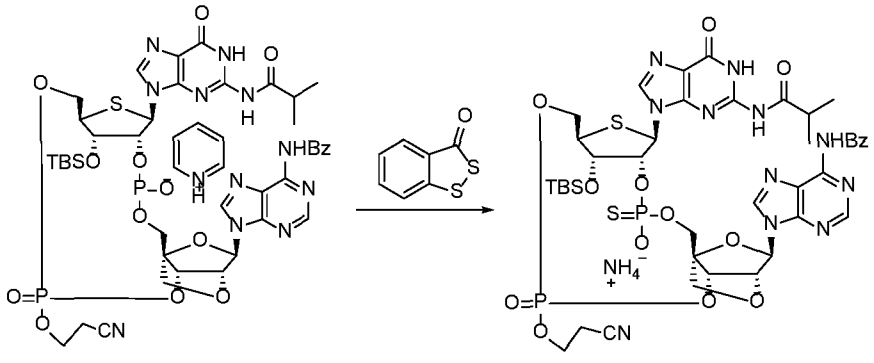
To the crude from Step 3 in CH_2Cl_2 (9mL) was added water (156mg, 8.65mmol) and 2,2-dichloroacetic acid in CH_2Cl_2 (0.6N, 10mL, 6mmol). The resulting mixture was stirred at rt for 15min. Then, triethylsilane (4mL, 0.21mmol) was added, and the stirring was continued for 40min. Pyridine (1232mg, 15.57mmol) was added. The mixture was concentrated, and the residue was purified by reverse phase (C18) chromatography eluted with 0 to 95% ACN in aq NH_4HCO_3 (5mM) to give the product. LCMS (ES, m/z): 1046.3 $[M + H]^+$.

Step 5: (5R,7R,8R,12aR,14R,15R,15aS,18S)-18-{[tert-butyl(dimethyl)silyl]oxy}-2-(2-cyanoethoxy)-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}hexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-10(12H)-olate 2-oxide



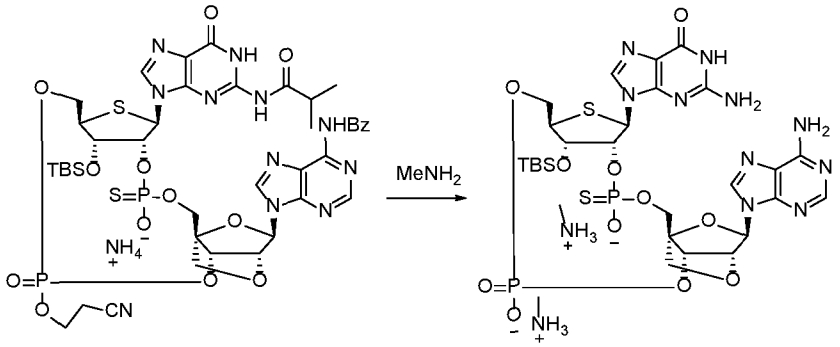
To pyridine (20mL) at -40°C under Ar was added diphenyl phosphorochloridate (1.28g, 4.78mmol) over 5min. To the solution at -40°C was added a solution of (2R,3R,4S,5R)-5-((((((1S,3R,4R,7S)-3-(6-benzamido-9H-purin-9-yl)-1-(hydroxymethyl)-2,5-dioxabicyclo [2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-((tert-butyldimethylsilyl) oxy)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrothiophen-3-yl phosphonate (250mg, 0.239mmol, co-evaporated with pyridine, 3x10mL) in CH_2Cl_2 (20mL) dropwise. The resulting mixture was stirred at -40°C for 30min. The reaction mixture was used for the next reaction step immediately without purification. LCMS (ES, m/z): 1028.4 $[M + H]^+$.

Step 6: (5R,7R,8R,12aR,14R,15R,15aS,18S)-18-{[tert-butyl(dimethyl)silyl]oxy}-2-(2-cyanoethoxy)-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}hexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-10(12H)-olate 2-oxide 10-sulfide



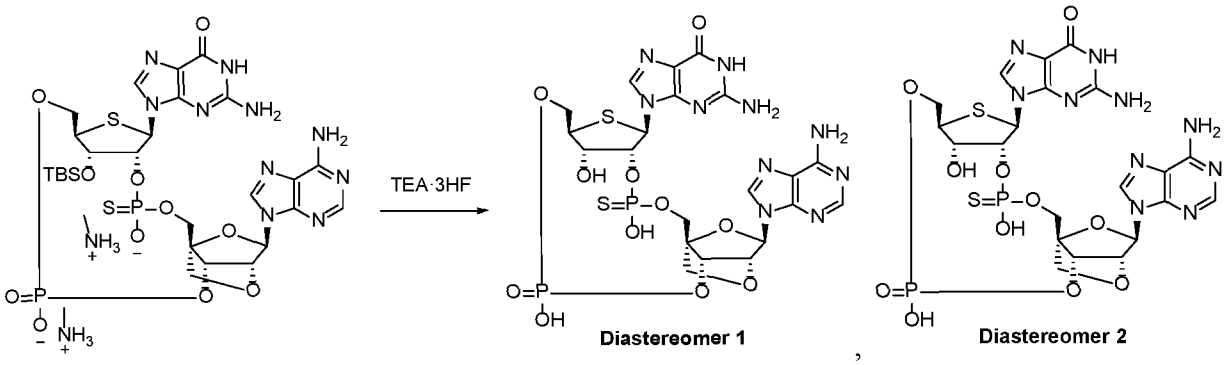
To the reaction mixture at rt from Step 5 was added 3*H*-benzo[c][1,2]dithiol-3-one (121mg, 0.717mmol). After stirring at 25°C for 40min, water (431mg, 23.9mmol) was added. The reaction mixture was concentrated under reduced pressure, and the residue was purified by reverse phase (C18) chromatography eluted with 0 to 95% ACN in aq NH_4HCO_3 (5mM) to give the product. LCMS (ES, m/z): 1060.3 $[M + H]^+$. ^{31}P-NMR: (121MHz, D_2O) δ 54.91, 54.61 (m, 1P), δ-6.08, -6.48 (m, 1P).

Step 7: (5R,7R,8R,12aR,14R,15R,15aS,18S)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-18-{[tert-butyl(dimethyl)silyl]oxy}hexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecine-2,10(12H)-diolate 2-oxide 10-sulfide



(5R,7R,8R,12aR,14R,15R,15aS,18S)-18-{[tert-butyl(dimethyl)silyl]oxy}-2-(2-cyanoethoxy)-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}hexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-10(12H)-olate 2-oxide 10-sulfide (190mg, 0.178mmol) was dissolved in a solution of methylamine in EtOH (30%, 1mL), and the resulting solution was stirred at rt for 2h. Then, it was concentrated to give a crude sample containing the product. LCMS (ES, m/z): 833.2 $[M + H]^+$.

Step 8: 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2-oxido-10-sulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one



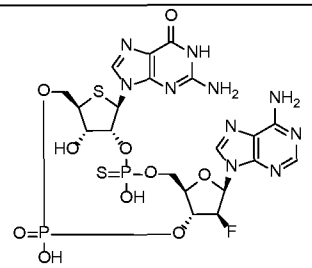
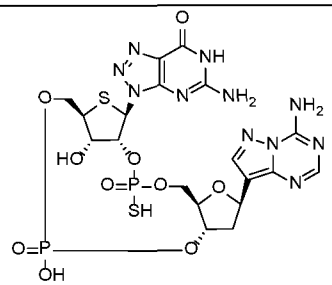
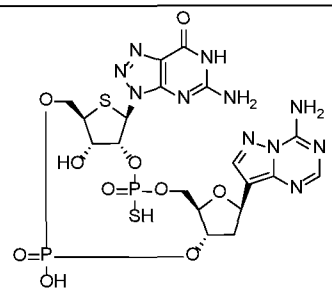
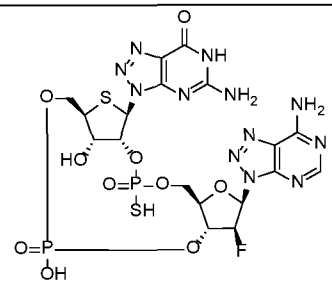
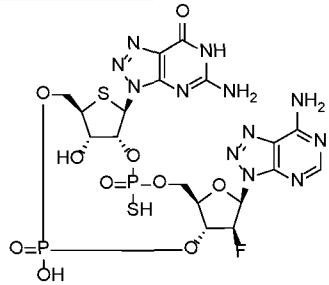
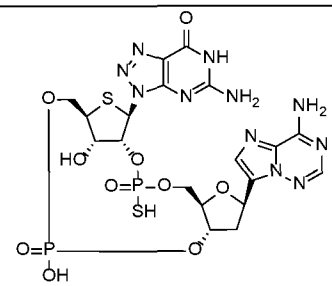
The crude from Step 7 was suspended in pyridine (5mL) under Ar. To the mixture was added Et_3N (1864mg, 18.42mmol) and triethylamine trihydrofluoride (742mg, 4.60mmol) dropwise. The mixture was heated to 50°C for 16h. Then, it was concentrated, and the residue was purified by preparative-HPLC (XBridge Shield RP18 OBD Column, 19×150mm) eluted with 0 to 14% ACN in aq NH_4HCO_3 (10mM) over 16min to give two products after concentration.

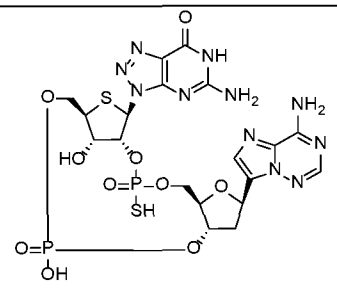
Example 264: 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2-oxido-10-sulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1): T_R: 11.00min. LCMS (ES, m/z): 719.0 $[M + H]^+$. ^{1}H-NMR: (300MHz, D_2O) :δ 8.13 (s, 1H), 7.94 (s, 1H), 7.64 (s, 1H), 6.11 - 5.97 (m, 2H), 5.92-5.85 (m, 1H), 5.25-5.05 (m, 1H), 4.87 (s, 1H), 4.75 (s, 2H), 4.30-4.21 (m, 1H), 4.12 -3.78 (m, 4H), 3.50-3.45 (m, 1H). ^{31}P-NMR: (121MHz, D_2O) δ 52.06 (s, 1P), δ-0.86 (s, 1P)

Example 265: 2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-2,10,18-trihydroxy-2-oxido-10-sulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2): T_R: 12.32min. LCMS (ES, m/z): 719.0 $[M + H]^+$. ^{1}H-NMR: (300MHz, D_2O) : δ 8.13 -8.03 (m, 2H), 7.64 (s, 1H), 6.11 - 5.97 (m, 2H), 5.92-5.90 (m, 1H), 5.20-5.15 (m, 1H), 4.87- 4.75 (m,3H), 4.33-4.20 (m, 1H), 4.12-4.00 (m, 2H),3.90-3.78(m, 2H), 3.45-3.40 (m, 1H). ^{31}P-NMR: (121MHz, D_2O) δ 54.88 (s, 1P), δ-0.97 (s, 1P).

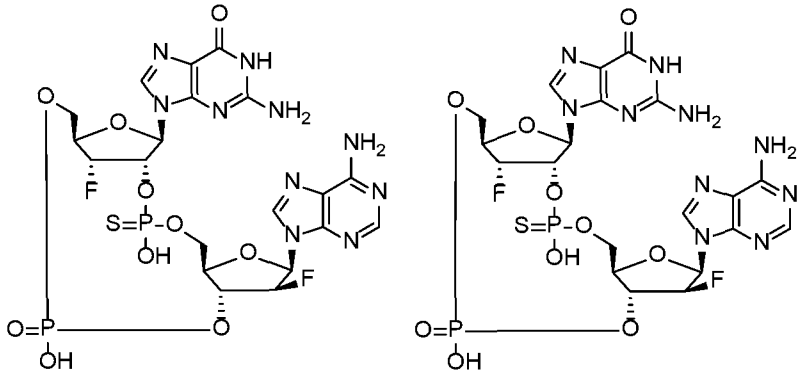
Examples 266 through 272, as shown in Table 9 below, were prepared according to procedures analogous to those outlined for Examples 264 and 265 above using the appropriate monomeric nucleosides, decribed as Preparations or as obtained from commercial sources.

Table 9

Ex.	Structure	Name	Mass [M-H]⁻
266		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(6-amino-9H-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	707
267		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-2,16-dihydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	690
268		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)-2,16-dihydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	690
269		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-2,16-dihydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	709
270		5-amino-3-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15-fluoro-2,16-dihydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	709
271		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,16-dihydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	690

Ex.	Structure	Name	Mass [M-H]⁻
272		5-amino-3-[(5R,7R,8R,12aR,14R,15aS,16S)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2,16-dihydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	690

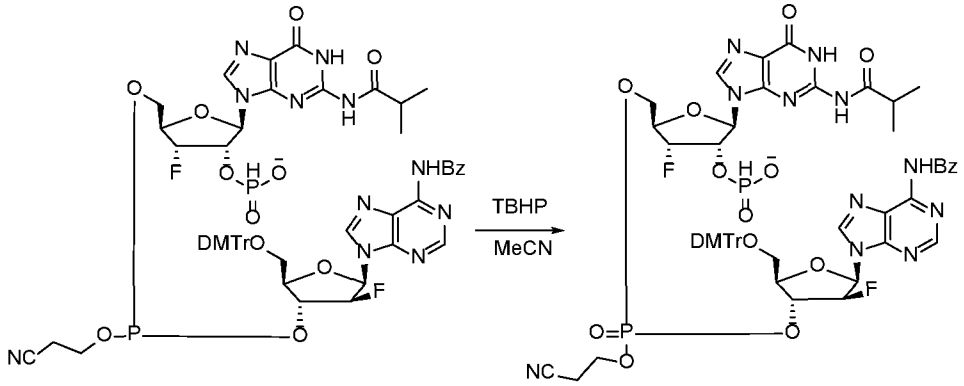
Examples 273 and 274: 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1) and 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)



Diastereomer 1 Diastereomer 2

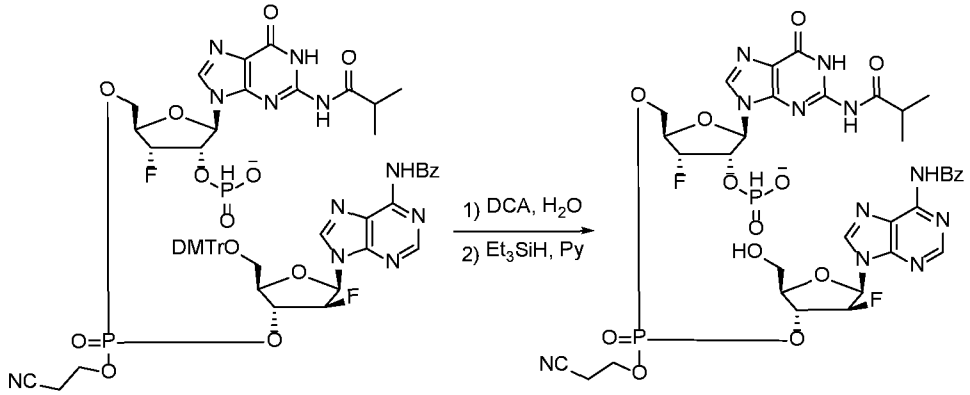
,

Step 1: (2R,3S,4R,5R)-5-((((((2R,3R,4S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



To a mixture containing the crude (2R,3S,4R,5R)-5-((((((2R,3R,4S,5R)-5-(6-benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphanyl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (product of Step 1, Examples 244 – 247, crude, assumed 0.75mmol) was added *tert*-butyl hydroperoxide in decane (5.5M, 0.48mL, 2.6mmol) dropwise, and the mixture was stirred at rt for 1h. Then, it was cooled to 0°C, and a solution of $Na_2S_2O_3$ (553mg) in water (2mL) was added slowly. The mixture was stirred at rt for 5min and then, concentrated to give the product. LCMS (ES, m/z): 1208.5 $[M - H]^-$.

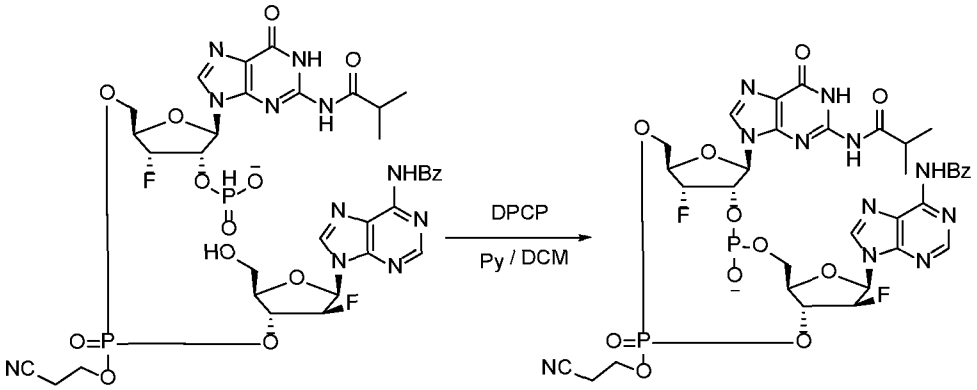
Step 2: (2R,3S,4R,5R)-5-((((((2R,3R,4S,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate



To a solution of the crude from Step 1 in CH_2Cl_2 (10mL) was added water (130mg, 7.5mmol) and 2,2-dichloroacetic acid (0.77g, 6mmol) in CH_2Cl_2 (10mL). After 20min, triethylsilane (20mL) was added, and stirring was continued for 2h. Pyridine (1mL) was added, and the reaction mixture was concentrated. The residue was purified by reverse phase (C18) chromatography eluted with 0 to 95% ACN in aq NH_4HCO_3 (0.04%) to give the product. LCMS (ES, m/z): 908.2 $[M + H]^+$. ^{1}H-NMR: (400MHz, CD_3OD) δ 8.76 (d, J = 7.7Hz, 2H), 8.65 (s, 1H), 8.56 (s, 1H), 8.21 – 8.08 (m, 6H), 7.69 (t, J = 7.4Hz, 2H), 7.64 – 7.50 (m, 5H), 6.67 (ddd, J =

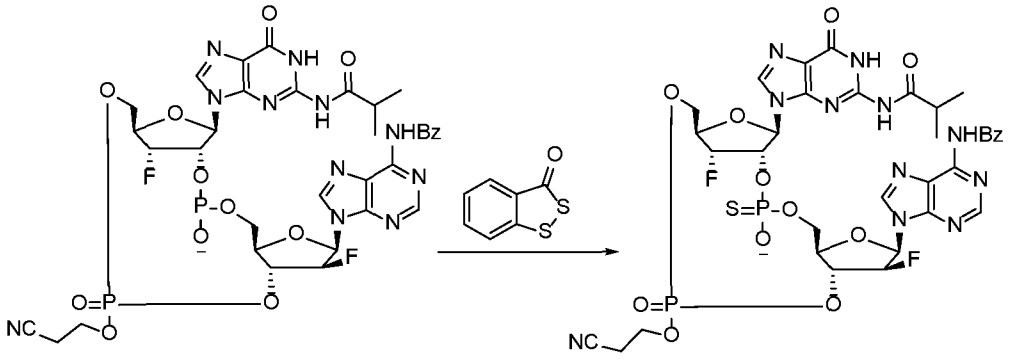
32.7, 14.9, 4.4Hz, 2H), 6.21 – 6.10 (m, 2H), 5.99 – 5.91 (m, 1H), 5.64 – 5.57 (m, 1H), 5.56 – 5.45 (m, 3H), 5.38 (s, 3H), 4.71 – 4.54 (m, 6H), 4.47 – 4.23 (m, 5H), 3.92 (d, J = 3.8Hz, 1H), 3.89 – 3.80 (m, 2H), 2.94 (dt, J = 31.1, 5.9Hz, 4H), 2.83 – 2.74 (m, 2H), 1.36 – 1.19 (m, 13H), 1.12 (s, 2H). ^{31}P-NMR: (162MHz, CD_3OD) δ 2.55, -1.33, -3.09, -3.11, -155.89.

Step 3: (5R,7R,8S,12aR,14R,15S,15aR,16R)-2-(2-cyanoethoxy)-15,16-difluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-10-olate 2-oxide



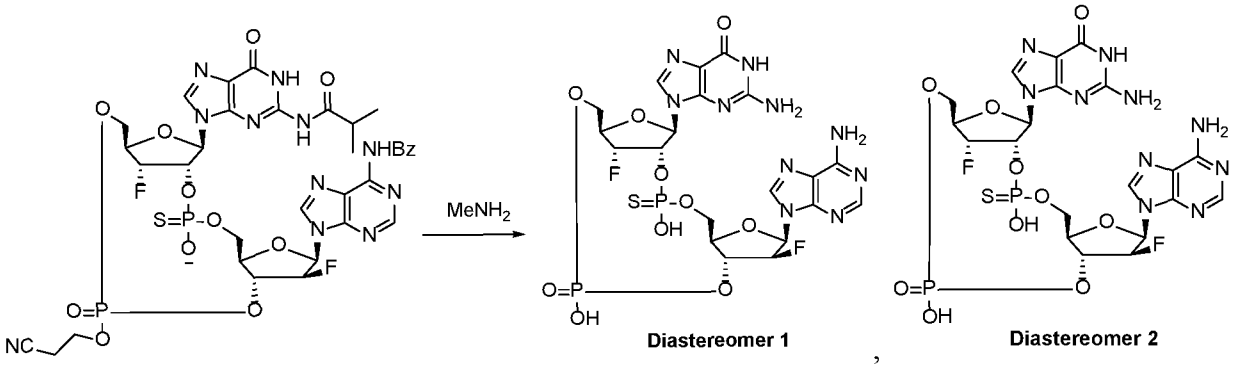
To pyridine (50mL) under Ar was added diphenyl chlorophosphate (2.66g, 9.92mmol). It was cooled at -40°C, and then, a solution of (2R,3S,4R,5R)-5-((((((2R,3R,4S,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl)oxy)(2-cyanoethoxy)phosphoryl)oxy)methyl)-4-fluoro-2-(2-isobutyramido-6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-3-yl phosphonate (450mg, 496mmol, co-evaporated with pyridine 3x5mL) in CH_2Cl_2 (50mL) was added dropwise over 20min. The resulting mixture was stirred at -40°C for 20min. The reaction mixture was immediately used in the next step without purification. LCMS (ES, m/z): 891.1 $[M + H]^+$.

Step 4: (5R,7R,8S,12aR,14R,15S,15aR,16R)-2-(2-cyanoethoxy)-15,16-difluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-10-olate 2-oxide 10-sulfide



To the reaction mixture of Step 3 at -40°C was added 3*H*-benzo[c][1,2]dithiol-3-one (32.76mg, 0.195mmol) and water (125mg, 0.744mmol). After stirring at rt for 40min, the reaction mixture was concentrated under reduced pressure. The residue was purified by reverse phase (C18) chromatography eluted with 0 to 95% ACN in aq NH_4HCO_3 (0.04%) to give the product. LCMS (ES, m/z): 922.1 $[M + H]^+$. ^{1}H-NMR: (400MHz, CD_3OD) δ 8.81 – 8.73 (m, 2H), 8.63 – 8.48 (m, 2H), 8.24 (s, 1H), 8.21 – 8.10 (m, 5H), 7.73 – 7.56 (m, 7H), 6.83 – 6.72 (m, 2H), 6.28 (d, J = 8.6Hz, 1H), 6.14 (d, J = 8.5Hz, 1H), 5.83 – 5.56 (m, 4H), 5.49 (d, J = 11.1Hz, 1H), 4.76 – 4.46 (m, 11H), 4.37 (s, 1H), 4.29 (d, J = 6.8Hz, 1H), 4.18 (d, J = 10.2Hz, 1H), 3.04 (dd, J = 6.5, 5.1Hz, 4H), 2.83 – 2.68 (m, 1H), 2.06 (s, 2H), 1.23 (dd, J = 22.5, 6.9Hz, 9H), 1.14 – 1.05 (m, 3H). ^{31}P-NMR: (162MHz, CD_3OD) δ 62.41, 56.84, 56.29, -3.14, -3.35, -4.77, -5.06, -60.84.

Step 5: 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomers 1 and 2)



The (5R,7R,8S,12aR,14R,15S,15aR,16R)-2-(2-cyanoethoxy)-15,16-difluoro-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-14-{6-[(phenylcarbonyl)amino]-9H-purin-9-yl}octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-10-olate 2-oxide 10-sulfide (380mg, 0.377mmol) was

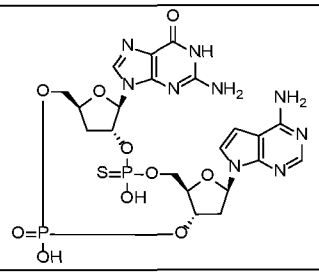
dissolved in a solution of $MeNH_2$ in EtOH (30%, 30mL), and the resulting solution was stirred at rt for 2h. Then, it was concentrated, and the residue was purified by Prep-HPLC (XBridge Shield RP18 OBD Column, 19×150mm) eluted with 4 to 10% ACN in aq NH_4HCO_3 (10mM) over 20min.

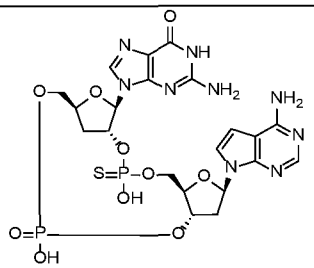
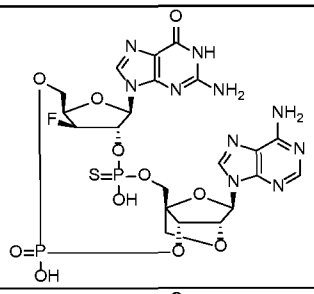
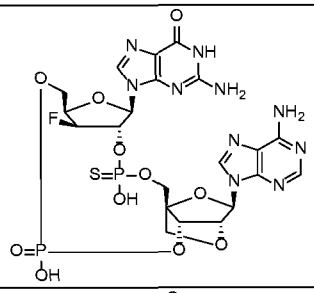
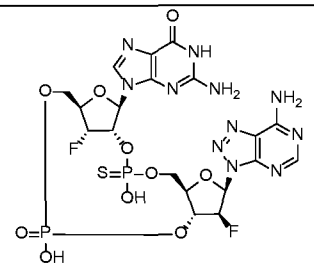
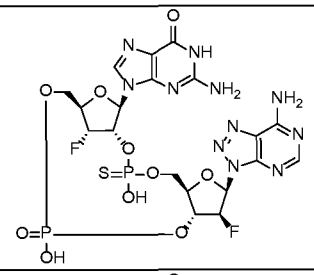
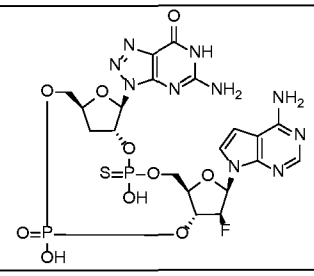
Example 273: 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1): T_R = 10.10min. LCMS (ES, m/z): 693.0 $[M - H]^-$. ^{1}H-NMR: (400MHz, $CDCl_3$) δ 10.84 (d, J = 2.4Hz, 1H), 10.72 (s, 1H), 10.59 (d, J = 3.9Hz, 1H), 9.01 (dd, J = 19.4, 3.0Hz, 1H), 8.56 (d, J = 8.5Hz, 1H), 8.14 – 7.96 (m, 2H), 7.93 – 7.82 (m, 1H), 7.73 (dd, J = 12.0, 6.5Hz, 2H), 7.31 – 7.19 (m, 2H), 7.15 (d, J = 2.8Hz, 1H), 6.94 – 6.74 (m, 3H), 6.71 – 6.53 (m, 2H). ^{31}P-NMR: (162MHz, D_2O): δ 62.301 (s, 1P), 0.976 (s, 1P).

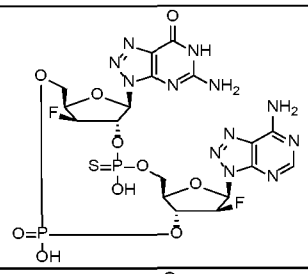
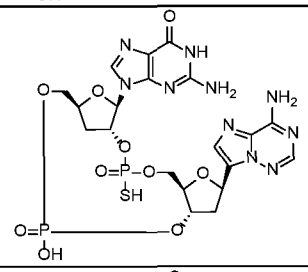
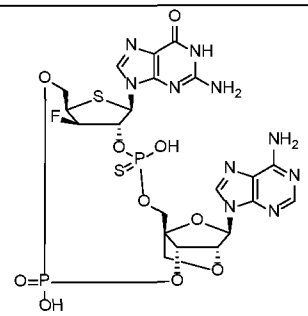
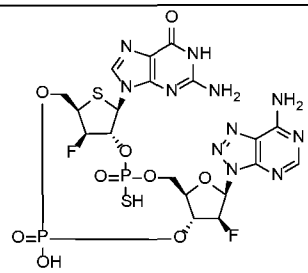
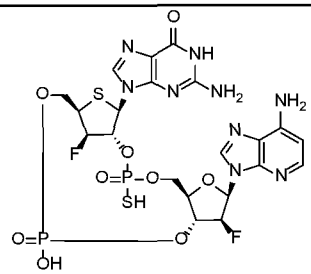
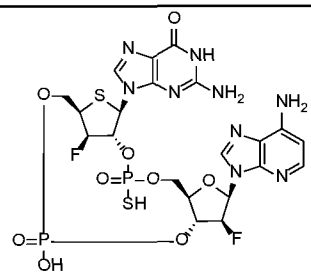
Example 274: 2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2): T_R: 14.27min. LCMS (ES, m/z): 692.9 $[M - H]^-$. ^{1}H-NMR: (400MHz, DMSO-d6) δ 8.31 (d, J = 11.0Hz, 2H), 8.18 (s, 1H), 6.40 (dd, J = 22.6, 2.6Hz, 1H), 5.88 (d, J = 8.8Hz, 1H), 5.44 (d, J = 33.7Hz, 2H), 5.35 – 5.23 (m, 1H), 5.24 – 5.05 (m, 2H), 4.48 – 4.34 (m, 1H), 4.31 (dd, J = 10.6, 4.5Hz, 1H), 4.16 – 3.87 (m, 3H). ^{31}P-NMR: (162MHz, DMSO-d6): δ 55.096 (s, 1P), -2.731 (s, 1P).

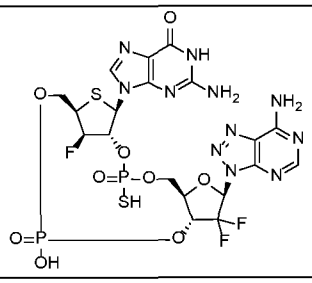
Examples 275 through 288, as shown in Table 10 below, were prepared according to procedures analogous to those outlined in Examples 274 and 275 above using the appropriate monomeric nucleosides, decribed as Preparations or as obtained from commercial sources.

Table 10

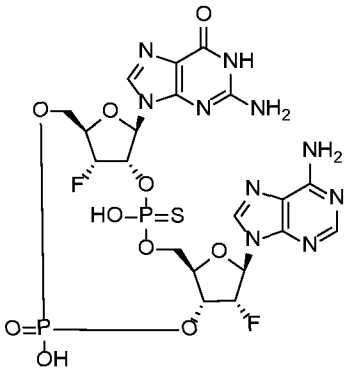
Ex.	Structure	Name	Mass [M-H]
275		2-amino-9-[(5S,7R,8R,12aR,14R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	656

Ex.	Structure	Name	Mass [M-H]
276		2-amino-9-[(5S,7R,8R,12aR,14R,15aS)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	656
277		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dihydroxy-2-oxido-10-sulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	703
278		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aS,18S)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dihydroxy-2-oxido-10-sulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	703
279		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	694
280		2-amino-9-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	694
281		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-14-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-15-fluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one	675

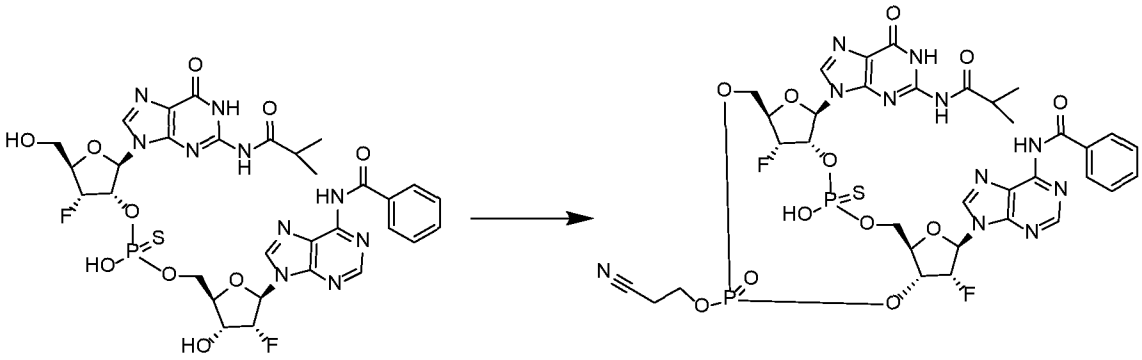
Ex.	Structure	Name	Mass [M-H]
282		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one	695
283		2-amino-9-[(5S,7R,8R,12aR,14R,15aS)-14-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2-hydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	657
284		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,18R)-14-(6-amino-9H-purin-9-yl)-18-fluoro-2,10-dihydroxy-2-oxido-10-sulfidohexahydro-14H-15,12a-(epoxymethano)-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7(12H)-yl]-1,9-dihydro-6H-purin-6-one	719
285		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,16-difluoro-2-hydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	710
286		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-imidazo[4,5-b]pyridin-3-yl)-15,16-difluoro-2-hydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	708
287		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16R)-14-(7-amino-3H-imidazo[4,5-b]pyridin-3-yl)-15,16-difluoro-2-hydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	708

Ex.	Structure	Name	Mass [M-H]
288		2-amino-9-[(5R,7R,8R,12aR,14R,15aR,16R)-14-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-15,15,16-trifluoro-2-hydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	728

Example 289: 2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2,10-disulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one



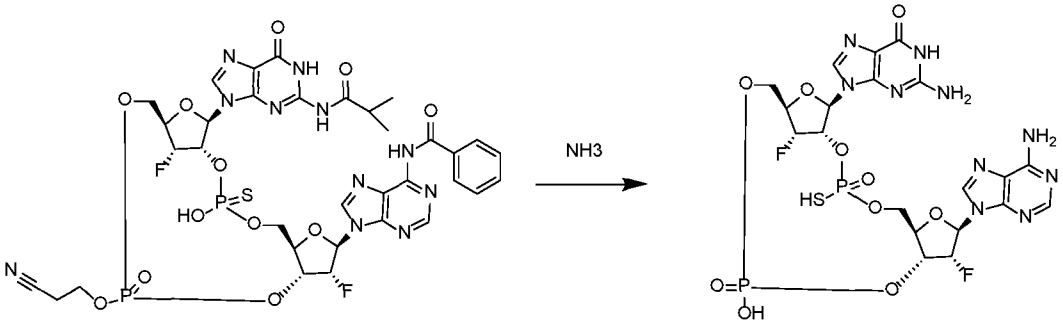
Step 1: N-{9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-2-(2-cyanoethoxy)-15,16-difluoro-10-hydroxy-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-9H-purin-6-yl}benzamide



O-(((2R,3R,4R,5R)-5-(6-benzamido-9H-purin-9-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methyl) O-((2R,3S,4R,5R)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1H-purin-9(6H)-yl)tetrahydrofuran-3-yl) O-hydrogen phosphorothioate (product of Step 3, Example 261, 100mg, 0.124mmol) and diisopropylammonium tetrazolide (31.8mg, 0.186mmol) were azeotrope with dry CH_3CN (3x10ml) and dried under high vacuum for 30min.

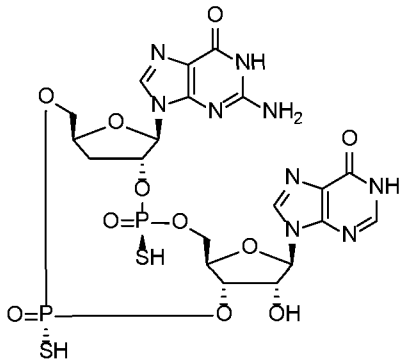
The above mixture was dissolved in DMF (1mL) and acetonitrile (7mL) and added 200mg active MS 4A and a solution of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite (51.1mg, 0.161mmol) in 1ml dry CH_3CN. The resulting mixture was strirred at rt for 30min, followed by addition of 1H-tetrazole (43.4mg, 0.620mmol). The reaction stayed at rt for 1h and added tert-butyl hydroperoxide (5.0M in Decane)(0.074mL, 0.372mmol). The stirring continued for 1h and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with 0-8% MeOH/DCM to give the desired product. LCMS (ES, m/z): 922 $[M + H]^+$

Step 2: 2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one



N-{9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-2-(2-cyanoethoxy)-15,16-difluoro-10-hydroxy-7-{2-[(2-methylpropanoyl)amino]-6-oxo-1,6-dihydro-9H-purin-9-yl}-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-14-yl]-9H-purin-6-yl}benzamide (90mg, 0.098mmol) and ammonia (7.0M in MeOH) (2ml, 14.00mmol) were sealed in a microwave tube. The mixture was heated to 50°C and stirred for 4h. The reaction mixture was concentrated, and purified using mass-directed reverse phase HPLC (X-Bridge BEH 150 Prep C18) using a gradient solvent system with MeCN and 100mM aqueous triethylammonium acetate to give three desired diastereomers. Lyophilization of the product fractions furnished 2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-14-(6-amino-9H-purin-9-yl)-15,16-difluoro-2,10-dihydroxy-2-oxido-10-sulfidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one. LCMS (ES, m/z): 693 $[M - H]^-$. ^1H NMR (H2O-d2, 500MHz): δ_H 8.23 (2H, d, J = 5.2Hz), 7.81 (1H, s), 6.42 (1H, d, J = 14.2Hz), 5.99 (1H, d, J = 8.6Hz), 5.70 (1H, m), 5.40 (2H, m), 5.20 (1H, m), 4.62 (2H, m), 4.54 (1H, s), 4.15-4.30 (3H, m). ^{31}P NMR: (H_2O-d_2, 202MHz): δ -1.6, 52.1.

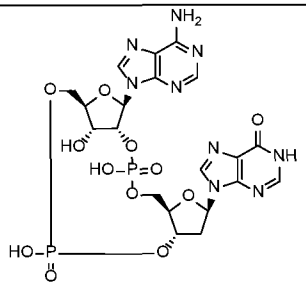
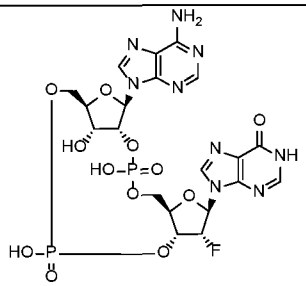
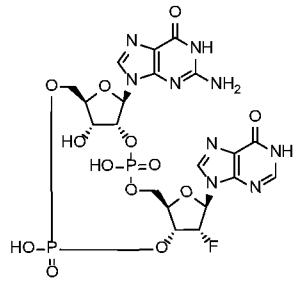
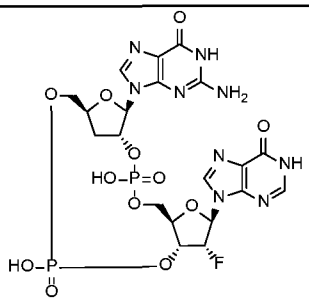
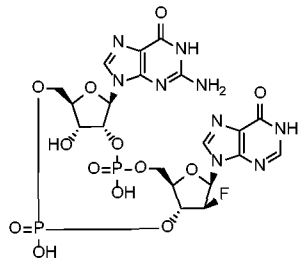
Example 290: 2-amino-9-[(2R,5S,7R,8R,10R,12aR,14R,15R,15aS)-15-hydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one

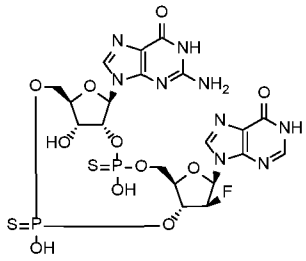
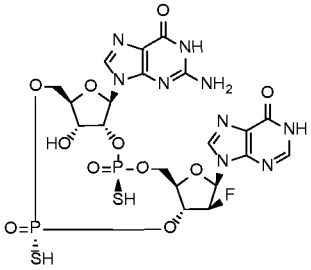
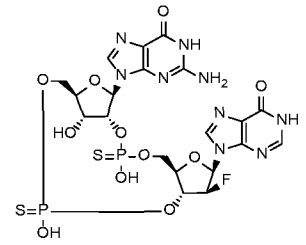
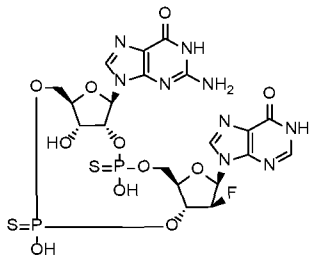
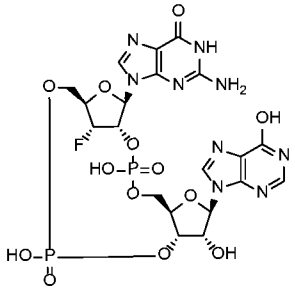


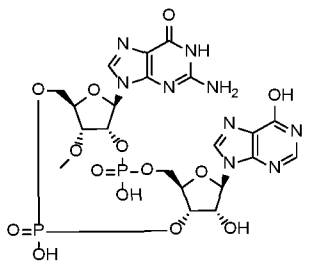
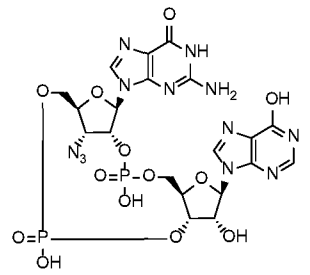
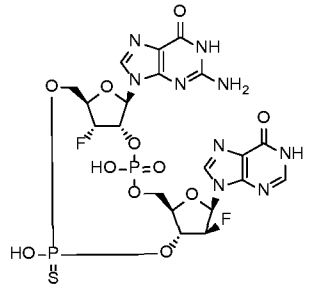
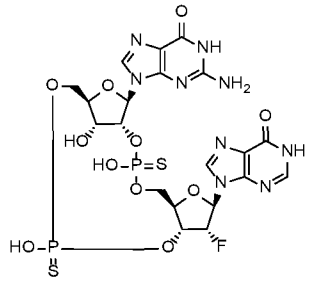
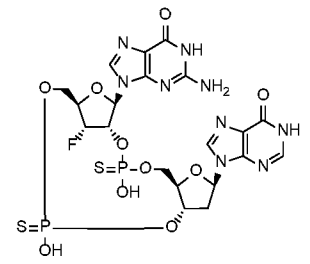
To (2R,5S,7R,8R,10R,12aR,14R,15R,15aS)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-14-(6-amino-9H-purin-9-yl)-15-hydroxyoctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-2,10-bis(thiolate) 2,10-dioxide (Example 86, 5.0mg, 0.0071mmol) were added sodium phosphate buffer (pH 6.8, 50mM, 0.5mL) and adenosine monphosphate deaminase (3mg). The reaction mixutre was left to stir overnight, filtered, and purified by reverse phase HPLC (1-10% MeCN in aq NH_4HCO_3 (100mM)) to afford the title compound as a diammonium salt. LCMS (ES, m/z): 690 $[M - H]^-$. ^1H NMR (500MHz, D_2O): δ 8.30 (s, 1H), 8.20 (s, 1H), 7.95 (s, 1H), 6.19 (s, 1H), 5.82 (d, J = 6.0Hz, 1H), 5.64 (m, 1H), 5.15 (m, 1H), 4.86 (d, J = 4.5Hz, 1H), 4.60 (m, 1H), 4.48-4.53 (m, 2H), 4.38 (m, 1H), 4.11 (dd, J = 11.5, 4.0Hz, 1H), 4.05 (m, 1H), 2.63 (m, 1H), 2.52 (m, 1H). ^{31}P NMR: (202MHz, D_2O): δ 55.78 (s), 52.44 (s).

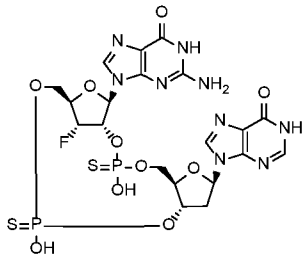
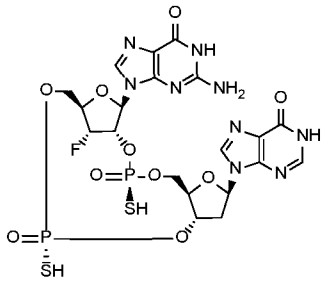
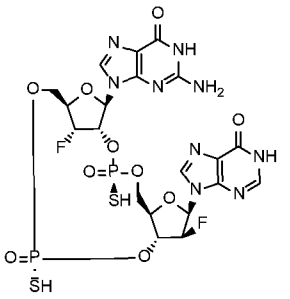
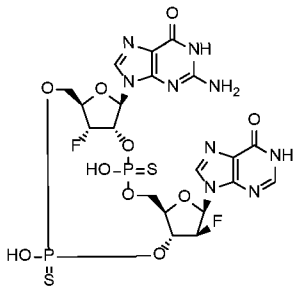
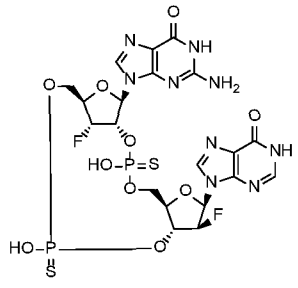
Examples 291 through 348 as shown in Table 11 below, were prepared according to procedures analogous to those outlined in Example 290 above, from the starting compound ("St. Cmpd.") indicated.

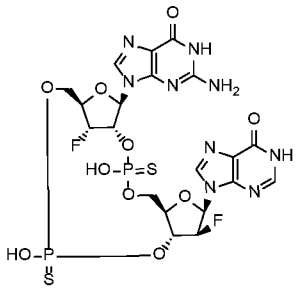
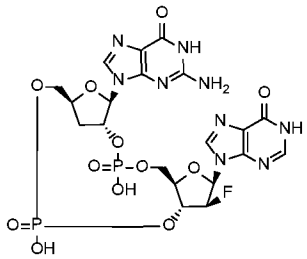
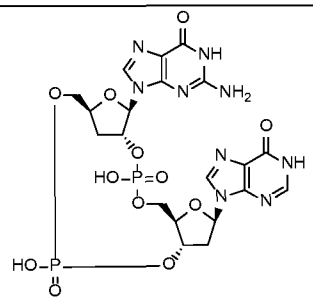
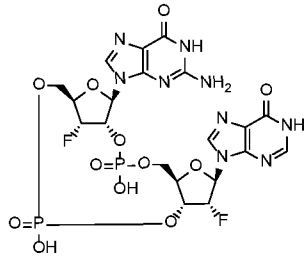
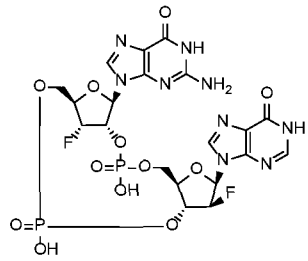
Table 11

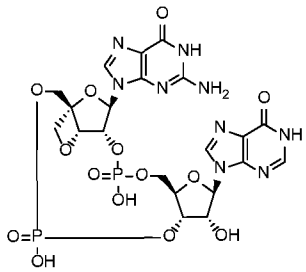
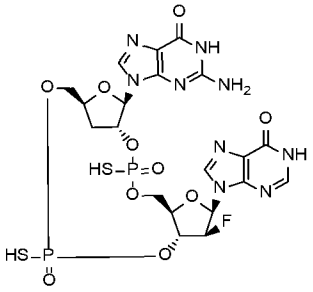
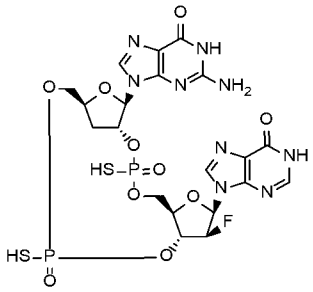
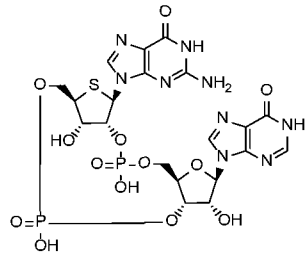
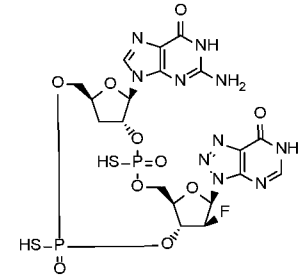
Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
291		9-[(5*R*,7*R*,8*R*,12a*R*,14*R*,15a*S*,16*R*)-7-(6-amino-9*H*-purin-9-yl)-2,10,16-trihydroxy-2,10-dioxidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-14-yl]-1,9-dihydro-6*H*-purin-6-one	642	3
292		9-[(5*R*,7*R*,8*R*,12a*R*,14*R*,15*R*,15a*R*,16*R*)-7-(6-amino-9*H*-purin-9-yl)-15-fluoro-2,10,16-trihydroxy-2,10-dioxidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10] pentaoxadiphosphacyclotetradecin-14-yl]-1,9-dihydro-6*H*-purin-6-one	660	4
293		2-amino-9-[(5*R*,7*R*,8*R*,12a*R*,14*R*,15*R*,15a*R*,16*R*)-15-fluoro-2,10,16-trihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)octahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	676	2
294		2-amino-9-[(5*S*,7*R*,8*R*,12a*R*,14*R*,15*R*,15a*R*)-15-fluoro-2,10-dihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)octahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	660	21
295		2-amino-9-[(5*R*,7*R*,8*R*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-15-fluoro-2,10,16-trihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)octahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	676	7

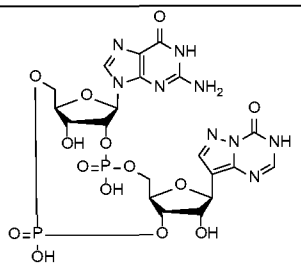
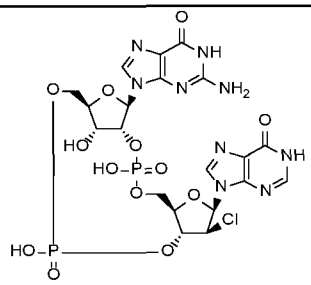
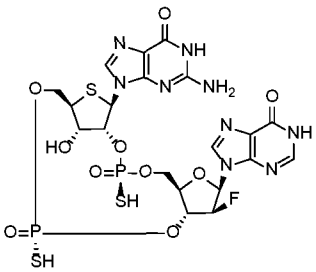
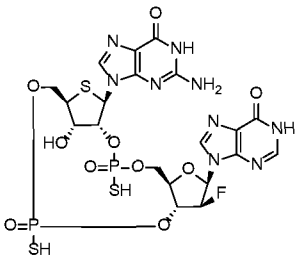
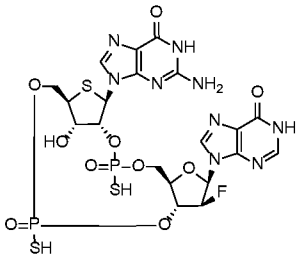
Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
296		2-amino-9-[(5*R*,7*R*,8*R*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-15-fluoro-2,10,16-trihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 1)	708	90
297		2-amino-9-[(2R,5*R*,7*R*,8*R*,10R,12a*R*,14*R*,15*S*,15a*R*,16*R*)-15-fluoro-2,10,16-trihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 2)	708	89
298		2-amino-9-[(5*R*,7*R*,8*R*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-15-fluoro-2,10,16-trihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 3)	708	91
299		2-amino-9-[(5*R*,7*R*,8*R*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-15-fluoro-2,10,16-trihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 4)	708	92
300		2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*R*,15a*S*,16*R*)-16-fluoro-2,10,15-trihydroxy-14-(6-hydroxy-9*H*-purin-9-yl)-2,10-dioxidooctahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	676	5

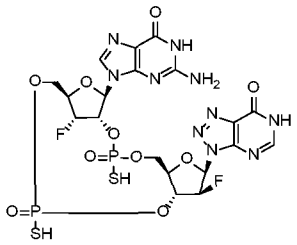
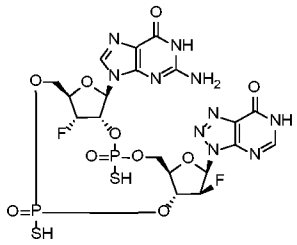
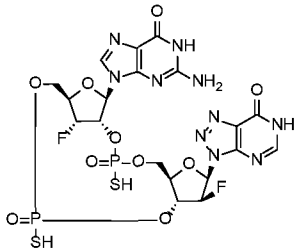
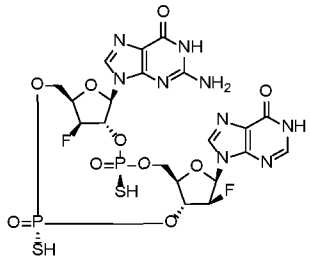
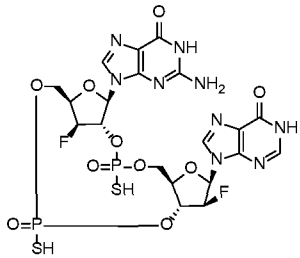
Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
301		2-amino-9-[(5*R*,7*R*,8*R*,12a*R*,14*R*,15*R*,15a*S*,16*R*)-2,10,15-trihydroxy-14-(6-hydroxy-9*H*-purin-9-yl)-16-methoxy-2,10-dioxidooctahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	688	13
302		2-amino-9-[(5*S*,7*R*,8*R*,12a*R*,14*R*,15*R*,15a*S*,16*R*)-16-azido-2,10,15-trihydroxy-14-(6-hydroxy-9*H*-purin-9-yl)-2,10-dioxidooctahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	699	14
303		2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-15,16-difluoro-2,10-dihydroxy-10-oxido-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2-sulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	694	143
304		2-amino-9-[(5*R*,7*R*,8*R*,12a*R*,14*R*,15*R*,15a*R*,16*R*)-15-fluoro-2,10,16-trihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	708	81
305		2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15a*S*,16*R*)-16-fluoro-2,10-dihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 1)	692	120

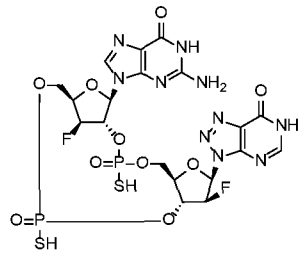
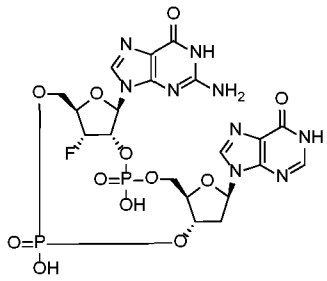
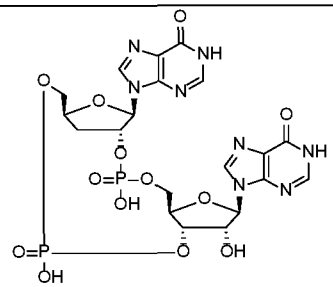
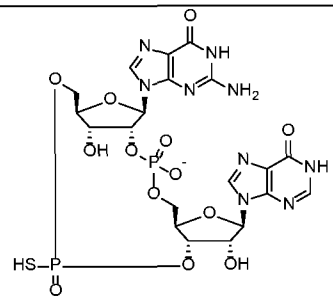
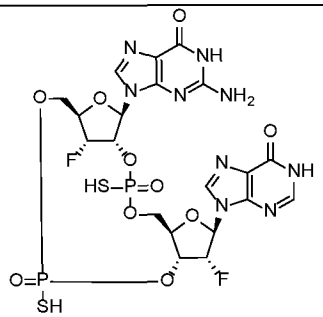
Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
306		2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15a*S*,16*R*)-16-fluoro-2,10-dihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 2)	692	118
307		2-amino-9-[(2R, 5*R*,7*R*,8*S*,10R, 12a*R*,14*R*,15a*S*,16*R*)-16-fluoro-2,10-dihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 3)	692	117
308		2-amino-9-[(2R, 5*R*,7*R*,8*S*,10R, 12a*R*,14*R*,15*S*,15a*R*,16*R*)-15,16-difluoro-2,10-dihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 1)	710	247
309		2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-15,16-difluoro-2,10-dihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 2)	710	245
310		2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-15,16-difluoro-2,10-dihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 3)	710	246

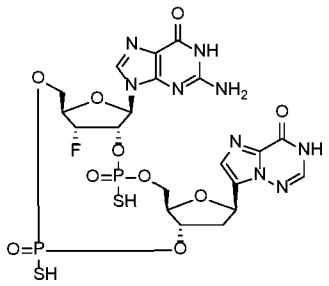
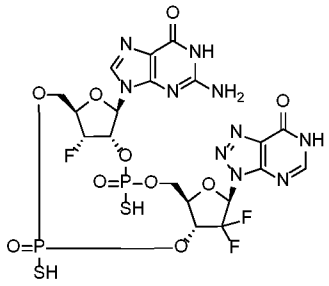
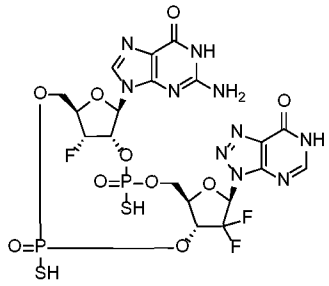
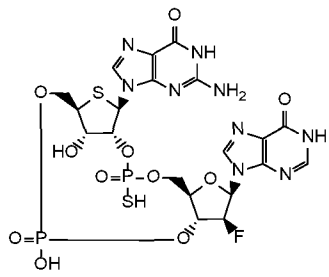
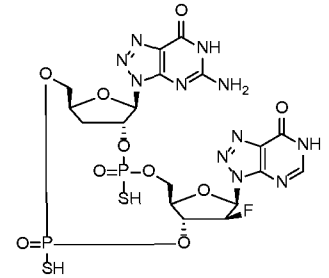
Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
311		2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-15,16-difluoro-2,10-dihydroxy-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,10-disulfidooctahydro-12*H*-5,8-methanofuro[3,2-*l*][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one (Diastereomer 4)	710	244
312		2-amino-9-[(5*S*,7*R*,8*R*,12a*R*,14*R*,15*S*,15a*R*)-15-fluoro-2,10-dihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)octahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	660	25
313		2-amino-9-[(5*S*,7*R*,8*R*,12a*R*,14*R*,15aS)-2,10-dihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)octahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	642	27
314		2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*R*,15a*R*,16*R*)-15,16-difluoro-2,10-dihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)octahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	678	23
315		2-amino-9-[(5*R*,7*R*,8*S*,12a*R*,14*R*,15*S*,15a*R*,16*R*)-15,16-difluoro-2,10-dihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)octahydro-12*H*-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6*H*-purin-6-one	678	26

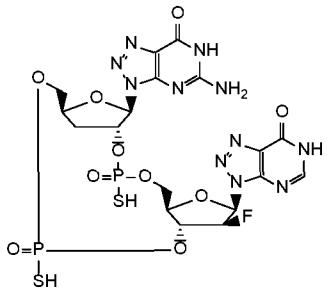
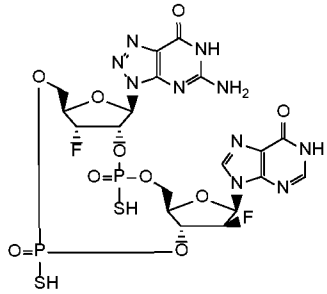
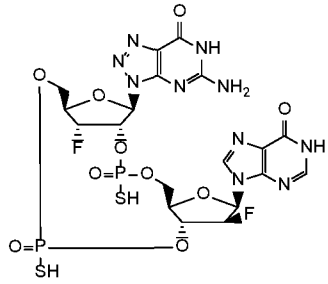
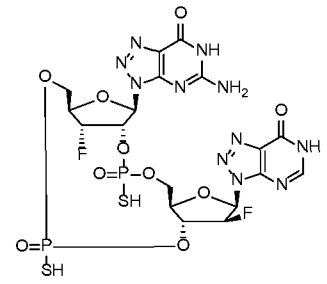
Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
316		2-amino-9-[(2a*R*,6a*S*,7*R*,8*R*,9a*R*,14*R*,14a*S*,15*R*)-5,7,12-trihydroxy-5,12-dioxido-8-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)hexahydro-6a*H*-2a,14-(epoxymethano)furo[3,2-d]oxeto[2,3-k][1,3,7,9,2,8]tetraoxadiphosphacyclotridecin-15(2H,3H)-yl]-1,9-dihydro-6*H*-purin-6-one	686	17
317		2-amino-9-[(*2R,5S,7R,8R,10R,12aR,14R,15S,15aR*)-15-fluoro-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	692	130
318		2-amino-9-[(5S,7R,8R,12aR,14R,15S,15aR)-15-fluoro-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	692	131
319		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16S)-2,10,15,16-tetrahydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)octahydro-12H-5,8-methanofuro [3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	690	11
320		3-[(5S,7R,8R,12aR,14R,15S, 15aR)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-15-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one	693	133

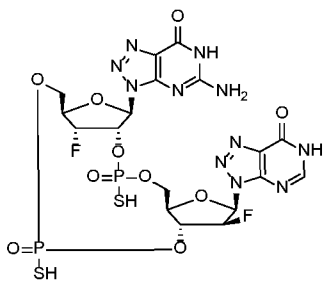
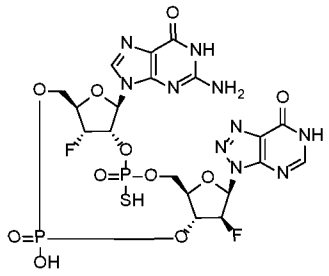
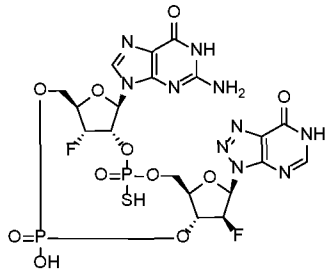
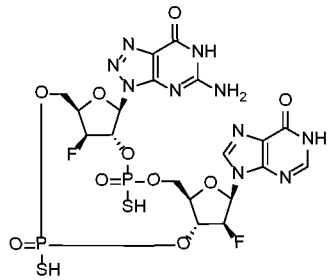
Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
321		8-[(5R,7R,8R,12aR,14S,15S,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10,15,16-tetrahydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]pyrazolo[1,5-a][1,3,5]triazin-4(3H)-one	674	54
322		2-amino-9-[(5R,7R,8R,,12aR,14R,15S,15aR,16R)-15-chloro-2,10,16-trihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	692	15
323		2-amino-9-[(2R, 5R,7R,8R,10R,12aR,14R,15S, 15aR,16S)-15-fluoro-16-hydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	724	97
324		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-15-fluoro-16-hydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	724	99
325		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-15-fluoro-16-hydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 3)	724	96

Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
326		3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	711	130
327		3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	711	128
328		3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 3)	711	127
329		2-amino-9-[(2R, 5R,7R,8S,10R, 12aR,14R,15S, 15aR,16S)-15,16-difluoro-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 1)	710	144
330		2-amino-9-[(5R,7R,8S,12aR,14R,15S, 15aR,16S)-15,16-difluoro-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one (Diastereomer 2)	710	145

Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
331		3-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-15,16-difluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one	711	147
332		2-amino-9-[(5R,7R,8S,12aR,14R,15aS,16R)-16-fluoro-2,10-dihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)octahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	660	22
333		9,9'-[(5S,7R,8R, 12aR,14R,15R,15aS)-2,10,15-trihydroxy-2,10-dioxidooctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecine-7,14-diyl]bis(1,9-dihydro-6H-purin-6-one)	643	50
334		2-amino-9-[(5R,7R,8R,12aR,14R,15R,15aS,16R)-10,15,16-trihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	690	38
335		2-amino-9-[(5R,7R,8S,12aR,14R,15R,15aR,16R)-15,16-difluoro-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	710	260

Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
336		7-[(5R,7R,8S,12aR,14R,15aS,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-16-fluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]imidazo[2,1-f][1,2,4]triazin-4(3H)-one (Diastereomer 1)	692	249
337		3-[5R,7R,8S,12aR,14R,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-15,15,16-trifluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	729	165
338		3-[(5R,7R,8S,12aR,14R,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-15,15,16-trifluoro-2,10-dioxido-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	729	167
339		2-amino-9-[(5R,7R,8R,12aR,14R,15S,15aR,16S)-15-fluoro-2,16-dihydroxy-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,9,11,6,2,10]tetraoxathiadiphosphacyclotetradecin-7-yl]-1,9-dihydro-6H-purin-6-one	708	266
340		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-15-fluoro-2,10-dioxido-14-(7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	694	181

Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
341		5-amino-3-[(5S,7R,8R,12aR,14R,15S,15aR)-15-fluoro-2,10-dioxido-14-(7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	694	182
342		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-15,16-difluoro-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	711	199
343		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-15,16-difluoro-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	711	197
344		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-15,16-difluoro-2,10-dioxido-14-(7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	712	200

Ex.	Structure	Name	Mass [M-H]⁻	St. Cmpd.
345		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-15,16-difluoro-2,10-dioxido-14-(7-oxo-6,7-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	712	201
346		3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-15,16-difluoro-2-hydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 1)	695	279
347		3-[(5R,7R,8S,12aR,14R,15S,15aR,16R)-7-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-15,16-difluoro-2-hydroxy-2,10-dioxido-10-sulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-14-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one (Diastereomer 2)	695	280
348		5-amino-3-[(5R,7R,8S,12aR,14R,15S,15aR,16S)-15,16-difluoro-2,10-dioxido-14-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2,10-disulfanyloctahydro-12H-5,8-methanofuro[3,2-l][1,3,6,9,11,2,10]pentaoxadiphosphacyclotetradecin-7-yl]-3,6-dihydro-7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one	711	179

BIOLOGICAL EVALUATION

The individual compounds described in the Examples are defined as STING agonists by demonstrating binding to the STING protein with an EC_{50} of 20uM or less in the STING Biochemical [^{3}H]cGAMP Competition Assay (using either HAQ or wild type (WT) STING) and demonstrating interferon production with a 5% or greater luminescence induction at 30uM in the INF-β secretion in the THP1 cell assay. The methods below describe each of these assays.

[^{3}H]-cGAMP Synthesis

2.3mL of buffer solution containing 80mM tris Cl, 200mM $MgCl_2$ and 20mM NaCl followed by 0.32mL of a 10mM aq solution of GTP was added to a plastic 50mL AMICON tube. A solution of [^{3}H]ATP (21Ci/mmol, 45mCi) in 0.5mL H_2O was then added followed by 1mL of a 1mg/mL solution of DNA (Herring testes activator DNA, Sigma, #D6898) and 53uL of a 47mM solution of cGAS enzyme. Additional H_2O was added to bring the total volume to 10mL.

The reaction was stirred for 2h at 37°C and then added directly to an Amicon Ultra-15 10K centrifuge tube and spun for 1h at 4,000g. The collected solution was then purified on a semi-prep Mono Q column using the following mobile phases:

A: 0.05M TrisCl pH 8.5 adjusted with 1M NaOH

B: 0.05M TrisCl, 0.5M NaCl pH 8.5 adjusted with 1M NaOH

Gradient: 100% A for 5min followed by a linear gradient to 50:50 (A:B) over 25min, 3mL/min, 254nm.

The collected product fractions were pooled and adjusted to a total volume of 30mL with buffer A. A total yield of 15.5mCi of [^{3}H]cGAMP was isolated at a radiochemcial purity of 98.0% at a specific activity of 21.5Ci/mmol.

cGAS Enzyme

A recombinant DNA vector was chemically synthesized to express the truncated human cGAS enzyme (residues 161-522). To aid in expression and purification, the amino terminus contains a hexahistidine tag, SUMO tag and TEV cleavage site. The recombinant enzyme was overexpressed in ROSETTA™ 2(DE3) Single Competent Cells (Novagen). Affinity purification was carried out using HIS-Select HF Nickel Affinity Gel (Sigma) followed by size exclusion chromatography using a Hi-Load 26/60 SUPERDEX200 prep grade column (GE Healthcare). Fractions were pooled, concentrated, flash frozen in liquid nitrogen and stored at -80°C until needed for research applications.

Example 349: ^{3}H-cGAMP filtration binding assay (HAQ STING)

The ability of compounds to bind STING is quantified by their ability to compete with tritiated cGAMP ligand for human STING receptor membrane using a radioactive filter-binding assay. The binding assay employs STING receptor obtained from *Trichoplusia ni* cell membranes (*T.ni*; Expression Systems, cat # 94-002F, www.expressionsystems.com)

overexpressing full-length HAQ STING prepared in-house and tritiated cGAMP ligand also purified in-house.

The basic HAQ STING filtration assay protocol is as follows:

The compounds were serially titrated by the Hamilton STARPlus CORE in a 96-well plate (Greiner, # 651201) using a 1:3 ten-point dose response format. After compound preparation, a 2.2ug/ml working concentration of STING membrane (SEQ. ID. No. 2) was prepared by diluting concentrated membrane into assay buffer (1x PBS; Invitrogen # SH30028.02) and douncing 7x using a manual tissue homogenizer (Wheaton, # 357546). 148uL of prepared membrane was then manually added to each well of a 96-well deep-well polypropylene plate (Fisher Scientific, # 12-566-121). Following membrane addition, 2uL of either titrated test compound, DMSO control (Sigma # 276855), or cold cGAMP control (prepared in-house) was added to the appropriate wells using a Biomek FX. Compound and membrane then preincubated for 60min at RT to allow compound binding to equilibrate. Following equilibration, 8nM of [^{3}H] c-GAMP ligand was prepared by diluting into assay buffer, and 50uL of this working stock was then manually added to each well of the assay plate. Plates were then incubated at RT for 60min, and the contents of each assay plate were then filtered through a 96-well GF/B filter plate (PerkinElmer, # 6005250) using a TomTec MachIII Cell Harvester equipped with 20mM HEPES buffer (Fisher Scientific, # BP299500). The filter plates were then dried at 55°C for 30min using a pressurized VWR oven before 30uL of Ultima GoldF scintillate was added to each well. Tritium levels for each reaction well were then measured using a PerkinElmer TopCount plate reader.

After normalization to controls, the percent activity for each compound concentration was calculated by measuring the amount of remaining radioactivity. The plot of percent activity versus the log of compound concentration was fit with a 4-parameter dose response equation to calculate EC_{50} values.

The final reaction conditions were:

Component	**Volume (uL)**	**Final Concentration**
STING membrane	148	1.5ug/ml
^{3}H-cGAMP	50	2.0nM
Low Control (cold cGAMP)	2	10uM
Test compound/DMSO	2	10uM

Compound concentrations tested were 20.000, 637.00, 2.200, 0.740, 0.247, 0.082, 0.027, 0.009, 0.003, and 0.001μM with 1.0% residual DMSO.

Full-Length STING (HAQ) Virus Generation

STING virus was generated using an insect cell baculovirus system. *Spodoptera frugiperda Sf21* cells (Kempbio, Inc.) were diluted to 5e5 cells/ml in Sf-900II SFM media (LifeTechnologies # 10902088) without antibiotics. The cell suspension was added to each well of a treated 6-well plate (2mL per well, 1e6 cells total), and the cells were allowed to adhere for at least 30min. Meanwhile, a 1mL co-transfection mix was assembled by combining 500ng of HAQ STING [STING(1-379)R71H,G230A,H232R,R293Q-GG-AviTag-GS-HRV3C-HIS8/pBAC1] DNA (Genewiz custom synthesis) with 1mL Sf-900II SFM media containing 10μL Cellfectin® II Reagent (Invitrogen # 10362100) and 100ng viral backbone BestBac 2.0, v-cath/chiA Deleted Linearized Baculovirus DNA (Expression Systems # 91-002). The transfection mixtures were allowed to incubate for 30min. After incubation, media was gently removed from the adhered cells in the 6-well plate, the 1mL transfection mixtures were added (1mL per well), and the plate was placed in a humidified incubator at 27°C. The following day, 1mL Sf-900II SFM media (no antibiotics) was added to each well of the 6-well plate. After media addition, the cells were allowed to incubate with DNA (SEQ. ID. No. 3) at 27°C for 5-7 days to generate the P0 viral stock. To generate P1 viral stocks, 0.5mL of P0 viral supernatant was added to 50mL uninfected *Sf21* cells (seeded the day prior to infection at a density of $5x10^5$ cells/mL to allow for one overnight doubling) in Sf-900II SFM media containing 5μg/mL gentamicin (Invitrogen #15710072). The infected cells were then incubated at 27°C for 3d while shaking at 110rpm (ATR Biotech Multitron Infors HT #AJ118). On day 3, P1 cultures were counted using a ViCell XR (Beckman Coulter Life Sciences # 383556) to confirm infection had occurred (cell size ≥3μm larger than uninfected cells and viability approximately 85-95%). Cultures were harvested in 50mL conical tubes and centrifuged at 2000xg for 10min at 4°C. The P1 viral supernatants were poured off into clean 50ml centrifuge tubes, and the remaining P1 cell pellets were used to generate Baculovirus Infected Insect Cells (BIICs) according to in-house validated SOP. Cryopreservation media containing Sf-900II SFM media with 10% heat inactivated FBS, 10% DMSO (Sigma #D2650), and 5μg/ml gentamicin was prepared in-house and sterilized through 0.22μM filter immediately prior to use. P1 cell pellets were resuspended to a density of 2e7 cells/ml and aliquoted into cryovials (1mL per vial). Cryovials were placed in Mr. Frosty cell freezers O/N at -80°C and transferred to liquid nitrogen for long term storage

the following day. To generate P2 viral stock, 0.5mL of the P1 viral supernatant was added to 50mL uninfected *Sf21* cells (seeded the day prior to infection at a density of $5x10^5$ cells/mL to allow for one overnight doubling) in Sf-900II SFM media containing 5µg/mL gentamicin. These cells were incubated at 27°C for 3d while shaking at 110rpm before harvesting P2 stock with centrifugation at 2000xg for 10min at 4°C. The P2 viral supernatants were poured off and discarded, while the P2 cell pellets were used to generate P2 BIICs following the same protocol described above. The baculovirus generation protocol has been validated to consistently produce P1/P2 BIICs with titers of 2e9 pfu/mL (2e7 cells/mLx100 pfu/cell).

Full-Length STING (HAQ) Expression

To generate STING membranes, P1/P2 BIICs were amplified overnight by adding thawed BIICs to *Sf21* cells seeded at a density of $1.0x10^6$cells/mL. The volume of BIIC used to infect the culture was calculated using an assumed BIIC titer of 2e9 pfu/ml to achieve an MOI of 10 in the overnight amplification. After culturing overnight, the cells were counted on a ViCell XR to confirm infection had occurred (cell size ≥3µm larger than uninfected cells and viability approximately 80-90%). The volume of infected *Sf21* cells from the overnight amplification used to infect the large-scale expression of *Trichoplusia ni* (*T.ni*; Expression Systems, cat # 94-002F, www.expressionsystems.com) seeded at a density of $1.0x10^6$ in cell media (ESF921 SFM containing 5µg/mL gentamicin) at MOI = 2.0 was calculated based on (100 pfu/infected *Sf21* cell). The cells were allowed to express for 48h at 27°C before harvesting the cell pellet, by centrifugation at 3,400xg for 10min at 4°C. *T. ni* cells were counted on a ViCell XR to confirm infection had occurred (cell size ≥3µm larger than uninfected cells and viability approximately 80-90%) prior to harvest.

Full-Length STING (HAQ) Membrane Generation

Buffer stock reagents:

1) 1 M HEPES pH 7.5, Teknova, Cat#H1035
2) 5 M NaCl, Sigma Aldrich, Cat#S5150-1L
3) KCl, Sigma Aldrich, Cat#319309-500ML
4) Complete EDTA-free protease inhibitor tablets, Roche Diagnostics, Cat#11873580001
5) Benzonase, Universal Nuclease, Pierce, Cat#88702

Lysis buffer [25mM HEPES pH 7.5, 10mM $MgCl_2$, 20mM KCl, (Benzonase 1:5000, Complete Protease Inhibitor tab/50mL)] was added to the pellet of cells expressing full-length STING (HAQ) prepared above at 5mL Lysis buffer/g of cell pellet. The pellet was resuspended and dounced twenty times using a Wheaton Dounce Homogenizer to disrupt the cell membrane. Homogenized lysate was then passed through the emulsiflex-C5 microfluidizer at a pressure close to 5000PSI. The resuspended pellet was centrifuged at 36,000rpm (100,000xg) in a 45Ti rotor in the ultra-high speed centrifuge for 45min, 4°C. The supernatant was removed. The pellet then was resuspended in wash buffer [(25mM HEPES pH7.5, 1mM $MgCl_2$, 20mM KCl, 1M NaCl (Complete Protease Inhibitor tab/50mL)] at a volume of 50mL pellet/centrifuge tube. The pellet/wash buffer mixture was then homogenized, using a glass homogenizer on ice (20 strokes), followed by centrifugation at 36,000rpm for 45min at 4°C. The supernatant was removed. The wash step was repeated once more. The resulting membrane was resuspended in 20mM HEPES pH 7.5, 500mM NaCl, 10% glycerol, EDTA-free Protease Inhibitors (1tablet/50mL). The protein concentration was measured by Bradford assay (Bio-Rad Protein Assay, Cat# 500-0006), and protein enrichment was determined by SDS-PAGE and confirmed by Western blot. The resuspended membranes were stored at -80°C.

Full-Length HAQ STING [STING(1-379)R71H,G230A,H232R,R293Q-GG-AviTag-GS-HRV3C-HIS8]Amino Acid Sequence:

MPHSSLHPSIPCPRGHGAQKAALVLLSACLVTLWGLGEPPEHTLRYLVLHLASLQLGLL
LNGVCSLAEELHHIHSRYRGSYWRTVRACLGCPLRRGALLLLSIYFYYSLPNAVGPPFT
WMLALLGLSQALNILLGLKGLAPAEISAVCEKGNFNVAHGLAWSYYIGYLRLILPELQA
RIRTYNQHYNNLLRGAVSQRLYILLPLDCGVPDNLSMADPNIRFLDKLPQQTADRAGIK
DRVYSNSIYELLENGQRAGTCVLEYATPLQTLFAMSQYSQAGFSREDRLEQAKLFCQTL
EDILADAPESQNNCRLIAYQEPADDSSFSLSQEVLRHLRQEEKEEVTVGSLKTSAVPSTST
MSQEPELLISGMEKPLPLRTDFSGGGLNDIFEAQKIEWHEGSLEVLFQGPHHHHHHHH
(SEQ. ID. No. 2)

Full-length HAQ [STING(1-379)R71H,G230A,H232R,R293Q-GG-AviTag-GS-HRV3C-HIS8/pBAC1] Plasmid DNA Sequence:

GGAACGGCTCCGCCCACTATTAATGAAATTAAAAATTCCAATTTTAAAAAACGCAG
CAAGAGAAACATTTGTATGAAAGAATGCGTAGAAGGAAAGAAAAATGTCGTCGAC
ATGCTGAACAACAAGATTAATATGCCTCCGTGTATAAAAAAAATATTGAACGATTTG

AAAGAAAACAATGTACCGCGCGGCGGTATGTACAGGAAGAGGTTTATACTAAACTG
TTACATTGCAAACGTGGTTTCGTGTGCCAAGTGTGAAAACCGATGTTTAATCAAGGC
TCTGACGCATTTCTACAACCACGACTCCAAGTGTGTGGGTGAAGTCATGCATCTTTT
AATCAAATCCCAAGATGTGTATAAACCACCAAACTGCCAAAAAATGAAAACTGTCG
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AATAATAAAACAATTATAAATGCTAAATTTGTTTTTTATTAACGATACAAACCAAAC
GCAACAAGAACATTTGTAGTATTATCTATAATTGAAAACGCGTAGTTATAATCGCTG
AGGTAATATTTAAAATCATTTTCAAATGATTCACAGTTAATTTGCGACAATATAATT
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CAAATAAATAAGTATTTTACTGTTTTCGTAACAGTTTTGTAATAAAAAAACCTATAA
ATATAGGATCCATGCCCCACTCCAGCCTGCATCCATCCATCCCGTGTCCCAGGGGTC
ACGGGGCCCAGAAGGCAGCCTTGGTTCTGCTGAGTGCCTGCCTGGTGACCCTTTGGG
GGCTAGGAGAGCCACCAGAGCACACTCTCCGGTACCTGGTGCTCCACCTAGCCTCCC
TGCAGCTGGGACTGCTGTTAAACGGGGTCTGCAGCCTGGCTGAGGAGCTGCACCAC
ATCCACTCCAGGTACCGGGGCAGCTACTGGAGGACTGTGCGGGCCTGCCTGGGCTG
CCCCCTCCGCCGTGGGGCCCTGTTGCTGCTGTCCATCTATTTCTACTACTCCCTCCCA
AATGCGGTCGGCCCGCCCTTCACTTGGATGCTTGCCCTCCTGGGCCTCTCGCAGGCA
CTGAACATCCTCCTGGGCCTCAAGGGCCTGGCCCCAGCTGAGATCTCTGCAGTGTGT
GAAAAAGGGAATTTCAACGTGGCCCATGGGCTGGCATGGTCATATTACATCGGATA
TCTGCGGCTGATCCTGCCAGAGCTCCAGGCCCGGATTCGAACTTACAATCAGCATTA
CAACAACCTGCTACGGGGTGCAGTGAGCCAGCGGCTGTATATTCTCCTCCCATTGGA
CTGTGGGGTGCCTGATAACCTGAGTATGGCTGACCCCAACATTCGCTTCCTGGATAA
ACTGCCCCAGCAGACCGCTGACCGTGCTGGCATCAAGGATCGGGTTTACAGCAACA
GCATCTATGAGCTTCTGGAGAACGGGCAGCGGGCGGGCACCTGTGTCCTGGAGTAC

GCCACCCCCTTGCAGACTTTGTTTGCCATGTCACAATACAGTCAAGCTGGCTTTAGC
CGGGAGGATAGGCTTGAGCAGGCCAAACTCTTCTGCCAGACACTTGAGGACATCCT
GGCAGATGCCCCTGAGTCTCAGAACAACTGCCGCCTCATTGCCTACCAGGAACCTGC
AGATGACAGCAGCTTCTCGCTGTCCCAGGAGGTTCTCCGGCACCTGCGGCAGGAGG
AAAAGGAAGAGGTTACTGTGGGCAGCTTGAAGACCTCAGCGGTGCCCAGTACCTCC
ACGATGTCCCAAGAGCCTGAGCTCCTCATCAGTGGAATGGAAAAGCCCCTCCCTCTC
CGCACGGATTTCTCTGGCGGTGGCCTGAACGACATCTTCGAAGCCCAGAAAATCGA
ATGGCATGAAGGCAGCCTGGAAGTGCTGTTCCAGGGCCCACACCACCATCATCACC
ATCACCATTAATGAGCGGCCGCACTCGAGCACCACCACCACCACCACTAACCTAGG
TAGCTGAGCGCATGCAAGCTGATCCGGGTTATTAGTACATTTATTAAGCGCTAGATT
CTGTGCGTTGTTGATTTACAGACAATTGTTGTACGTATTTTAATAATTCATTAAATTT
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TCTGAATTTAAATATTAAATCCTCAATAGATTTGTAAAATAGGTTTCGATTAGTTTCA
AACAAGGGTTGTTTTTCCGAACCGATGGCTGGACTATCTAATGGATTTTCGCTCAAC
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GTTTTGTTTTGTAATAAAGGTTCGACGTCGTTCAAAATATTATGCGCTTTTGTATTTC
TTTCATCACTGTCGTTAGTGTACAATTGACTCGACGTAAACACGTTAAATAGAGCTT
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AACCCTCGTCGTTAGAAGTTGCTTCCGAAGACGATTTTGCCATAGCCACACGACGCC
TATTAATTGTGTCGGCTAACACGTCCGCGATCAAATTTGTAGTTGAGCTTTTTGGAAT
TATTTCTGATTGCGGGCGTTTTTGGGCGGGTTTCAATCTAACTGTGCCCGATTTTAAT
TCAGACAACACGTTAGAAAGCGATGGTGCAGGCGGTGGTAACATTTCAGACGGCAA
ATCTACTAATGGCGGCGGTGGTGGAGCTGATGATAAATCTACCATCGGTGGAGGCG
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CGGTTTAGGCTCAAATGTCTCTTTAGGCAACACAGTCGGCACCTCAACTATTGTACT
GGTTTCGGGCGCCGTTTTTGGTTTGACCGGTCTGAGACGAGTGCGATTTTTTTCGTTT
CTAATAGCTTCCAACAATTGTTGTCTGTCGTCTAAAGGTGCAGCGGGTTGAGGTTCC
GTCGGCATTGGTGGAGCGGGCGGCAATTCAGACATCGATGGTGGTGGTGGTGGTGG
AGGCGCTGGAATGTTAGGCACGGGAGAAGGTGGTGGCGGCGGTGCCGCCGGTATAA
TTTGTTCTGGTTTAGTTTGTTCGCGCACGATTGTGGGCACCGGCGCAGGCGCCGCTG
GCTGCACAACGGAAGGTCGTCTGCTTCGAGGCAGCGCTTGGGGTGGTGGCAATTCA
ATATTATAATTGGAATACAAATCGTAAAAATCTGCTATAAGCATTGTAATTTCGCTA
TCGTTTACCGTGCCGATATTTAACAACCGCTCAATGTAAGCAATTGTATTGTAAAGA

GATTGTCTCAAGCTCGGATCGATCCCGCACGCCGATAACAAGCCTTTTCATTTTTACT
ACAGCATTGTAGTGGCGAGACACTTCGCTGTCGTCGAGGTTTAAACGCTTCCTCGCT
CACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAA
GGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAG
CAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTC
CATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTG
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TGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTC
GGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGT
CGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGC
CTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACT
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AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCT
GCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCA
AACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCA
GAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTC
ACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAG
TAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATC
TGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATAC
GGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCA
CCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAG
TGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAG
AGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCAT
CGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCA
AGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCT
CCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCA
CTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGT
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CGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTG
GAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTT
CGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGT
TTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCG

ACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATC
AGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAA
TAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGCGCCCTGTAGCGGCG
CATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGC
GCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTT
TCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACG
GCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCC
CTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTC
TTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAG
GGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTA
ACGCGAATTTTAACAAAATATTAACGTTTACAATTTCCCATTCGCCATTCAGGCTGC
GCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCA
(SEQ. ID. No. 3)

Certain compounds of the disclosure were evaluated in HAQ STING *in vitro* binding assay as described above. The following table tabulates the biological data for these compounds as EC_{50} values.

Table 12: ^{3}H-cGAMP filtration binding assay for HAQ STING

Compound	EC_{50} (nM)	Compound	EC_{50} (nM)	Compound	EC_{50} (nM)
Example 1	<1	Example 55	55.3	Example 134	<1
Example 2	1.3	Example 56	89.4	Example 135	253.4
Example 3	257.1	Example 57	437.9	Example 136	3.7
Example 4	22	Example 58	3.1	Example 137	11.4
Example 5	1.1	Example 59	11.8	Example 138	738.8
Example 6	<1	Example 60	42.5	Example 139	<1
Example 7	1.2	Example 61	18.2	Example 140	12.9
Example 8	13.6	Example 66	117.1	Example 141	<1
Example 9	7.2	Example 67	6.4	Example 142	6.2
Example 10	4.9	Example 68	63.8	Example 143	1.05
Example 11	1.2	Example 69	<1	Example 144	<1
Example 12	<1	Example 70	2	Example 145	<1
Example 13	1.5	Example 71	<1	Example 146	5.6

Compound	EC_{50} (nM)	Compound	EC_{50} (nM)	Compound	EC_{50} (nM)
Example 14	<1	Example 72	<1	Example 147	1.4
Example 15	378.1	Example 73	834	Example 148	33.3
Example 16	12.4	Example 74	5.8	Example 149	8.4
Example 17	121	Example 75	2.6	Example 150	<1
Example 18	53.9	Example 76	75.4	Example 151	<1
Example 19	173	Example 77	160.5	Example 152	<1
Example 20	<1	Example 78	14.6	Example 244	156.6
Example 21	1.3	Example 79	103.2	Example 245	2.7
Example 22	4822	Example 80	1.3	Example 246	21.3
Example 23	<1	Example 81	<1	Example 247	<1
Example 24	2.4	Example 82	21.5	Example 290	2.5
Example 25	3.4	Example 83	3.7	Example 291	64.7
Example 26	<1	Example 85	<1	Example 292	2.6
Example 27	1.3	Example 86	<1	Example 293	1.9
Example 28	18.7	Example 87	<1	Example 294	6.6
Example 30	91	Example 88	<1	Example 295	29.6
Example 31	94	Example 89	8272	Example 296	4.1
Example 32	11.1	Example 90	1.7	Example 297	<1
Example 33	11.9	Example 91	15.8	Example 298	52.9
Example 34	24.6	Example 92	114	Example 299	1325
Example 35	7.9	Example 93	<1	Example 300	5.1
Example 36	21.5	Example 94	24.6	Example 301	4.8
Example 37	619.6	Example 95	13.9	Example 302	2.3
Example 38	3	Example 96	5.3	Example 303	<1
Example 69	<1	Example 97	<1	Example 304	3.3
Example 70	2	Example 98	1.8	Example 305	326.2
Example 71	<1	Example 99	<1	Example 306	3.6
Example 72	<1	Example 100	16.7	Example 307	<1
Example 73	834	Example 101	8	Example 308	1.6
Example 74	5.8	Example 102	<1	Example 309	4

Compound	EC_{50} (nM)	Compound	EC_{50} (nM)	Compound	EC_{50} (nM)
Example 39	24.4	Example 117	1	Example 310	27.6
Example 40	3.2	Example 118	1.4	Example 311	1595
Example 41	11.8	Example 119	17.8	Example 312	122.5
Example 42	15.2	Example 120	45.1	Example 313	210.6
Example 43	162.2	Example 121	<1	Example 314	<1
Example 44	17.1	Example 123	5.1	Example 315	49.9
Example 45	24.8	Example 124	<1	Example 316	2324
Example 46	11.7	Example 125	21.7	Example 317	<1
Example 47	39.2	Example 126	9.7	Example 318	6.2
Example 48	1.2	Example 127	24.4	Example 319	7.3
Example 49	30	Example 128	<1	Example 320	6.3
Example 50	2.7	Example 129	88.6	Example 321	112.2
Example 51	287.1	Example 130	<1	Example 322	68.8
Example 52	1.5	Example 131	2.4	Example 332	8.2
Example 53	1.7	Example 132	25.9	Example 333	45.9
Example 54	<1	Example 133	61.4		

Example 350: ^{3}H-cGAMP filtration binding assay (WT STING)

The ability of compounds to bind STING is quantified by their ability to compete with tritiated cGAMP ligand for human STING receptor membrane using a radioactive filter-binding assay. The binding assay employs STING receptor obtained from *Trichoplusia ni* cell membranes (*T.ni*; Expression Systems, cat # 94-002F, www.expressionsystems.com) overexpressing full-length WT STING prepared in-house and tritiated cGAMP ligand also purified in-house.

The basic WT STING filtration assay protocol is as follows:

16nM of [^{3}H] c-GAMP ligand was prepared by diluting into assay buffer, and 50uL of this working stock was manually added to each well of the assay plate. After ligand addition, 2uL of either titrated test compound, DMSO control (Sigma # 276855), or cold cGAMP control (prepared in-house) was added to the appropriate wells using a Biomek FX. The serially titrated compound was prepared on a Hamilton STARPlus CORE in a 96-well plate (Greiner, # 651201)

using a 1:3 ten-point dose response format. Following compound addition, a 2.2ug/ml working concentration of STING membrane (SEQ. ID. No. 4) was prepared by diluting concentrated membrane into assay buffer (1x PBS; Invitrogen # SH30028.02) and douncing 7x using a manual tissue homogenizer (Wheaton, # 357546). 148uL of this prepared membrane was then manually added to each well of a 96-well deep-well polypropylene plate (Fisher Scientific, # 12-566-121). Compound, ligand, and membrane then incubated for 60min at RT before the contents of each assay plate were filtered through a 96-well GF/B filter plate (PerkinElmer, # 6005250) using a TomTec MachIII Cell Harvester equipped with 20mM HEPES buffer (Fisher Scientific, # BP299500). The filter plates were then dried at 55°C for 30min using a pressurized VWR oven before 30uL of Ultima GoldF scintillate was added to each well. Tritium levels for each reaction well were then measured using a PerkinElmer TopCount plate reader.

After normalization to controls, the percent activity for each compound concentration was calculated by measuring the amount of remaining radioactivity. The plot of percent activity versus the log of compound concentration was fit with a 4-parameter dose response equation to calculate EC_{50} values.

The final reaction conditions were:

Component	**Volume (uL)**	**Final Concentration**
STING membrane	148	1.5ug/ml
^{3}H-cGAMP	50	4.0nM
Low Control (cold cGAMP)	2	10uM
Test compound/DMSO	2	10uM

Compound concentrations tested were 20.000, 637.00, 2.200, 0.740, 0.247, 0.082, 0.027, 0.009, 0.003, and 0.001μM with 1.0% residual DMSO.

Full-Length STING (WT) Virus Generation

STING virus was generated using an insect cell baculovirus system. *Spodoptera frugiperda Sf21* cells (Kempbio, Inc.) were diluted to 5e5 cells/ml in Sf-900II SFM media (LifeTechnologies # 10902088) without antibiotics. The cell suspension was added to each well of a treated 6-well plate (2mL per well, 1e6 cells total), and the cells were allowed to adhere for at least 30min. Meanwhile, a 1mL co-transfection mix was assembled by combining 500ng of WT STING[STING(1-379)H232R-gg-AviTag-gs-HRV3C-HIS8/pBAC1] (Genewiz custom synthesis) with 1mL Sf-900II SFM media containing 10μL Cellfectin® II Reagent (Invitrogen # 10362100) and 100ng viral backbone BestBac 2.0, v-cath/chiA Deleted Linearized Baculovirus

DNA (Expression Systems # 91-002). The transfection mixtures were allowed to incubate for 30min. After incubation, media was gently removed from the adhered cells in the 6-well plate, the 1mL transfection mixtures were added (1mL per well), and the plate was placed in a humidified incubator at 27°C. The following day, 1mL Sf-900II SFM media (no antibiotics) was added to each well of the 6-well plate. After media addition, the cells were allowed to incubate with DNA [(SEQ. ID. No. 5) and linearized viral backbone BestBac 2.0] at 27°C for 5-7 days to generate the P0 viral stock. To generate P1 viral stocks, 0.5mL of P0 viral supernatant was added to 50mL uninfected *Sf21* cells (seeded the day prior to infection at a density of $5x10^5$ cells/mL to allow for one overnight doubling) in Sf-900II SFM media containing 5µg/mL gentamicin (Invitrogen #15710072). The infected cells were then incubated at 27°C for 3d while shaking at 110rpm (ATR Biotech Multitron Infors HT #AJ118). On day 3, P1 cultures were counted using a ViCell XR (Beckman Coulter Life Sciences # 383556) to confirm infection had occurred (cell size ≥3µm larger than uninfected cells and viability approximately 85-95%). Cultures were harvested in 50mL conical tubes and centrifuged at 2000xg for 10min at 4°C. The P1 viral supernatants were poured off into clean 50ml centrifuge tubes, and the remaining P1 cell pellets were used to generate Baculovirus Infected Insect Cells (BIICs) according to in-house validated SOP. Cryopreservation media containing Sf-900II SFM media with 10% heat inactivated FBS, 10% DMSO (Sigma #D2650), and 5µg/ml gentamicin was prepared in-house and sterilized through 0.22µM filter immediately prior to use. P1 cell pellets were resuspended to a density of 2e7 cells/ml and aliquoted into cryovials (1mL per vial). Cryovials were placed in Mr. Frosty cell freezers O/N at -80°C and transferred to liquid nitrogen for long term storage the following day. To generate P2 viral stock, 0.5mL of the P1 viral supernatant was added to 50mL uninfected *Sf21* cells (seeded the day prior to infection at a density of $5x10^5$ cells/mL to allow for one overnight doubling) in Sf-900II SFM media containing 5µg/mL gentamicin. These cells were incubated at 27°C for 3d while shaking at 110rpm before harvesting P2 stock with centrifugation at 2000xg for 10min at 4°C. The P2 viral supernatants were poured off and discarded, while the P2 cell pellets were used to generate P2 BIICs following the same protocol described above. The baculovirus generation protocol has been validated to consistently produce P1/P2 BIICs with titers of 2e9 pfu/mL (2e7 cells/mLx100 pfu/cell).

Full-Length STING (WT) Expression

To generate STING membranes, P1/P2 BIICs were amplified overnight by adding thawed BIICs to *Sf21* cells seeded at a density of $1.0x10^6$ cells/mL. The volume of BIIC used to

infect the culture was calculated using an assumed BIIC titer of 2e9 pfu/ml to achieve an MOI of 10 in the overnight amplification. After culturing overnight, the cells were counted on a ViCell XR to confirm infection had occurred (cell size ≥3µm larger than uninfected cells and viability approximately 80-90%). The volume of infected *Sf21* cells from the overnight amplification used to infect the large-scale expression of *Trichoplusia ni* (*T.ni*; Expression Systems, cat # 94-002F, www.expressionsystems.com) seeded at a density of $1.0x10^6$ in cell media (ESF921 SFM containing 5µg/mL gentamicin) at MOI = 2.0 was calculated based on (100pfu/infected *Sf21* cell). The cells were allowed to express for 48h at 27°C before harvesting the cell pellet, by centrifugation at 3,400xg for 10min at 4°C. *T. ni* cells were counted on a ViCell XR to confirm infection had occurred (cell size ≥3µm larger than uninfected cells and viability approximately 80-90%) prior to harvest.

Full-Length STING (WT) Membrane Generation

Buffer stock reagents:

1) 1 M HEPES pH 7.5, Teknova, Cat#H1035
2) 5 M NaCl, Sigma Aldrich, Cat#S5150-1L
3) KCl, Sigma Aldrich, Cat#319309-500ML
4) Complete EDTA-free protease inhibitor tablets, Roche Diagnostics, Cat#11873580001
5) Benzonase, Universal Nuclease, Pierce, Cat#88702

Lysis buffer [25mM HEPES pH 7.5, 10mM $MgCl_2$, 20mM KCl, (Benzonase 1:5000, Complete Protease Inhibitor tab/50mL)] was added to the pellet of cells expressing full-length STING (WT) prepared above at 5mL Lysis buffer/g of cell pellet. The pellet was resuspended and dounced twenty times using a Wheaton Dounce Homogenizer to disrupt the cell membrane. Homogenized lysate was then passed through the emulsiflex-C5 microfluidizer at a pressure close to 5000PSI. The resuspended pellet was centrifuged at 36,000rpm (100,000xg) in a 45Ti rotor in the ultra-high speed centrifuge for 45min, 4°C. The supernatant was removed. The pellet then was resuspended in wash buffer [(25mM HEPES pH 7.5, 1mM $MgCl_2$, 20mM KCl, 1M NaCl (Complete Protease Inhibitor tab/50mL)] at a volume of 50mL/pellet/centrifuge tube. The pellet/Wash buffer mixture was then homogenized, using a glass homogenizer on ice (20 strokes), followed by centrifugation at 36,000rpm for 45min at 4°C. The supernatant was removed. The wash step was repeated once more. The resulting membrane was resuspended in 20mM HEPES pH 7.5, 500mM NaCl, 10% glycerol, EDTA-free Protease Inhibitors

(1tablet/50mL). The protein concentration was measured by Bradford assay (Bio-Rad Protein Assay, Cat# 500-0006), and protein enrichment was determined by SDS-PAGE and confirmed by Western blot. The resuspended membranes were stored at -80°C.

Full-Length STING WT [STING(1-379)H232R-gg-AviTag-gs-HRV3C-HIS8] Amino Acid Sequence:

MPHSSLHPSIPCPRGHGAQKAALVLLSACLVTLWGLGEPPEHTLRYLVLHLASLQLGLL
LNGVCSLAEELRHIHSRYRGSYWRTVRACLGCPLRRGALLLLSIYFYYSLPNAVGPPFT
WMLALLGLSQALNILLGLKGLAPAEISAVCEKGNFNVAHGLAWSYYIGYLRLILPELQA
RIRTYNQHYNNLLRGAVSQRLYILLPLDCGVPDNLSMADPNIRFLDKLPQQTGDRAGIK
DRVYSNSIYELLENGQRAGTCVLEYATPLQTLFAMSQYSQAGFSREDRLEQAKLFCRTL
EDILADAPESQNNCRLIAYQEPADDSSFSLSQEVLRHLRQEEKEEVTVGSLKTSAVPSTST
MSQEPELLISGMEKPLPLRTDFSGGGLNDIFEAQKIEWHEGSLEVLFQGPHHHHHHHH
(SEQ. ID. No. 4)

Full-length WT STING [STING(1-379)H232R-gg-AviTag-gs-HRV3C-HIS8/pBAC1] plasmid sequence:

GGAACGGCTCCGCCCACTATTAATGAAATTAAAAATTCCAATTTTAAAAAACGCAG
CAAGAGAAACATTTGTATGAAAGAATGCGTAGAAGGAAAGAAAAATGTCGTCGAC
ATGCTGAACAACAAGATTAATATGCCTCCGTGTATAAAAAAAATATTGAACGATTTG
AAAGAAAACAATGTACCGCGCGGCGGTATGTACAGGAAGAGGTTTATACTAAACTG
TTACATTGCAAACGTGGTTTCGTGTGCCAAGTGTGAAAACCGATGTTTAATCAAGGC
TCTGACGCATTTCTACAACCACGACTCCAAGTGTGTGGGTGAAGTCATGCATCTTTT
AATCAAATCCCAAGATGTGTATAAACCACCAAACTGCCAAAAAATGAAAACTGTCG
ACAAGCTCTGTCCGTTTGCTGGCAACTGCAAGGGTCTCAATCCTATTTGTAATTATTG
AATAATAAAACAATTATAAATGTCAAATTTGTTTTTTATTAACGATACAAACCAAAC
GCAACAAGAACATTTGTAGTATTATCTATAATTGAAAACGCGTAGTTATAATCGCTG
AGGTAATATTTAAAATCATTTTCAAATGATTCACAGTTAATTTGCGACAATATAATT
TTATTTTCACATAAACTAGACGCCTTGTCGTCTTCTTCTTCGTATTCCTTCTCTTTTTC
ATTTTTCTCTTCATAAAAATTAACATAGTTATTATCGTATCCATATATGTATCTATCG
TATAGAGTAAATTTTTTGTTGTCATAAATATATATGTCTTTTTTAATGGGGTGTATAG
TACCGCTGCGCATAGTTTTTCTGTAATTTACAACAGTGCTATTTTCTGGTAGTTCTTC
GGAGTGTGTTGCTTTAATTATTAAATTTATATAATCAATGAATTTGGGATCGTCGGTT

TTGTACAATATGTTGCCGGCATAGTACGCAGCTTCTTCTAGTTCAATTACACCATTTT
TTAGCAGCACCGGATTAACATAACTTTCCAAAATGTTGTACGAACCGTTAAACAAAA
ACAGTTCACCTCCCTTTTCTATACTATTGTCTGCGAGCAGTTGTTTGTTGTTAAAAAT
AACAGCCATTGTAATGAGACGCACAAACTAATATCACAAACTGGAAATGTCTATCA
ATATATAGTTGCTGATCAGATCTGATCATGGAGATAATTAAAATGATAACCATCTCG
CAAATAAATAAGTATTTTACTGTTTTCGTAACAGTTTTGTAATAAAAAAACCTATAA
ATATAGGATCCATGCCCCACTCCAGCCTGCATCCATCCATCCCGTGTCCCAGGGGTC
ACGGGGCCCAGAAGGCAGCCTTGGTTCTGCTGAGTGCCTGCCTGGTGACCCTTTGGG
GGCTAGGAGAGCCACCAGAGCACACTCTCCGGTACCTGGTGCTCCACCTAGCCTCCC
TGCAGCTGGGACTGCTGTTAAACGGGGTCTGCAGCCTGGCTGAGGAGCTGCGCCAC
ATCCACTCCAGGTACCGGGGCAGCTACTGGAGGACTGTGCGGGCCTGCCTGGGCTG
CCCCCTCCGCCGTGGGGCCCTGTTGCTGCTGTCCATCTATTTCTACTACTCCCTCCCA
AATGCGGTCGGCCCGCCCTTCACTTGGATGCTTGCCCTCCTGGGCCTCTCGCAGGCA
CTGAACATCCTCCTGGGCCTCAAGGGCCTGGCCCCAGCTGAGATCTCTGCAGTGTGT
GAAAAAGGGAATTTCAACGTGGCCCATGGGCTGGCATGGTCATATTACATCGGATA
TCTGCGGCTGATCCTGCCAGAGCTCCAGGCCCGGATTCGAACTTACAATCAGCATTA
CAACAACCTGCTACGGGGTGCAGTGAGCCAGCGGCTGTATATTCTCCTCCCATTGGA
CTGTGGGGTGCCTGATAACCTGAGTATGGCTGACCCCAACATTCGCTTCCTGGATAA
ACTGCCCCAGCAGACCGGTGACCGTGCTGGCATCAAGGATCGGGTTTACAGCAACA
GCATCTATGAGCTTCTGGAGAACGGGCAGCGGGCGGGCACCTGTGTCCTGGAGTAC
GCCACCCCCTTGCAGACTTTGTTTGCCATGTCACAATACAGTCAAGCTGGCTTTAGC
CGGGAGGATAGGCTTGAGCAGGCCAAACTCTTCTGCCGGACACTTGAGGACATCCT
GGCAGATGCCCCTGAGTCTCAGAACAACTGCCGCCTCATTGCCTACCAGGAACCTGC
AGATGACAGCAGCTTCTCGCTGTCCCAGGAGGTTCTCCGGCACCTGCGGCAGGAGG
AAAAGGAAGAGGTTACTGTGGGCAGCTTGAAGACCTCAGCGGTGCCCAGTACCTCC
ACGATGTCCCAAGAGCCTGAGCTCCTCATCAGTGGAATGGAAAAGCCCCTCCCTCTC
CGCACGGATTTCTCTGGCGGTGGCCTGAACGACATCTTCGAAGCCCAGAAAATCGA
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TAGCTGAGCGCATGCAAGCTGATCCGGGTTATTAGTACATTTATTAAGCGCTAGATT
CTGTGCGTTGTTGATTTACAGACAATTGTTGTACGTATTTTAATAATTCATTAAATTT
ATAATCTTTAGGGTGGTATGTTAGAGCGAAAATCAAATGATTTTCAGCGTCTTTATA
TCTGAATTTAAATATTAAATCCTCAATAGATTTGTAAAATAGGTTTCGATTAGTTTCA

AACAAGGGTTGTTTTTCCGAACCGATGGCTGGACTATCTAATGGATTTTCGCTCAAC
GCCACAAAACTTGCCAAATCTTGTAGCAGCAATCTAGCTTTGTCGATATTCGTTTGT
GTTTTGTTTTGTAATAAAGGTTCGACGTCGTTCAAAATATTATGCGCTTTTGTATTTC
TTTCATCACTGTCGTTAGTGTACAATTGACTCGACGTAAACACGTTAAATAGAGCTT
GGACATATTTAACATCGGGCGTGTTAGCTTTATTAGGCCGATTATCGTCGTCGTCCC
AACCCTCGTCGTTAGAAGTTGCTTCCGAAGACGATTTTGCCATAGCCACACGACGCC
TATTAATTGTGTCGGCTAACACGTCCGCGATCAAATTTGTAGTTGAGCTTTTTGGAAT
TATTTCTGATTGCGGGCGTTTTTGGGCGGGTTTCAATCTAACTGTGCCCGATTTTAAT
TCAGACAACACGTTAGAAAGCGATGGTGCAGGCGGTGGTAACATTTCAGACGGCAA
ATCTACTAATGGCGGCGGTGGTGGAGCTGATGATAAATCTACCATCGGTGGAGGCG
CAGGCGGGGCTGGCGGCGGAGGCGGAGGCGGAGGTGGTGGCGGTGATGCAGACGG
CGGTTTAGGCTCAAATGTCTCTTTAGGCAACACAGTCGGCACCTCAACTATTGTACT
GGTTTCGGGCGCCGTTTTTGGTTTGACCGGTCTGAGACGAGTGCGATTTTTTTCGTTT
CTAATAGCTTCCAACAATTGTTGTCTGTCGTCTAAAGGTGCAGCGGGTTGAGGTTCC
GTCGGCATTGGTGGAGCGGGCGGCAATTCAGACATCGATGGTGGTGGTGGTGGTGG
AGGCGCTGGAATGTTAGGCACGGGAGAAGGTGGTGGCGGCGGTGCCGCCGGTATAA
TTTGTTCTGGTTTAGTTTGTTCGCGCACGATTGTGGGCACCGGCGCAGGCGCCGCTG
GCTGCACAACGGAAGGTCGTCTGCTTCGAGGCAGCGCTTGGGGTGGTGGCAATTCA
ATATTATAATTGGAATACAAATCGTAAAAATCTGCTATAAGCATTGTAATTTCGCTA
TCGTTTACCGTGCCGATATTTAACAACCGCTCAATGTAAGCAATTGTATTGTAAAGA
GATTGTCTCAAGCTCGGATCGATCCCGCACGCCGATAACAAGCCTTTTCATTTTTACT
ACAGCATTGTAGTGGCGAGACACTTCGCTGTCGTCGAGGTTTAAACGCTTCCTCGCT
CACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAA
GGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAG
CAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTC
CATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTG
GCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCG
TGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTC
GGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGT
CGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGC
CTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACT
GGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAG
AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCT

GCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCA
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GAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTC
ACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAG
TAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATC
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TGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAG
AGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCAT
CGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCA
AGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCT
CCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCA
CTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGT
ACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGG
CGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTG
GAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTT
CGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGT
TTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCG
ACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATC
AGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAA
TAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGCGCCCTGTAGCGGCG
CATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGC
GCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTT
TCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACG
GCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCC
CTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTC
TTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAG
GGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTA
ACGCGAATTTTAACAAAATATTAACGTTTACAATTTCCCATTCGCCATTCAGGCTGC
GCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCA
(SEQ. ID. No. 5)

Certain compounds of the disclosure were evaluated in WT STING *in vitro* binding assay as described above. The following table tabulates the biological data for these compounds as EC_{50} values.

Table 13: ^{3}H-cGAMP filtration binding assay for WT STING

Compound	EC_{50} (nM)	Compound	EC_{50} (nM)	Compound	EC_{50} (nM)
Example 1	3.5	Example 139	1.2	Example 226	27.8
Example 2	0.7	Example 141	<1	Example 227	260.6
Example 3	574.3	Example 142	174.8	Example 228	1005
Example 4	43.5	Example 144	4.6	Example 229	31% Inh @ 20 uM
Example 5	1.5	Example 145	14	Example 230	382.4
Example 6	2.9	Example 148	1932	Example 231	540.4
Example 7	<1	Example 149	110.8	Example 232	3283
Example 8	81.4	Example 152	5.7	Example 233	33.8
Example 9	12.4	Example 153	6.5	Example 234	275.2
Example 10	23.2	Example 154	60	Example 235	14.9
Example 13	0.7	Example 155	<1	Example 236	6963
Example 14	1.4	Example 156	<1	Example 237	307.2
Example 20	1.4	Example 157	<1	Example 238	66.3
Example 21	1.4	Example 158	40	Example 239	3.2
Example 25	16.1	Example 159	7.1	Example 240	73.6
Example 26	3.7	Example 160	10.3	Example 241	471.8
Example 27	10	Example 161	6.2	Example 242	1.1
Example 28	264.9	Example 162	100.7	Example 243	26.3
Example 29	5835	Example 163	14.3	Example 247	1.1
Example 32	25	Example 164	312.4	Example 248	31.7
Example 38	24.9	Example 165	2.9	Example 249	<1
Example 41	505.9	Example 166	463.2	Example 250	17.3
Example 42	219	Example 167	87.7	Example 251	<1
Example 43	1015	Example 168	8.6	Example 252	768.4
Example 44	1363	Example 169	186.6	Example 253	54.5

Compound	EC_{50} (nM)	Compound	EC_{50} (nM)	Compound	EC_{50} (nM)
Example 45	104.8	Example 170	25.5	Example 254	39.7
Example 46	337	Example 171	18.7	Example 255	301.9
Example 47	444.1	Example 172	105.9	Example 256	93.5
Example 51	995.8	Example 176	16.2	Example 257	135.9
Example 61	378.7	Example 177	7.9	Example 258	5
Example 62	2.6	Example 178	37.4	Example 259	1.5
Example 63	68.8	Example 179	111.7	Example 260	7.2
Example 64	61.6	Example 180	832.1	Example 261	21.8
Example 65	249.6	Example 181	459.2	Example 262	57.3
Example 70	19.4	Example 182	3276	Example 263	4.8
Example 71	4.4	Example 183	3180	Example 264	177.5
Example 72	<1	Example 184	<1	Example 265	5473
Example 81	3.1	Example 185	201.1	Example 266	1.9
Example 82	14.6	Example 186	30.2	Example 267	6267
Example 84	6.3	Example 187	6.5	Example 268	77
Example 86	2	Example 188	47.6	Example 269	4837
Example 89	<1	Example 189	423.9	Example 270	4383
Example 90	4.4	Example 190	408.7	Example 271	12.8
Example 93	1.9	Example 191	281.2	Example 272	749.6
Example 94	523.8	Example 192	3867	Example 273	216.4
Example 95	64	Example 193	190.5	Example 274	2.3
Example 96	57.8	Example 194	159.2	Example 275	21.8
Example 97	0.4	Example 195	1855	Example 276	321.5
Example 98	151.7	Example 196	1033	Example 277	28.7
Example 99	1.8	Example 197	1.2	Example 278	477.9
Example 100	552	Example 198	19.1	Example 279	1235
Example 101	74.4	Example 199	72	Example 280	1186
Example 84	6.3	Example 200	15	Example 281	2485
Example 86	2	Example 201	649.5	Example 282	12190
Example 102	1	Example 202	813.5	Example 283	17.2

Compound	EC_{50} (nM)	Compound	EC_{50} (nM)	Compound	EC_{50} (nM)
Example 103	<1	Example 203	7.3	Example 284	128.3
Example 104	11.6	Example 204	1233	Example 285	277.1
Example 105	3.9	Example 205	42.7	Example 286	4010
Example 106	<1	Example 206	259.7	Example 287	80.2
Example 107	17.9	Example 207	1.4	Example 288	24.9
Example 108	1784	Example 208	23.3	Example 289	5.3
Example 109	1393	Example 209	9.5	Example 308	3.3
Example 110	141.4	Example 210	28.5	Example 323	3.7
Example 111	155.1	Example 211	1045	Example 324	10.7
Example 112	1279	Example 212	11.8	Example 325	866.1
Example 113	44.2	Example 213	135	Example 326	89.7
Example 114	3.3	Example 214	2.1	Example 327	14
Example 115	67.9	Example 215	33.1	Example 328	74.4
Example 116	1624	Example 216	3.4	Example 329	31.7
Example 117	1.7	Example 217	66.2	Example 330	204
Example 118	1.6	Example 218	13.8	Example 331	182.9
Example 119	40	Example 219	530.5	Example 334	110.5
Example 120	393.7	Example 220	29% Inh @ 20 uM	Example 335	5.2
Example 122	179.2	Example 221	<1	Example 336	422.2
Example 124	4	Example 222	60	Example 337	29.2
Example 126	447.4	Example 223	302.5	Example 338	441.7
Example 128	19.5	Example 224	37.4		
Example 129	973.3	Example 225	779.3		

Example 351: IFN-β secretion in THP1 cell culture (5h)

The ability of compounds to stimulate the secretion of interferon-beta from THP1 cells was measured using a human IFN-β AlphaLISA kit (Perkin Elmer, Cat. No. AL265F). The basic protocol is as follows:

A Labcyte Echo 550 acoustic dispenser was used to transfer 120nL of compound dissolved in DMSO into the wells of an empty, sterile 384-well microplate, (Corning, Cat. No. 3712). THP1 cells (American Type Culture Collection, Cat. No. TIB202) previously frozen in Recovery Medium (Life Technologies, Cat. No. 12648-010) were thawed and immediately diluted 10-fold into 37°C assay medium (RPMI 1640 + L-Glutamine & phenol red, Life Technologies, Cat. No. 11875-085; 0.5% heat inactivated fetal bovine serum, Sigma Aldrich, Cat. No. F4135; 1mM Sodium Pyruvate, Life Technologies, Cat. No. 11360-070; 1x non-essential amino acids; Life Technologies, Cat. No. 11140-050). The cell viability and count was ascertained using a Beckman Coulter V-Cell XR cell counter. The cells suspension was centrifuged at 200xg for 5min at RT. Cells were resuspended to a density of $0.8x10^6$/mL in 37°C assay medium. Subsequent liquid transfers were performed using either a Matrix electronic multichannel pipette or an Agilent Bravo Automated Liquid Handling Platform.

The assay was started by dispensing 40µL of the previously prepared cell suspension into the wells of the plate containing compounds. After 5h incubation at 37°C, 5% CO_2 in a humidified atmosphere, the plate of cells and compounds was centrifuged at 200xg for 5min at RT. From each well, 5µL of supernatant was transferred into corresponding wells of a white 384-well plate (Perkin Elmer, Cat. No. 6005620). To these supernatant-containing wells was added 10µL of 5x Anti-Analyte Acceptor beads (50µg/mL of AlphaLISA HiBlock Buffer) and incubated for 30min at RT while shaking on an orbital plate shaker. To each well was added 10µL of 5x Biotinylated Antibody Anti-analyte (5nM in AlphaLISA HiBlock Buffer) and incubated on an orbital plate shaker for 60min at RT or overnight at 4°C. To each well was added 25µL of 2x SA-Donor beads (80µg/mL in AlphaLISA HiBlock Buffer) and incubated for 30-45min at RT in the dark while shaking on an orbital plate shaker. The plate was then read on a Perkin Elmer Envision (λ_{ex} = 680nm, λ_{em} = 570nm). The percent effect of the AlphaLISA signal at each compound concentration was calculated based on 30uM cGAMP positive controls and 0.3% DMSO negative controls. The plot of percent effect versus the log of compound concentration was fit with a 4-parameter concentration response equation to calculate EC_{50} values. The test compounds were tested at concentrations 30000, 10000, 3333, 1111, 370.4, 123.4, 41.2, 13.7, 4.6, and 1.5nM with 0.3% residual DMSO. The control compound, cGAMP was tested at concentrations 100000, 33333, 11111, 3704, 1235, 412, 137, 46, and 15nM with 0.3% residual DMSO.

Compounds of the disclosure were evaluated for IFN-β secretion in THP1 cell culture as described above. The following table tabulates the biological data for these compounds as percent activation relative to 2'3'-cGAMP at the 30μM concentration.

Table 14: IFN-β secretion in THP1 cell culture (5h)

Compound	% Effect at 30μM relative to 2'3'-cGAMP	Compound	% Effect at 30μM relative to 2'3'-cGAMP	Compound	% Effect at 30μM relative to 2'3'-cGAMP
Example 1	154	Example 117	211	Example 233	254
Example 2	148	Example 118	174	Example 234	75
Example 3	149	Example 119	151	Example 235	262
Example 4	171	Example 120	72	Example 236	5
Example 5	152	Example 121	108	Example 237	222
Example 6	114	Example 122	75	Example 238	183
Example 7	112	Example 123	128	Example 239	164
Example 8	98	Example 124	128	Example 240	141
Example 9	143	Example 125	138	Example 241	300
Example 10	126	Example 126	167	Example 242	156
Example 11	169	Example 127	145	Example 243	165
Example 12	89	Example 128	146	Example 244	96
Example 13	39	Example 129	33	Example 245	234
Example 14	139	Example 130	215	Example 246	217
Example 15	39	Example 131	238	Example 247	166
Example 16	57	Example 132	150	Example 248	42
Example 17	81	Example 133	117	Example 249	106
Example 18	74	Example 134	138	Example 250	6
Example 19	49	Example 135	43	Example 251	175
Example 20	159	Example 136	131	Example 252	23
Example 21	142	Example 137	124	Example 253	73
Example 22	109	Example 138	51	Example 254	131
Example 23	125	Example 139	110	Example 255	49
Example 24	90	Example 140	96	Example 256	149
Example 25	145	Example 141	111	Example 257	8

Compound	% Effect at 30μM relative to 2'3'-cGAMP	Compound	% Effect at 30μM relative to 2'3'-cGAMP	Compound	% Effect at 30μM relative to 2'3'-cGAMP
Example 26	150	Example 142	111	Example 258	120
Example 27	164	Example 143	157	Example 259	91
Example 28	60	Example 144	114	Example 260	113
Example 29	25	Example 145	141	Example 261	90
Example 30	131	Example 146	59	Example 262	108
Example 31	100	Example 147	146	Example 263	114
Example 32	125	Example 148	13	Example 264	123
Example 33	200	Example 149	8	Example 265	25
Example 34	125	Example 150	89	Example 266	168
Example 35	51	Example 151	57	Example 267	5
Example 36	120	Example 152	16	Example 268	181
Example 37	57	Example 153	234	Example 269	109
Example 38	123	Example 154	198	Example 270	235
Example 39	74	Example 155	139	Example 271	229
Example 40	77	Example 156	119	Example 272	44
Example 41	8	Example 157	151	Example 273	85
Example 42	101	Example 158	123	Example 274	124
Example 43	130	Example 159	140	Example 275	157
Example 44	135	Example 160	12	Example 276	45
Example 45	161	Example 161	130	Example 277	174
Example 46	143	Example 162	116	Example 278	68
Example 47	141	Example 163	127	Example 279	160
Example 48	104	Example 164	41	Example 280	191
Example 49	74	Example 165	245	Example 281	8
Example 50	85	Example 166	143	Example 282	39
Example 51	36	Example 167	278	Example 283	288
Example 52	143	Example 168	164	Example 284	260
Example 53	127	Example 169	117	Example 285	195
Example 54	83	Example 170	134	Example 286	16

Compound	% Effect at 30μM relative to 2'3'-cGAMP	Compound	% Effect at 30μM relative to 2'3'-cGAMP	Compound	% Effect at 30μM relative to 2'3'-cGAMP
Example 55	83	Example 171	142	Example 287	202
Example 56	64	Example 172	26	Example 288	153
Example 57	7	Example 173	142	Example 289	58
Example 58	92	Example 174	5	Example 290	90
Example 59	76	Example 175	120	Example 291	98
Example 60	12	Example 176	114	Example 292	114
Example 61	34	Example 177	180	Example 293	86
Example 62	68	Example 178	111	Example 294	84
Example 63	83	Example 179	66	Example 295	70
Example 64	93	Example 180	143	Example 296	92
Example 65	87	Example 181	178	Example 297	176
Example 66	54	Example 182	47	Example 298	121
Example 67	111	Example 183	23	Example 299	5
Example 68	72	Example 184	108	Example 300	86
Example 69	31	Example 185	61	Example 301	107
Example 70	100	Example 186	179	Example 302	103
Example 71	113	Example 187	134	Example 303	106
Example 72	102	Example 188	251	Example 304	104
Example 73	5	Example 189	93	Example 305	50
Example 74	57	Example 190	200	Example 306	153
Example 75	96	Example 191	516	Example 307	174
Example 76	39	Example 192	50	Example 308	132
Example 77	39	Example 193	399	Example 309	232
Example 78	162	Example 194	258	Example 310	117
Example 79	145	Example 195	61	Example 311	9
Example 80	234	Example 196	14	Example 312	90
Example 81	138	Example 197	237	Example 313	117
Example 82	111	Example 198	205	Example 314	143
Example 83	133	Example 199	139	Example 315	92

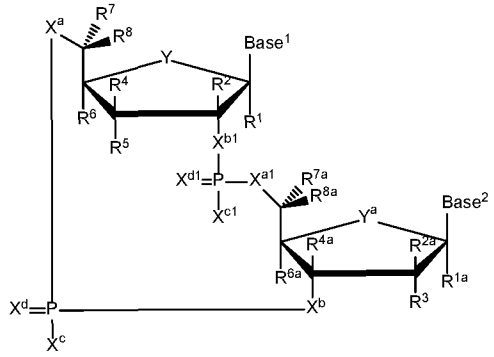
Compound	% Effect at 30μM relative to 2'3'-cGAMP	Compound	% Effect at 30μM relative to 2'3'-cGAMP	Compound	% Effect at 30μM relative to 2'3'-cGAMP
Example 84	128	Example 200	219	Example 316	12
Example 85	154	Example 201	222	Example 317	107
Example 86	137	Example 202	171	Example 318	150
Example 87	99	Example 203	194	Example 319	143
Example 88	54	Example 204	101	Example 320	115
Example 89	119	Example 205	132	Example 321	36
Example 90	117	Example 206	166	Example 322	41
Example 91	106	Example 207	189	Example 323	92
Example 92	62	Example 208	163	Example 324	99
Example 93	155	Example 209	170	Example 325	10
Example 94	138	Example 210	178	Example 326	34
Example 95	118	Example 211	114	Example 327	126
Example 96	67	Example 212	285	Example 328	99
Example 97	113	Example 213	255	Example 329	105
Example 98	54	Example 214	224	Example 330	67
Example 99	135	Example 215	277	Example 331	116
Example 100	35	Example 216	170	Example 332	113
Example 101	129	Example 217	70	Example 333	10
Example 102	132	Example 218	152	Example 334	66
Example 103	114	Example 219	142	Example 335	60
Example 104	154	Example 220	6	Example 336	85
Example 105	137	Example 221	405	Example 337	204
Example 106	208	Example 222	171	Example 338	219
Example 107	171	Example 223	11	Example 339	175
Example 108	15	Example 224	288	Example 340	306
Example 109	151	Example 225	467	Example 341	7
Example 110	181	Example 226	439	Example 342	23
Example 111	200	Example 227	290	Example 343	255
Example 112	33	Example 228	6	Example 344	337

Compound	% Effect at 30μM relative to 2'3'-cGAMP	Compound	% Effect at 30μM relative to 2'3'-cGAMP	Compound	% Effect at 30μM relative to 2'3'-cGAMP
Example 113	154	Example 229	6	Example 345	144
Example 114	163	Example 230	116	Example 346	182
Example 115	177	Example 231	189	Example 347	225
Example 116	149	Example 232	21	Example 348	8

It will be appreciated that various of the above-discussed and other features and functions, or alternatives thereof, may be desirably combined into many other different systems or applications. Also that various presently unforeseen or unanticipated alternatives, modifications, variations or improvements therein may be subsequently made by those skilled in the art which are also intended to be encompassed by the following claims.

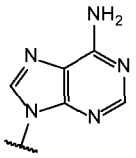
WHAT IS CLAIMED IS:

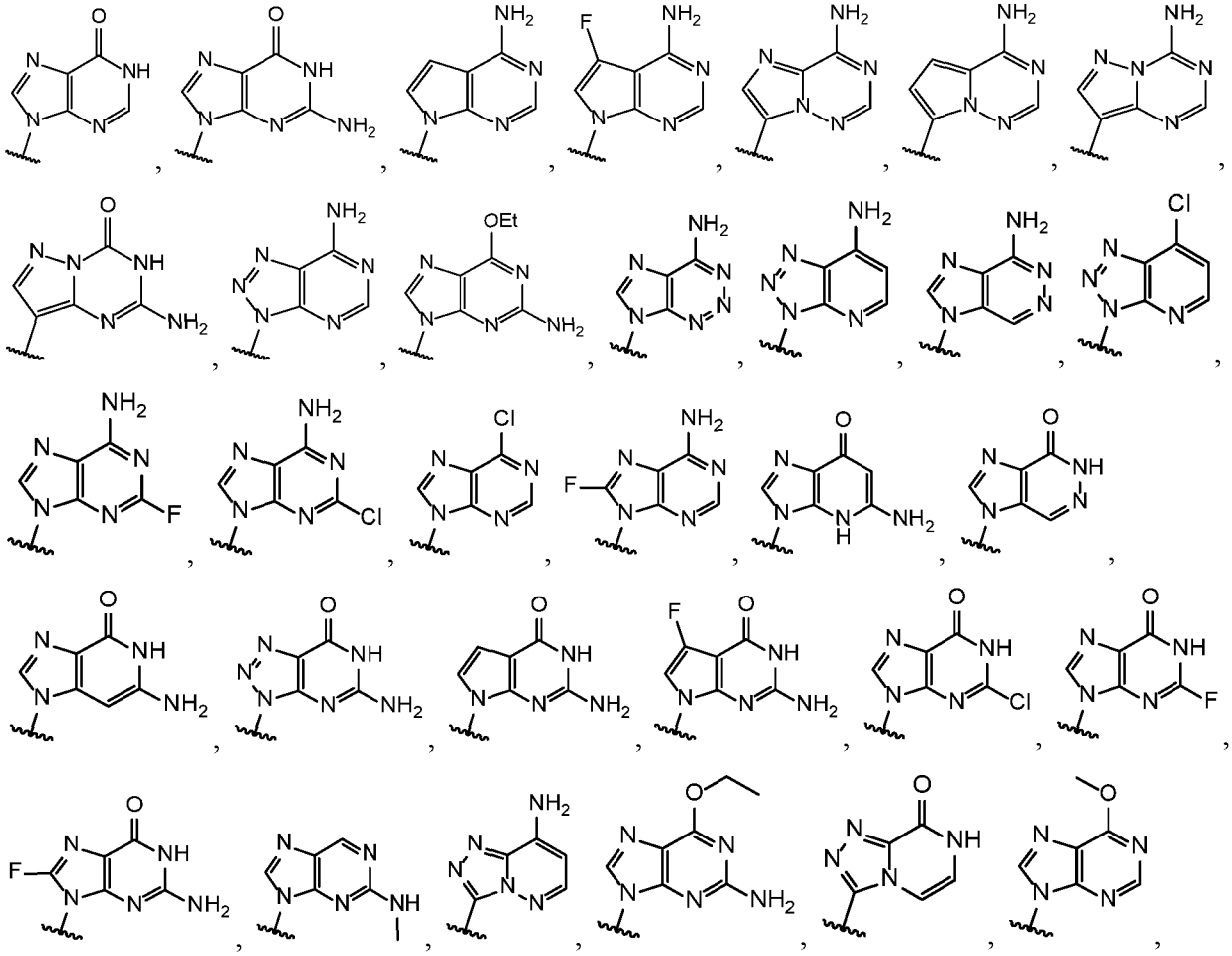
1. A compound of formula (I):

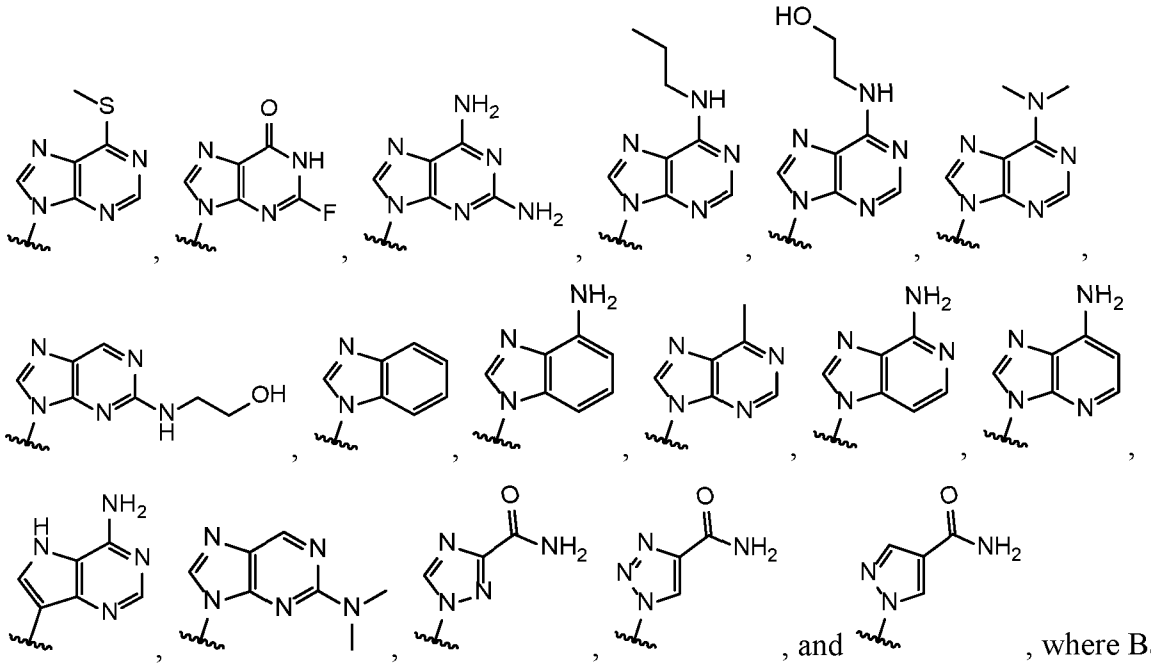


(I)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

Base[1] and Base[2] are each independently selected from the group consisting of  ,





and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$;

Y and Y^a are each independently selected from the group consisting of -O- and -S-;

X^a and X^{a1} are each independently selected from the group consisting of O, C, and S;

X^b and X^{b1} are each independently selected from the group consisting of O, C, and S;

X^c and X^{c1} are each independently selected from the group consisting of SR^9, OR^9, and NR^9R^9;

X^d and X^{d1} are each independently selected from the group consisting of O and S;

R^1 and R^{1a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^1 and R^{1a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^2 and R^{2a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6

haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^3 C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^4 and R^{4a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^4 and R^{4a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

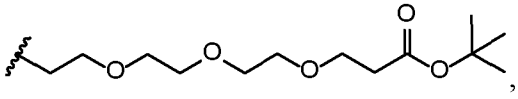
R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^5 C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^6 and R^{6a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^6 and R^{6a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

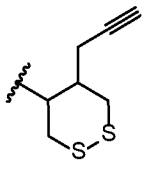
R^7 and R^{7a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^7 and R^{7a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^8 and R^{8a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^8 and R^{8a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

each R^9 is independently selected from the group consisting of H, C_1-C_{20} alkyl,



, and



, where each R^9 C_1-C_{20} alkyl is optionally substituted by 0 to 3 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl;

optionally R^{1a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{1a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position;

optionally R^{2a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{2a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position;

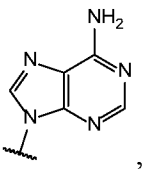
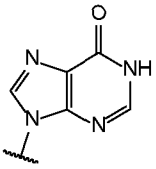
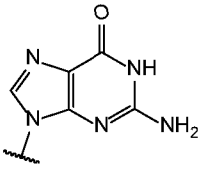
optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position;

optionally R^4 and R^5 are connected to form are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position;

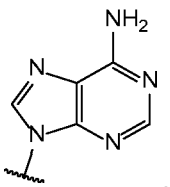
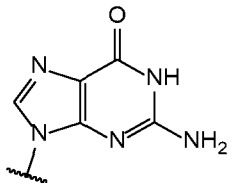
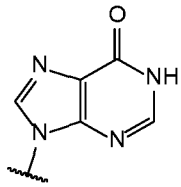
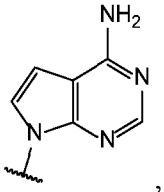
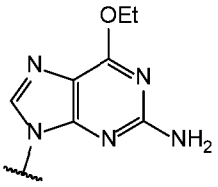
optionally R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position;

optionally R^7 and R^8 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene; and

optionally R^{7a} and R^{8a} are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene; and

providing that when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH or SH, X^d and X^{d1} are each O, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group consisting of  ,  and  , R^5 and R^3 are not both selected from the group consisting of H, F and OH.

2. The compound according to claim 1, or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

$Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  ,  ,  , and  , where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$;

Y and Y^a are each independently selected from the group consisting of -O- and -S-;

X^a and X^{a1} are each independently selected from the group consisting of O and S;

X^b and X^{b1} are each independently selected from the group consisting of O and S;

X^c and X^{c1} are each independently selected from the group consisting of SR^9, OR^9, and NR^9R^9;

X^d and X^{d1} are each independently selected from the group consisting of O and S;

R^1 and R^{1a} are each H;

R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^2 and R^{2a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^4 and R^{4a} are each independently selected from the group consisting of H, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^4 and R^{4a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

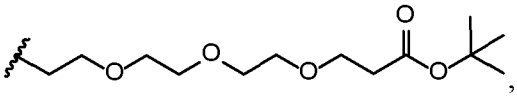
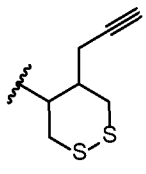
R^3 and R^5 are not both selected from the group consisting of: OH, C_1-C_6 alkyl substituted with OH, or C_1-C_6 haloalkyl substituted with OH; and

R^6 and R^{6a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, and C_1-C_6 haloalkyl, where said R^6 and R^{6a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^7 and R^{7a} are each H;

R^8 and R^{8a} are each H;

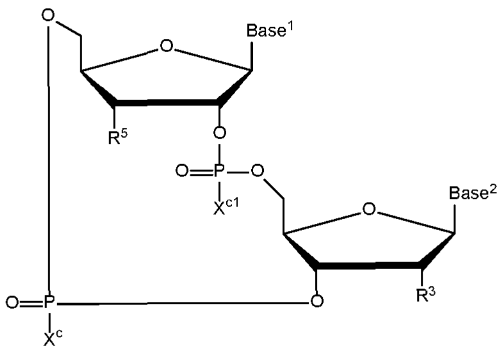
each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,


, and

, where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl;

optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, and -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position; and

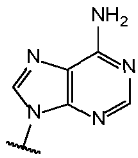
optionally R^4 and R^5 are connected by C_1-C_6 alkylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position.

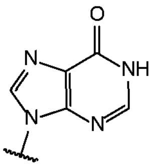
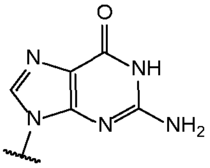
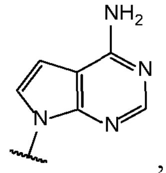
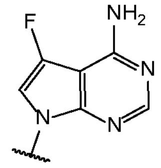
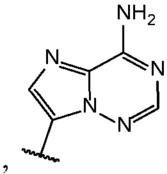
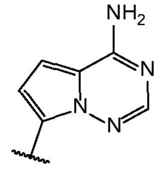
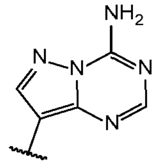
3. The compound according to claim 1, wherein the compound of formula (I) is a compound of formula (Ia):

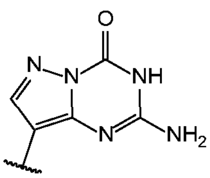
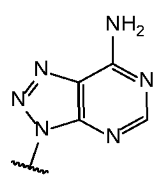
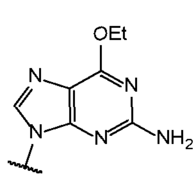
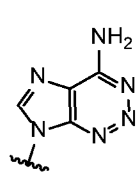
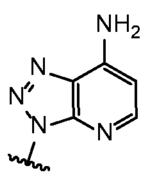
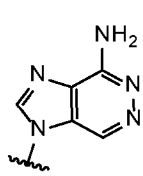
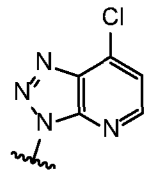


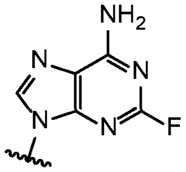
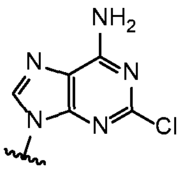
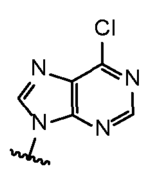
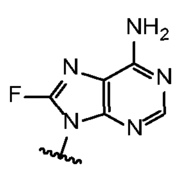
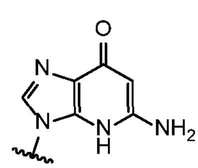
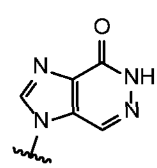
(Ia)

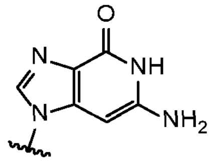
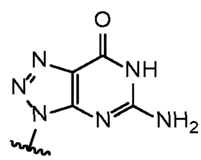
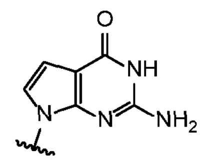
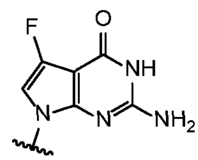
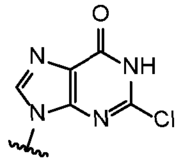
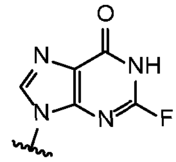
or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

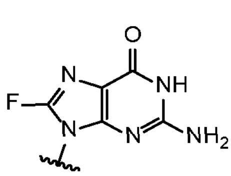
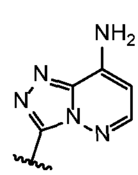
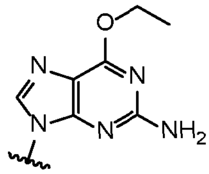
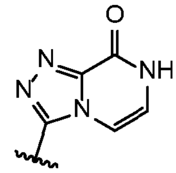
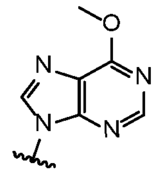
Base[1] and Base[2] are each independently selected from the group consisting of  ,

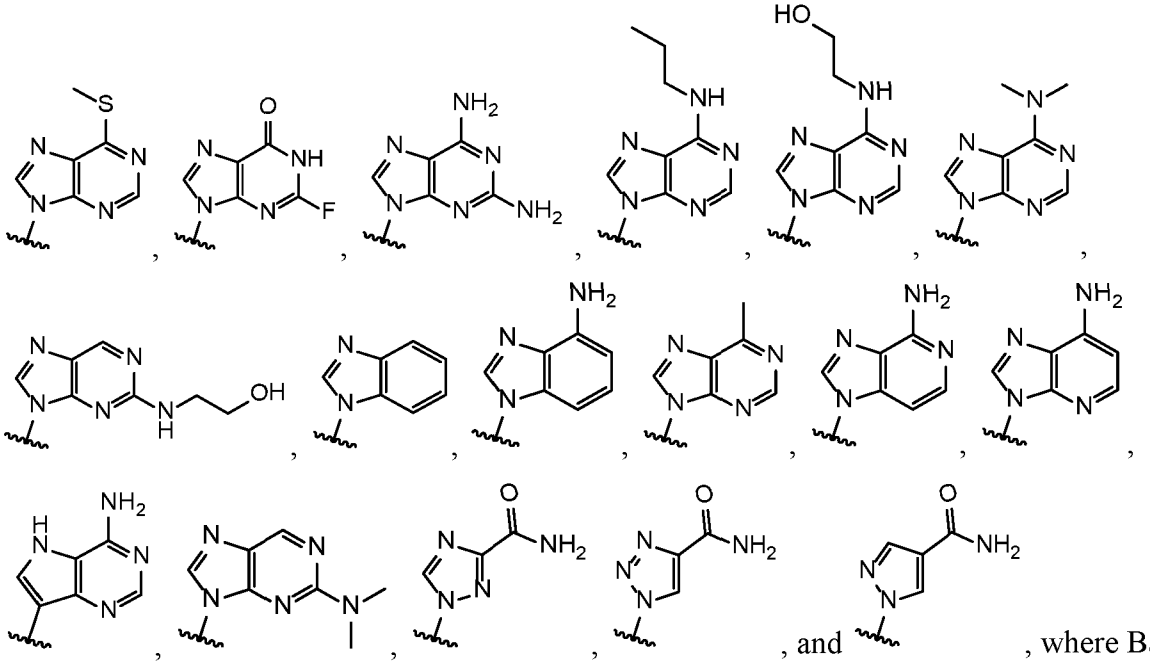
 ,  ,  ,  ,  ,  ,  ,

 ,  ,  ,  ,  ,  ,  ,

 ,  ,  ,  ,  ,  ,

 ,  ,  ,  ,  ,  ,

 ,  ,  ,  ,  ,  ,



and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$;

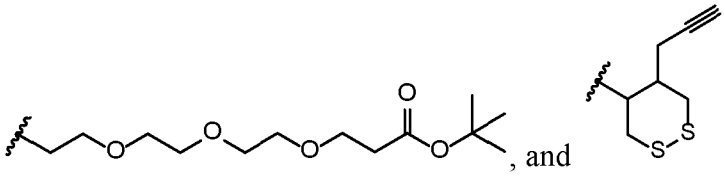
X^c and X^{c1} are each independently selected from the group consisting of SR^9, OR^9, and NR^9R^9;

R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

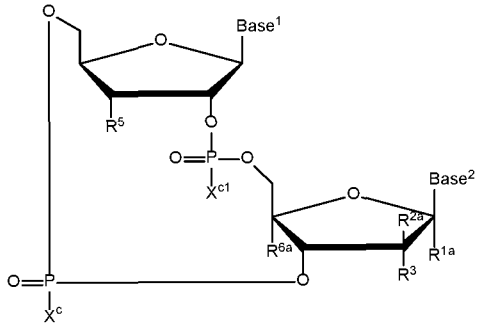
R^3 and R^5 are not both selected from the group consisting of: OH, R^5 C_1-C_6 alkyl substituted with OH, or C_1-C_6 haloalkyl substituted with OH; and

each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,



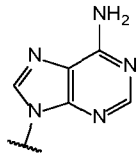
, where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl.

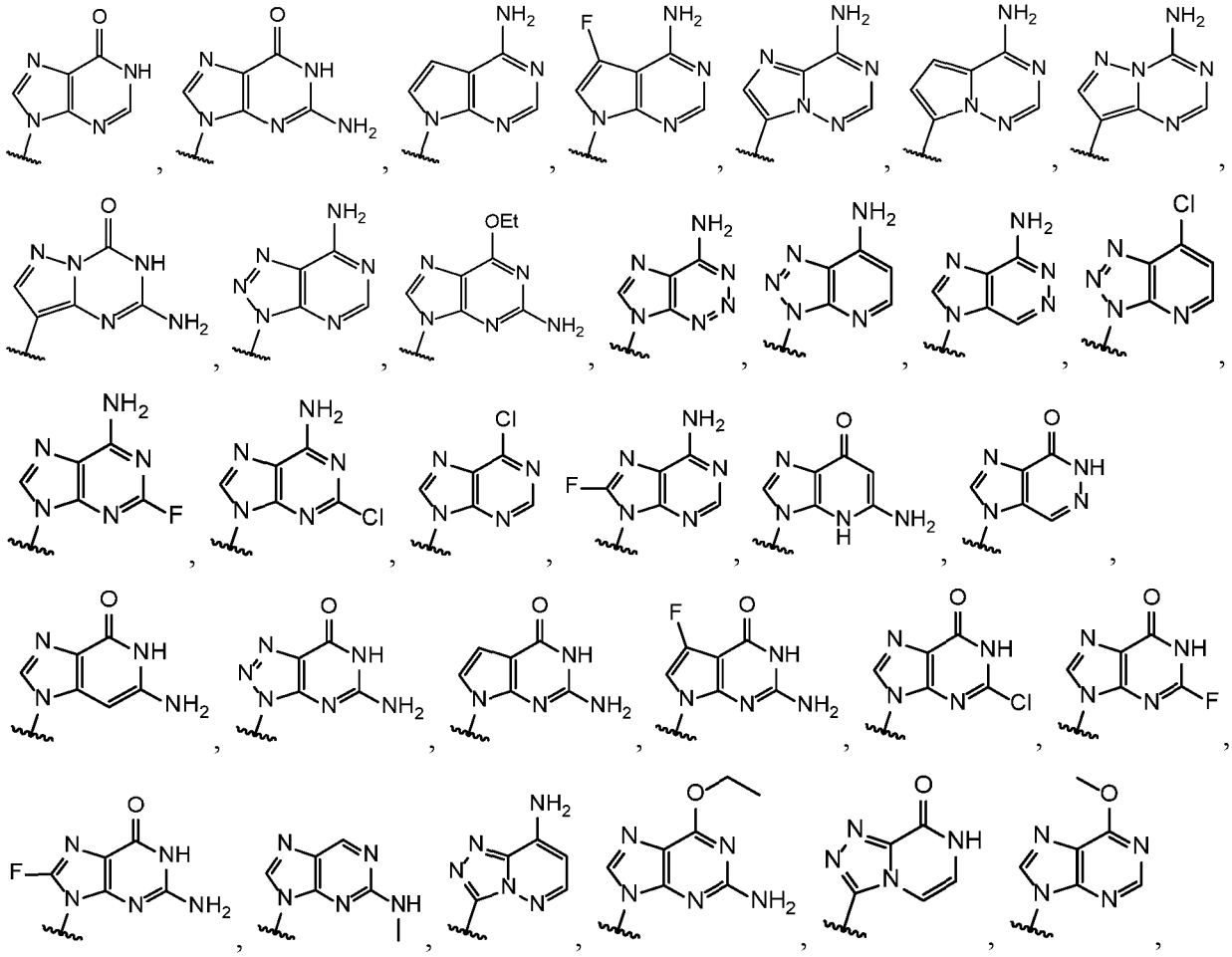
4. The compound according to claim 1, wherein the compound of formula (I) is a compound of formula (Ib):

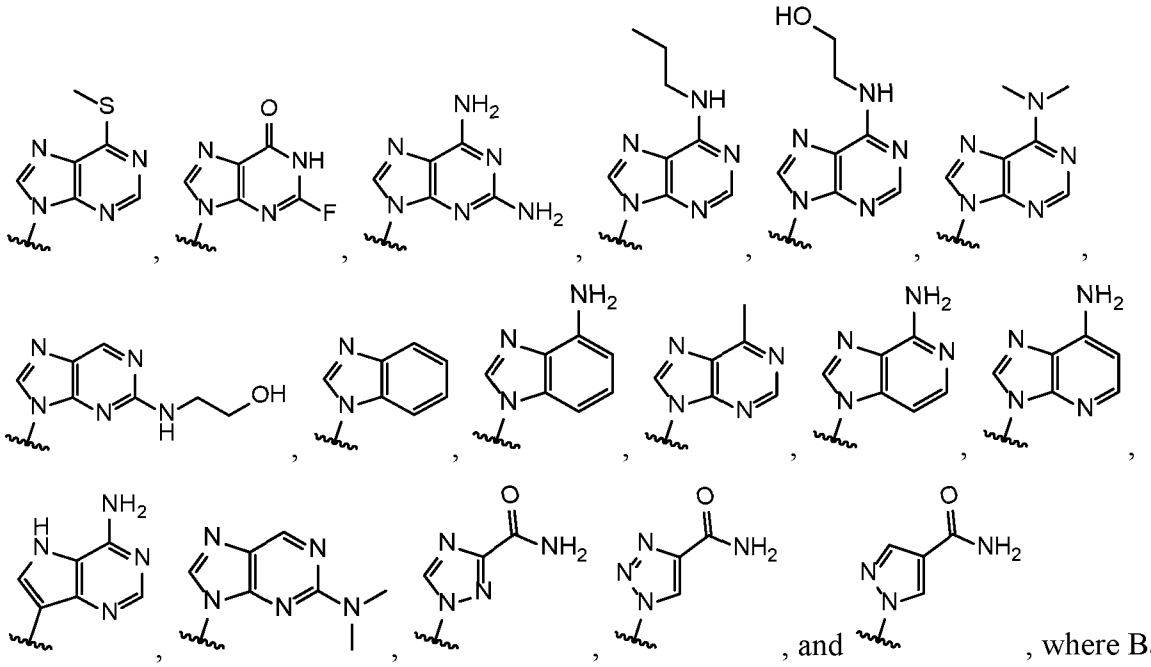


(Ib)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

$Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,





and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$;

X^c and X^{c1} are each independently selected from the group consisting of SR^9, OR^9, and NR^9R^9;

R^{1a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^{1a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^{2a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^{2a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

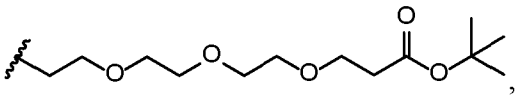
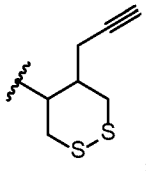
R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

R^3 and R^5 are not both selected from the group consisting of OH, C_1-C_6 alkyl substituted with OH, and C_1-C_6 haloalkyl substituted with OH;

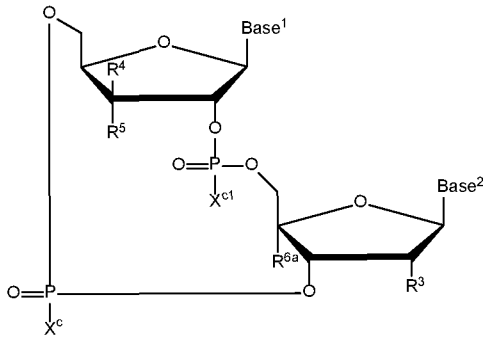
R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl;

each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,


, and

, where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; and

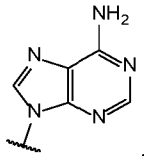
optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, and -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position.

5. The compound according to claim 1, wherein the compound of formula (I) is a compound of formula (Ic):



(Ic)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

Base^1 and Base^2 are each independently selected from the group consisting of

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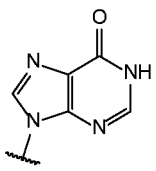
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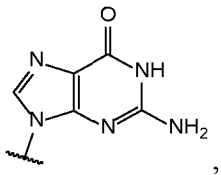
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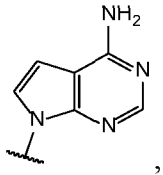
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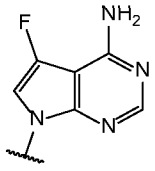
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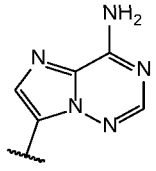
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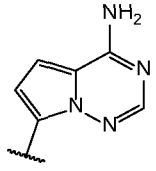
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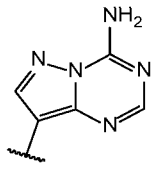
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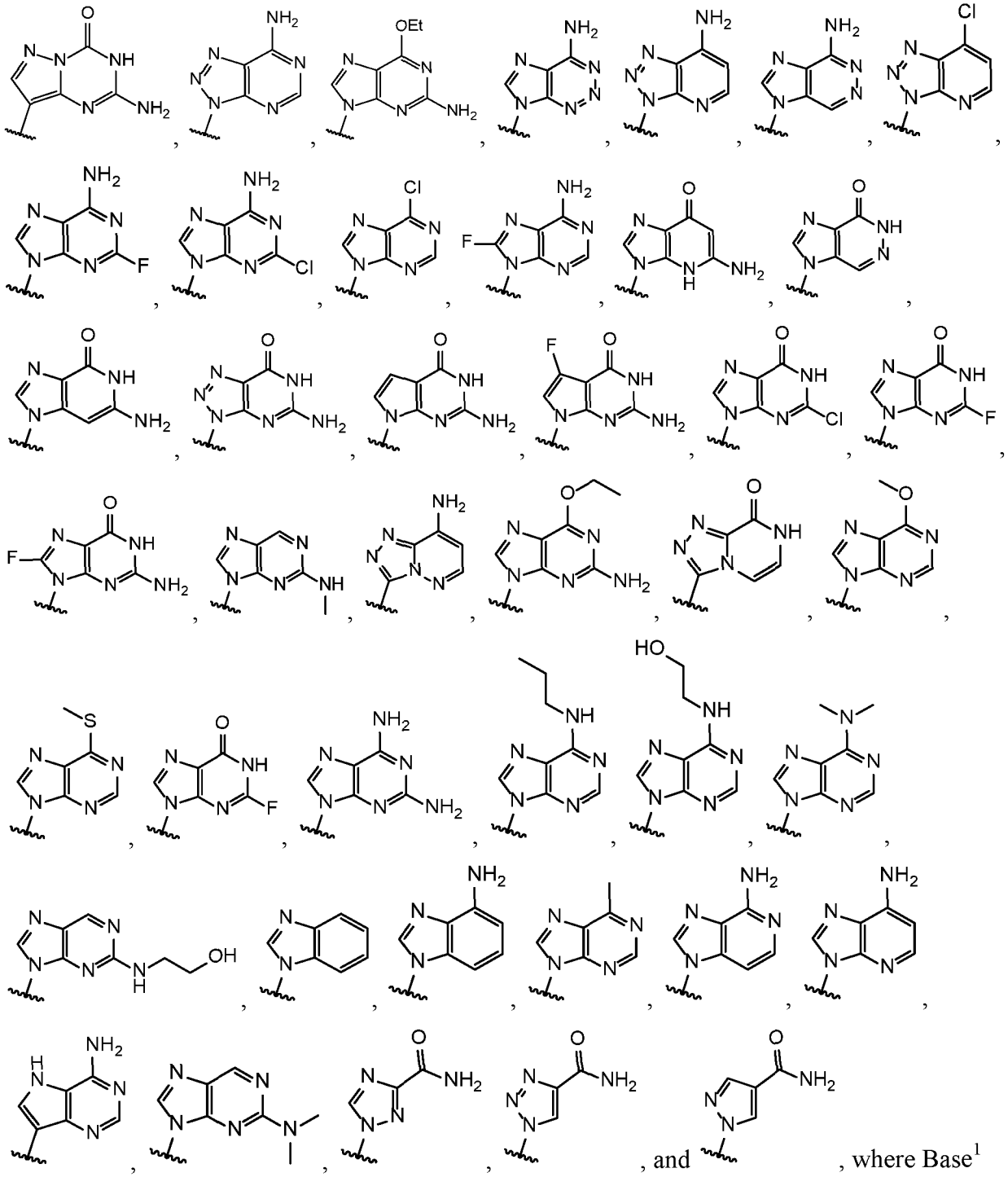


and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$;

X^c and X^{c1} are each independently selected from the group consisting of SR^9, OR^9, and NR^9R^9;

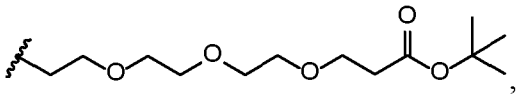
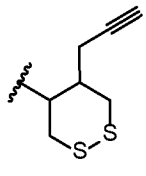
R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

R^4 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^4 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

R^3 and R^5 are not both selected from the group consisting of: OH, C_1-C_6 alkyl substituted with OH, or C_1-C_6 haloalkyl substituted with OH;

R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^{6a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,  , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; and

optionally R^4 and R^5 are connected by C_1-C_6 alkylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position.

6. A pharmaceutical composition, said pharmaceutical composition comprising:

(a) a compound according to any one of claims 1 to 5 or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof; and

(b) a pharmaceutically acceptable carrier.

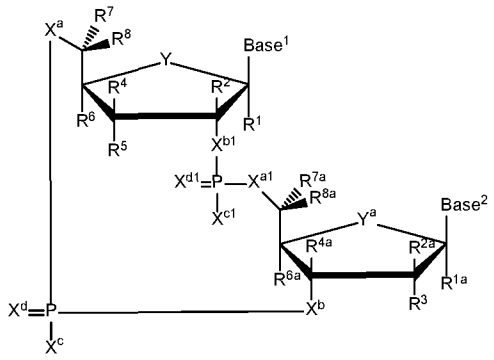
7. A method of inducing an immune response in a subject, said method comprising administering a therapeutically effective amount of a compound according to any one of claims 1 to 5 to the subject.

8. A method of inducing an immune response in a subject, said method comprising administering a therapeutically effective amount of a pharmaceutical composition according to claim 6 to the subject.

9. A method of inducing a STING-dependent type I interferon production in a subject, said method comprising administering a therapeutically effective amount of a compound according to any one of claims 1 to 5 to the subject.

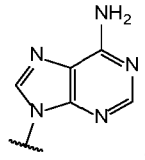
10. A method of inducing a STING-dependent type I interferon production in a subject, said method comprising administering a therapeutically effective amount of a pharmaceutical composition according to claim 6 to the subject.

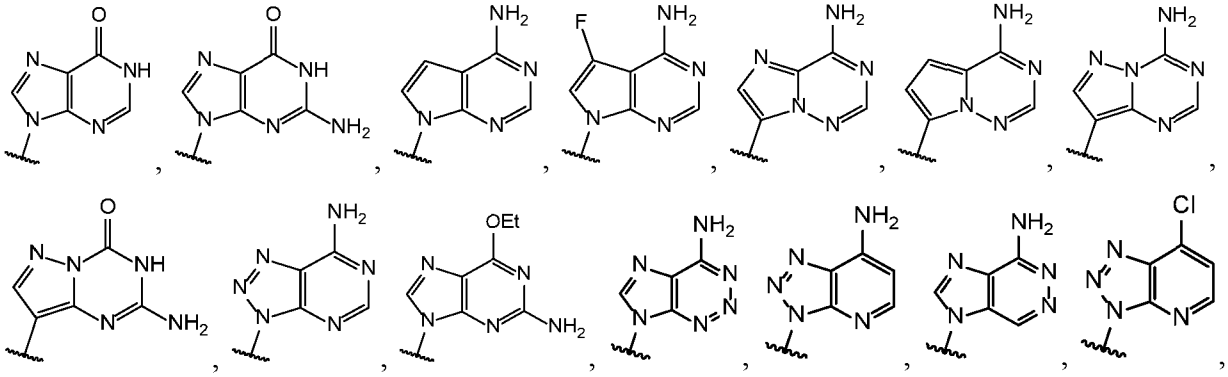
11. A compound of formula (I'):

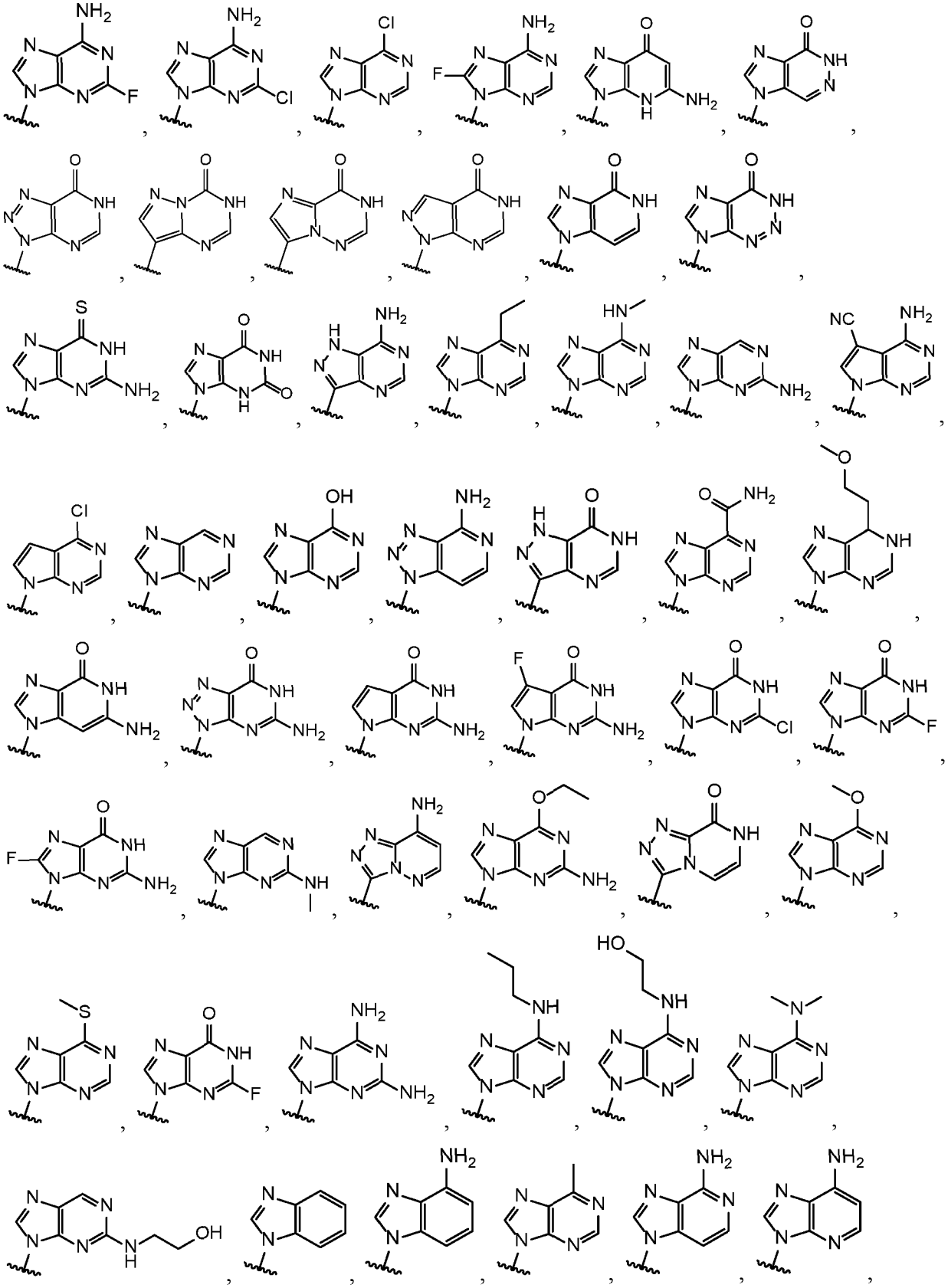


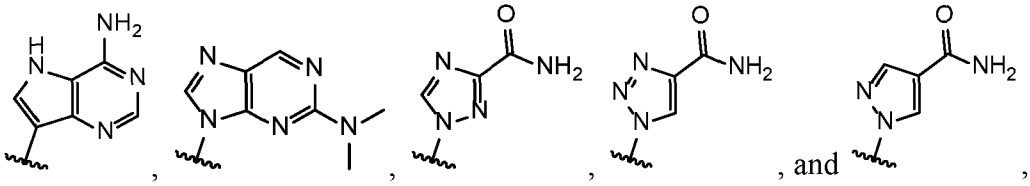
(I')

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

$Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,





 , , , , and , where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$;

Y and Y^a are each independently selected from the group consisting of -O- and -S-;

X^a and X^{a1} are each independently selected from the group consisting of O, and S;

X^b and X^{b1} are each independently selected from the group consisting of O, and S;

X^c and X^{c1} are each independently selected from the group consisting of OR^9, SR^9, and NR^9R^9;

X^d and X^{d1} are each independently selected from the group consisting of O and S;

R^1 and R^{1a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^1 and R^{1a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^2 and R^{2a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^3 C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

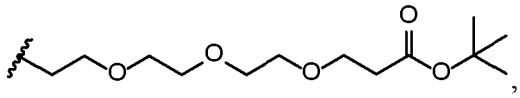
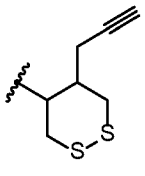
R^4 and R^{4a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^4 and R^{4a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^5 C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, NR^9R^9, and N_3;

R^6 and R^{6a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^6 and R^{6a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^7 and R^{7a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^7 and R^{7a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^8 and R^{8a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^8 and R^{8a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

each R^9 is independently selected from the group consisting of H, C_1-C_{20} alkyl,  , and  , where each R^9 C_1-C_{20} alkyl is optionally substituted by 0 to 3 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl;

optionally R^{1a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{1a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position;

optionally R^{2a} and R^3 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^{2a} and R^3 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position;

optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position;

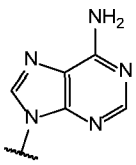
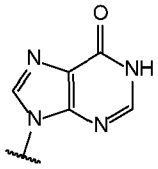
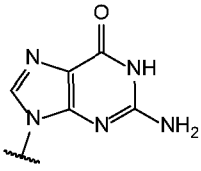
optionally R^4 and R^5 are connected to form are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position;

optionally R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^5 and R^6 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position;

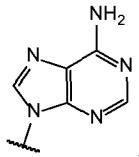
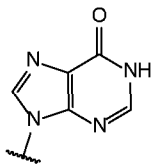
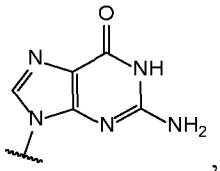
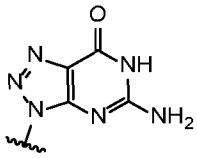
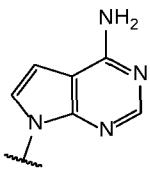
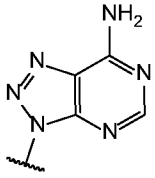
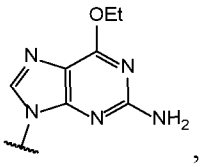
optionally R^7 and R^8 are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene; and

optionally R^{7a} and R^{8a} are connected to form C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene; and

providing that when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH or SH, X^d and X^{d1} are each O, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each

selected from the group consisting of  ,  and  , R^5 and R^3 are not both selected from the group consisting of H, F and OH.

12. The compound according to claim 11, or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

$Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  ,  ,  ,  ,  , and  ,

where $Base^1$ and $Base^2$ each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$;

Y and Y^a are each independently selected from the group consisting of -O- and -S-;

X^a and X^{a1} are each independently selected from the group consisting of O and S;

X^b and X^{b1} are each independently selected from the group consisting of O and S;

X^c and X^{c1} are each independently selected from the group consisting of OR^9, SR^9, and NR^9R^9;

X^d and X^{d1} are each independently selected from the group consisting of O and S;

R^1 and R^{1a} are each H;

R^2 and R^{2a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^2 and R^{2a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^4 and R^{4a} are each independently selected from the group consisting of H, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^4 and R^{4a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, NH_2, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, NR^9R^9, and N_3;

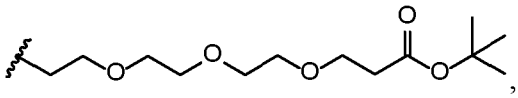
R^3 and R^5 are not both selected from the group consisting of OH, C_1-C_6 alkyl substituted with OH, and C_1-C_6 haloalkyl substituted with OH;

R^6 and R^{6a} are each independently selected from the group consisting of H, F, Cl, Br, I, OH, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, and C_1-C_6 haloalkyl, where said R^6 and R^{6a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

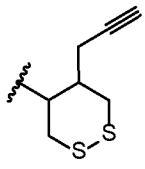
R^7 and R^{7a} are each H;

R^8 and R^{8a} are each H;

each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,



, and

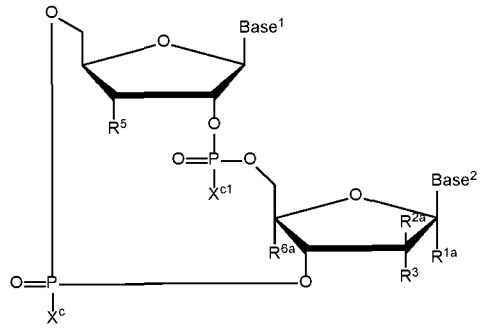


, where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl;

optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, and -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position; and

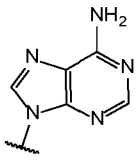
optionally R^4 and R^5 are connected by C_1-C_6 alkylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position.

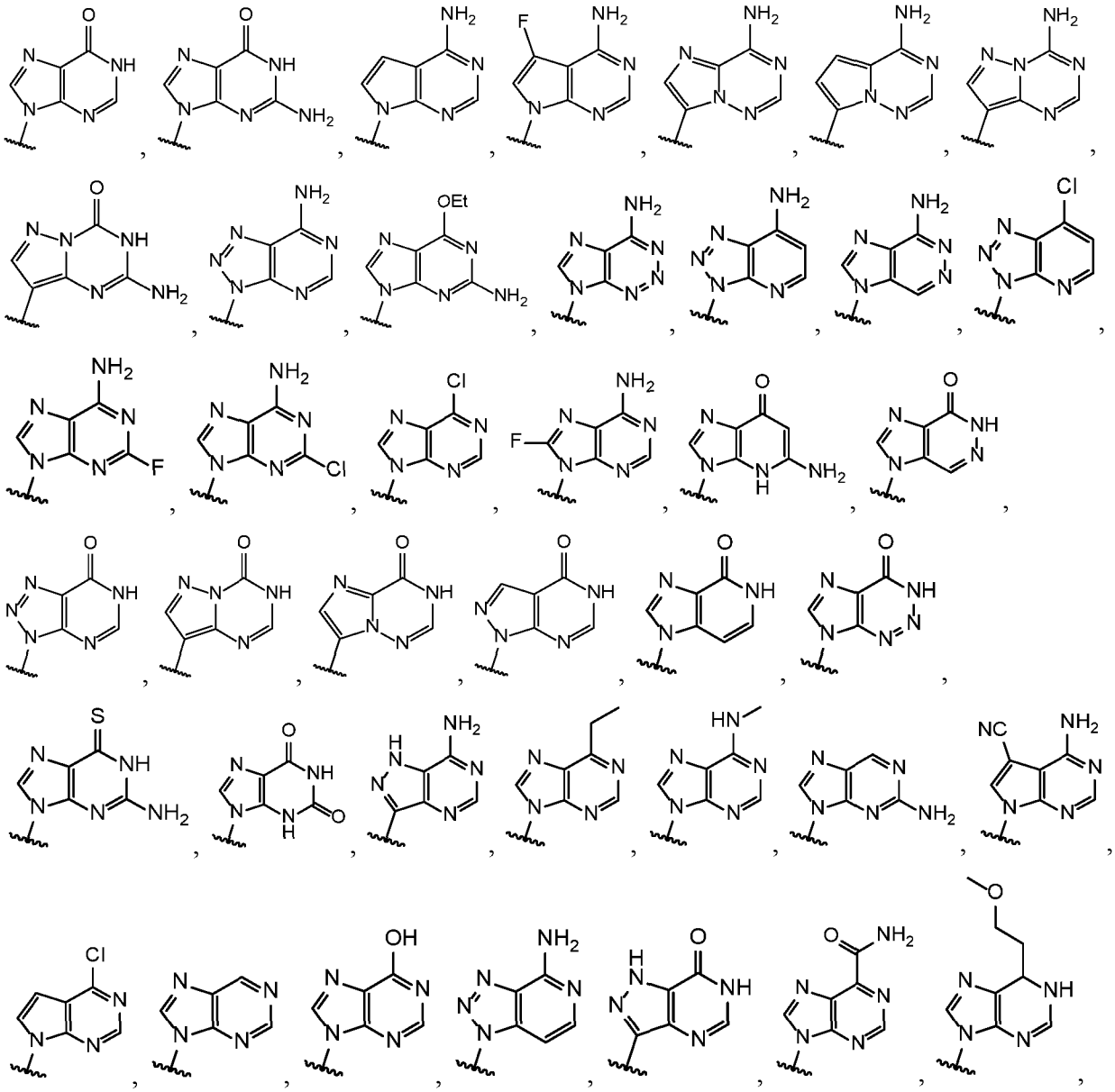
13. The compound according to claim 11, or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein the compound of formula (I') is a compound of formula (I'b):

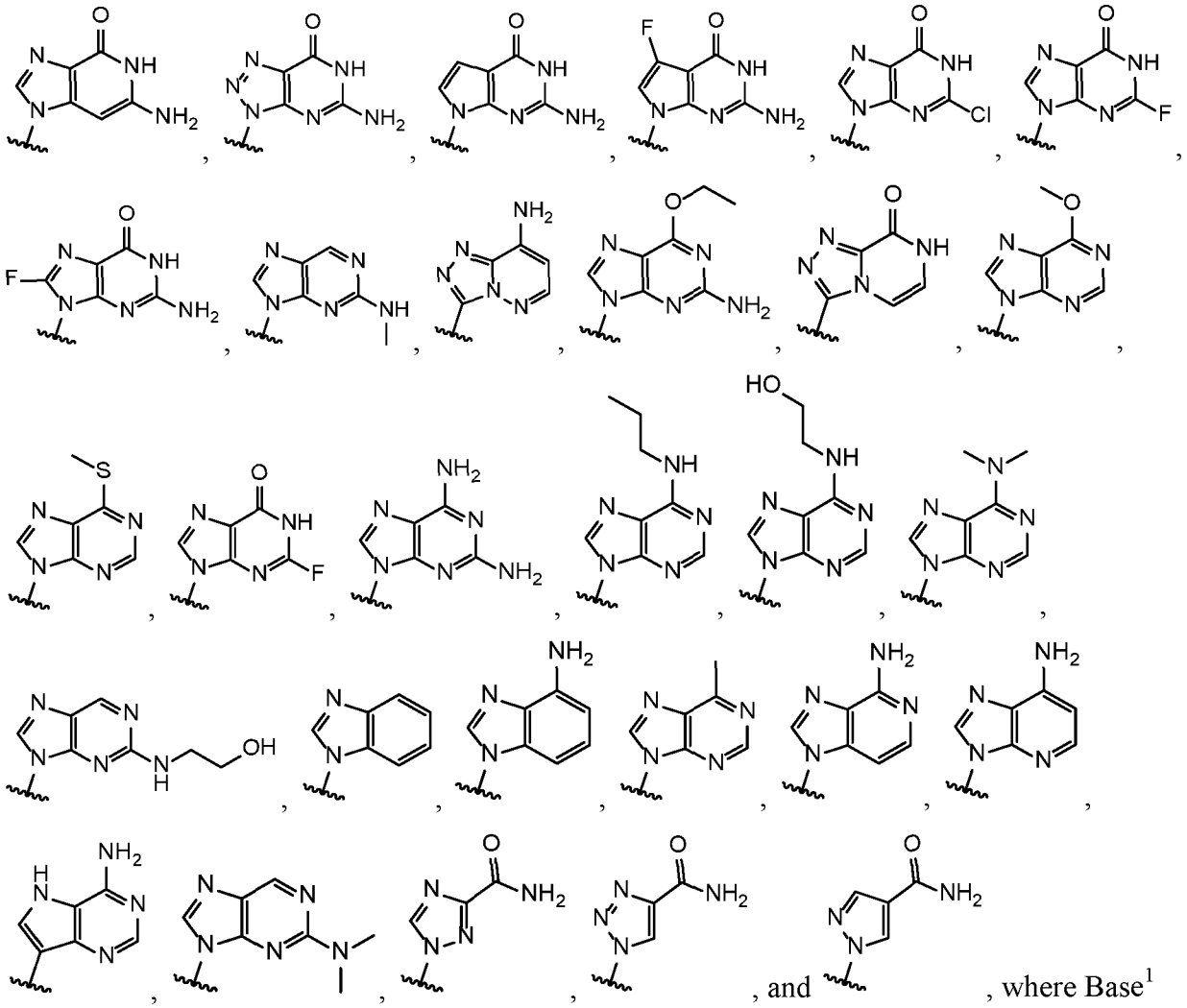


(I'b)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

Base[1] and Base[2] are each independently selected from the group consisting of  ,





and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$;

X^c and X^{c1} are each independently selected from the group consisting of OR^9, SR^9, and NR^9R^9;

R^{1a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl, where said R^{1a} C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, OH, CN, and N_3;

R^{2a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^{2a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

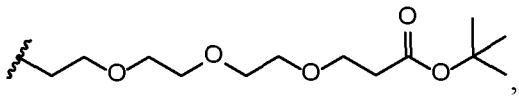
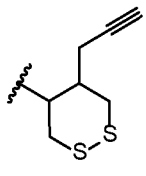
R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

R^3 and R^5 are not both selected from the group consisting of OH, C_1-C_6 alkyl substituted with OH, and C_1-C_6 haloalkyl substituted with OH;

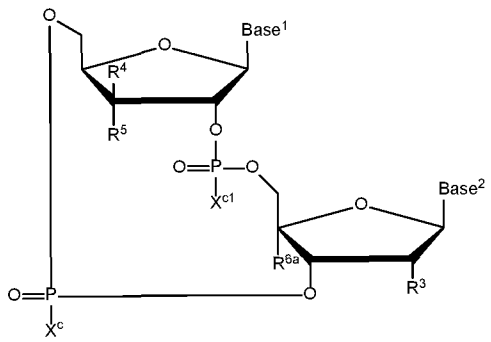
R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl;

each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,

 , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; and

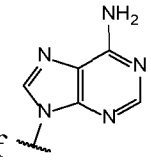
optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, and -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position.

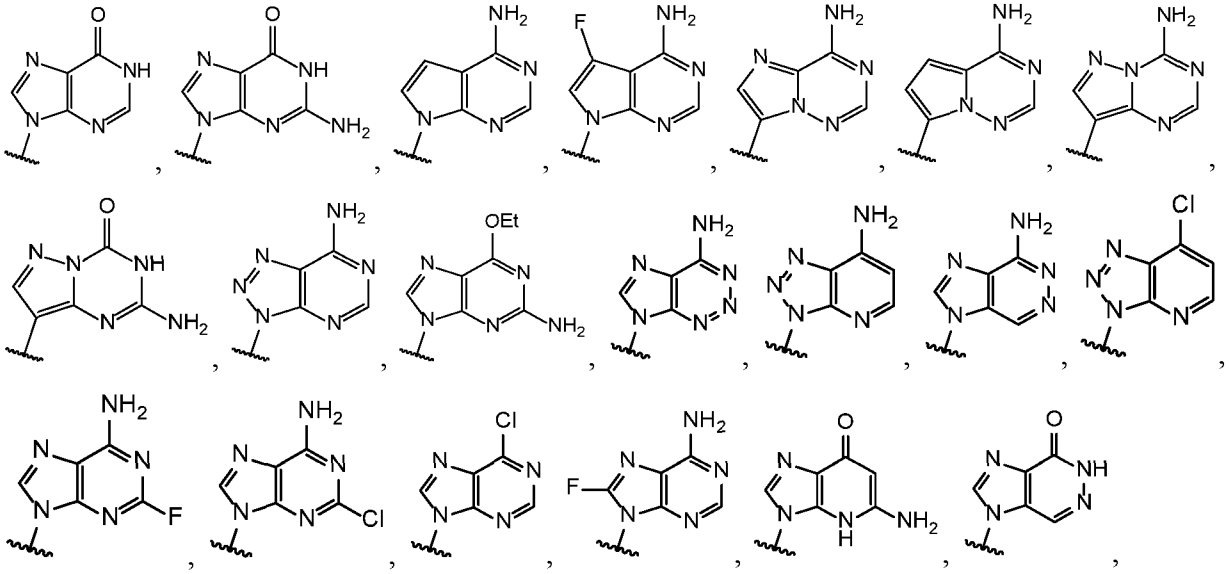
14. The compound according to claim 11, or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein the compound of formula (I') is a compound of formula (I'c):

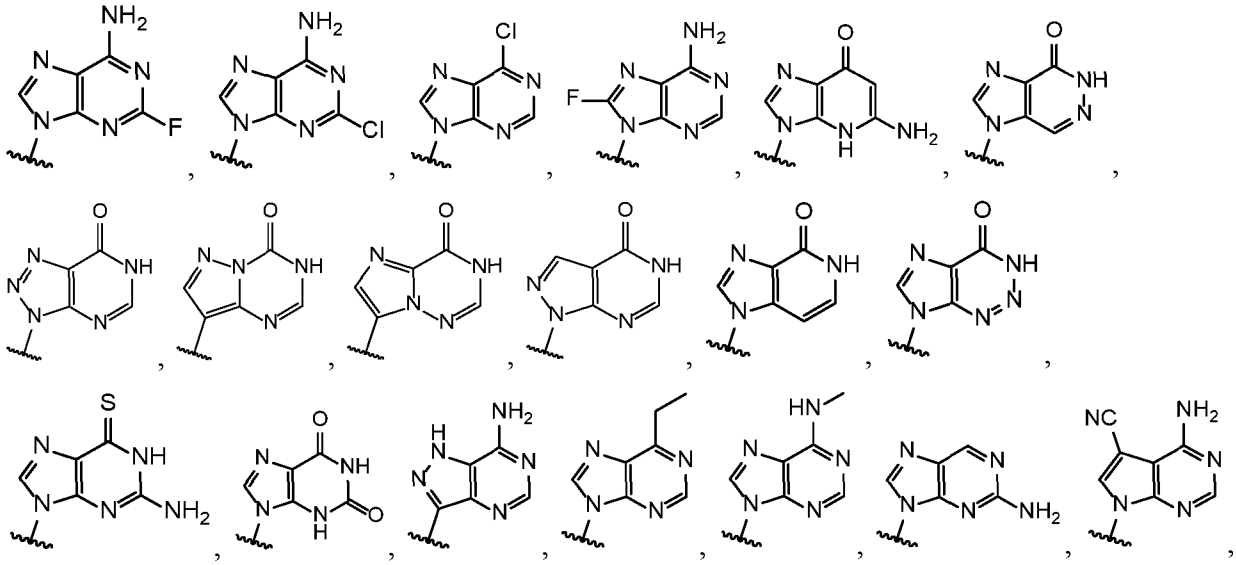


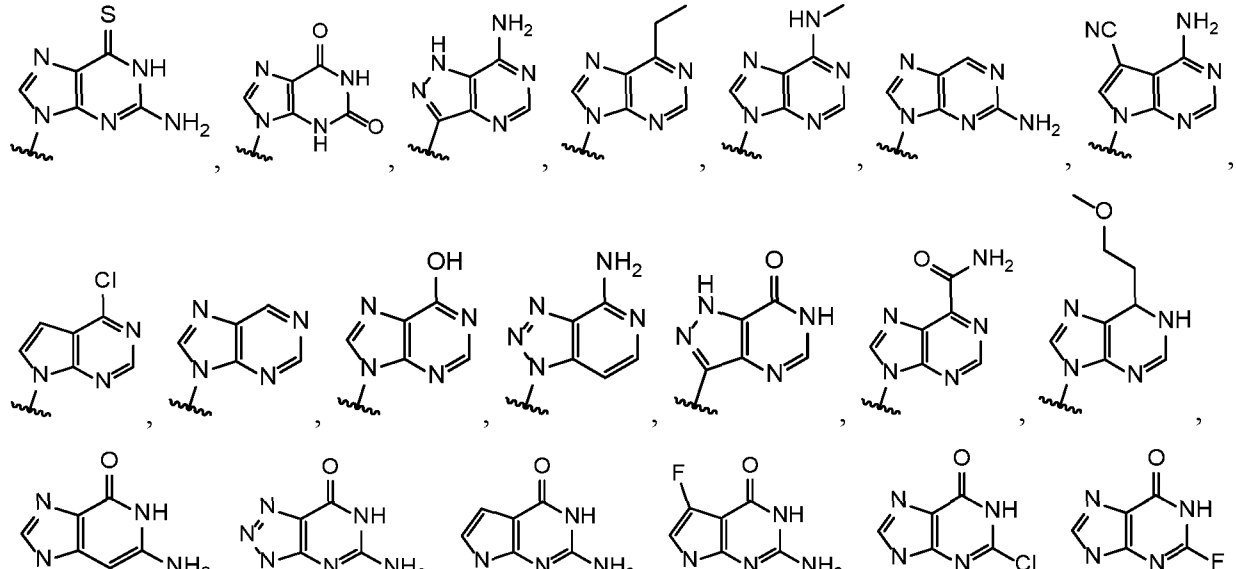
(I'c)

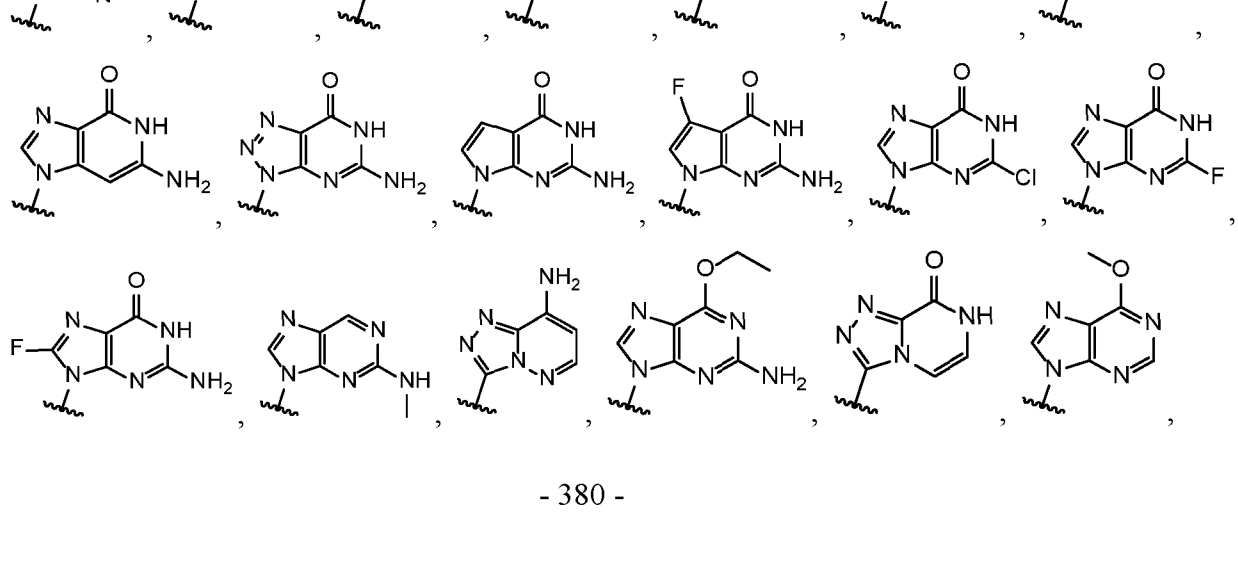
or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

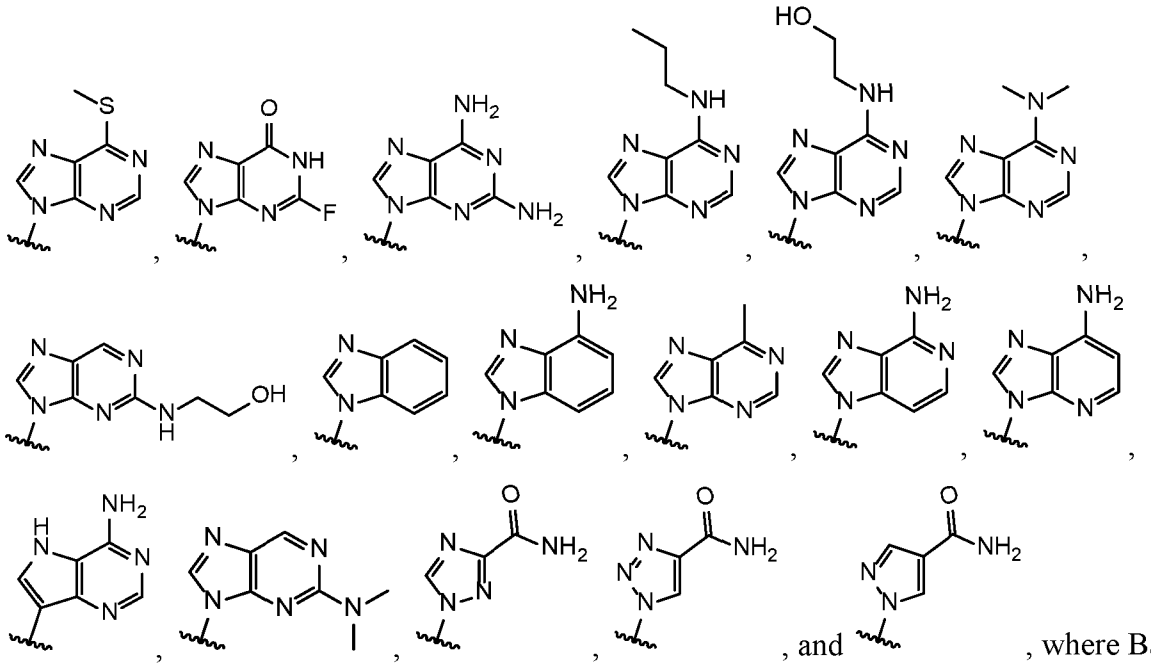
Base[1] and Base[2] are each independently selected from the group consisting of  ,











and Base[2] each may be independently substituted by 0-3 substituents R^{10}, where each R^{10} is independently selected from the group consisting of F, Cl, I, Br, OH, SH, NH_2, C_{1-3} alkyl, C_{3-6} cycloalkyl, $O(C_{1-3}$ alkyl), $O(C_{3-6}$ cycloalkyl), $S(C_{1-3}$ alkyl), $S(C_{3-6}$ cycloalkyl), $NH(C_{1-3}$ alkyl), $NH(C_{3-6}$ cycloalkyl), $N(C_{1-3}$ alkyl)$_2$, and $N(C_{3-6}$ cycloalkyl)$_2$;

X^c and X^{c1} are each independently selected from the group consisting of OR^9, SR^9, and NR^9R^9;

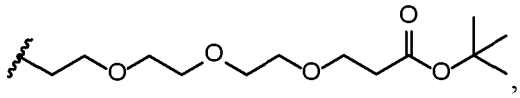
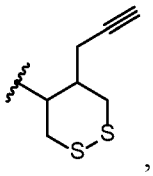
R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^3 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

R^4 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^4 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^5 C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

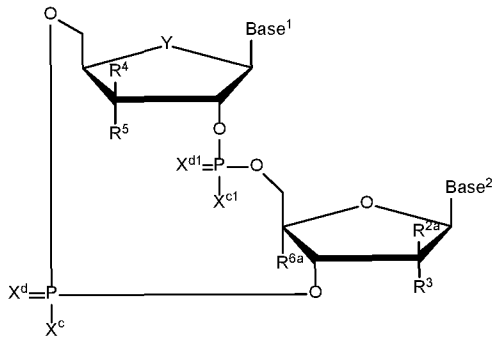
R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, and C_1-C_6 haloalkyl, where said R^{6a} C_1-C_6 alkyl or C_1-C_6 haloalkyl are substituted by 0 to 3 substituents selected from the group consisting of F, Cl, Br, I, and OH;

each R^9 is independently selected from the group consisting of H, C_2-C_3 alkyl,

 , and  , where each R^9 C_2-C_3 alkyl is optionally substituted by 1 to 2 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; and

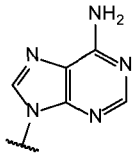
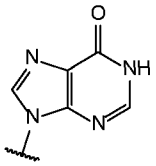
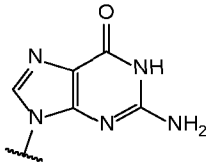
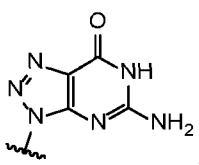
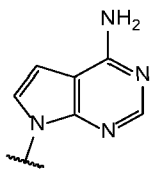
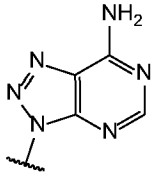
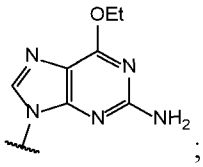
optionally R^4 and R^5 are connected by C_1-C_6 alkylene, -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^4 and R^5 are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^5 position.

15. A compound of formula (I''):



(I'')

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

$Base^1$ and $Base^2$ are each independently selected from the group consisting of  ,  ,  ,  ,  ,  , and  ;

Y is selected from the group consisting of -O- and -S-;

X^c and X^{c1} are each independently selected from the group consisting of OR^9 and SR^9;

X^d and X^{d1} are each independently selected from the group consisting of O and S;

R^{2a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

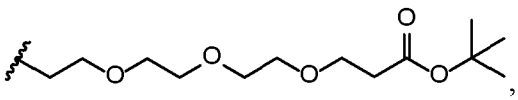
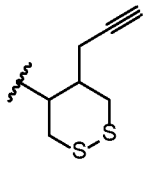
R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

R^4 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

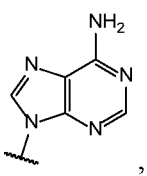
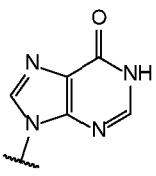
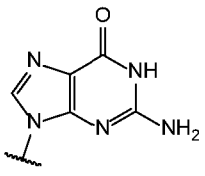
R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

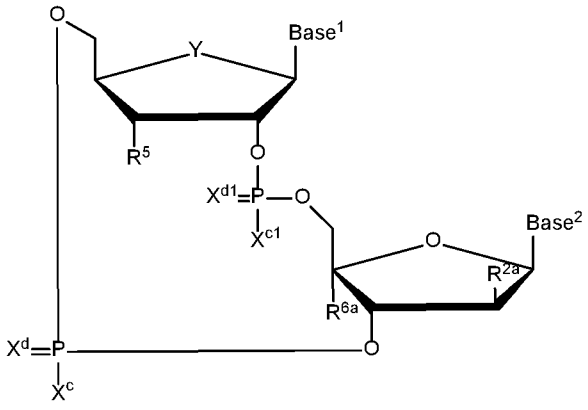
each R^9 is independently selected from the group consisting of H, C_1-C_{20} alkyl,


, and

, where each R^9 C_1-C_{20} alkyl is optionally substituted by 0 to 3 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl; and

optionally R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position; and

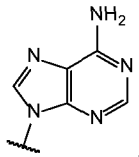
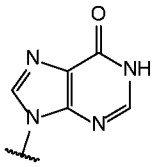
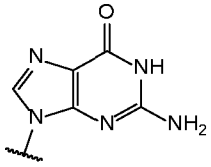
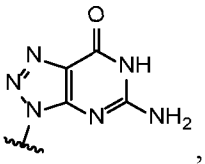
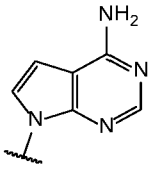
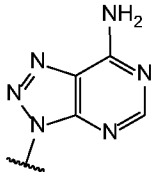
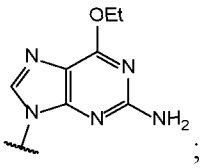
providing that when Y and Y^a are each O, X^a and X^{a1} are each O, X^b and X^{b1} are each O, and X^c and X^{c1} are each OH or SH, X^d and X^{d1} are each O, R^1 and R^{1a} are each H, R^2 is H, R^6 and R^{6a} are each H, R^7 and R^{7a} are each H, R^8 and R^{8a} are each H, and $Base^1$ and $Base^2$ are each selected from the group consisting of

,

and

, R^5 and R^3 are not both selected from the group consisting of H, F and OH.

16. The compound according to claim 15, or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein the compound of formula (I") is a compound of formula (I"a):



(I"a)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

Base^1 and Base^2 are each independently selected from the group consisting of  ,  ,  ,  ,  ,  , and  ;

Y is selected from the group consisting of -O- and -S-;

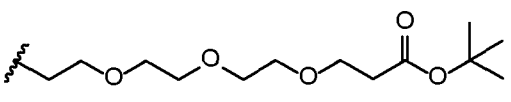
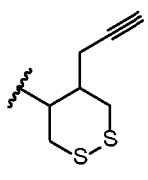
X^c and X^{c1} are each independently selected from the group consisting of OR^9 and SR^9;

X^d and X^{d1} are each independently selected from the group consisting of O and S;

R^{2a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

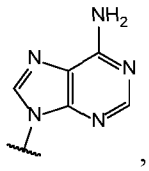
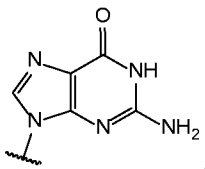
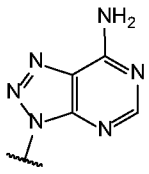
R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; and

each R^9 is independently selected from the group consisting of H, C_1-C_{20} alkyl,  , and  , where each R^9 C_1-C_{20} alkyl is

optionally substituted by 0 to 3 substituents independently selected from the group consisting of OH, -O-C_1-C_{20} alkyl,-S-C(O)C_1-C_6 alkyl, and C(O)OC_1-C_6 alkyl.

17. The compound according to claim 16, or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

Base[1] and Base[2] are each independently selected from the group consisting of  ,  , and  ;

Y is selected from the group consisting of -O- and -S-;

X^c and X^{c1} are each independently selected from the group consisting of OR^9 and SR^9;

X^d and X^{d1} are each independently selected from the group consisting of O and S;

R^{2a} is F;

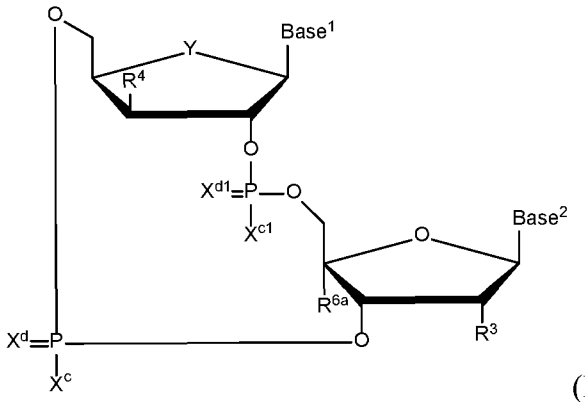
R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl; and

each R^9 is independently H.

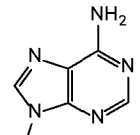
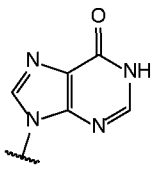
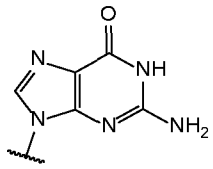
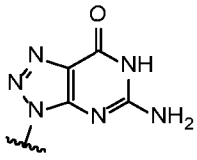
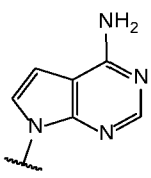
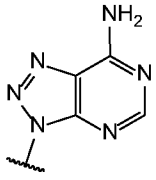
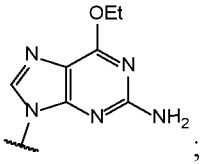
18. The compound according to claim 15, wherein R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position.

19. The compound according to claim 15, or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein the compound of formula (I") is a compound of formula (I"b):



(I"b)

or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein

Base[1] and Base[2] are each independently selected from the group consisting of  ,  ,  ,  ,  ,  , and  ;

Y is selected from the group consisting of -O- and -S-;

X^c and X^{c1} are each independently selected from the group consisting of OR^9 and SR^9;

X^d and X^{d1} are each independently selected from the group consisting of O and S;

R^3 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

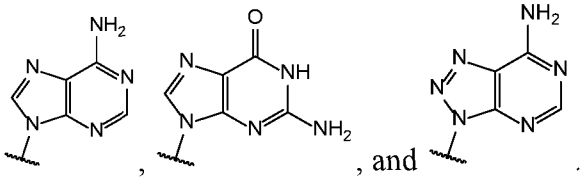
R^4 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

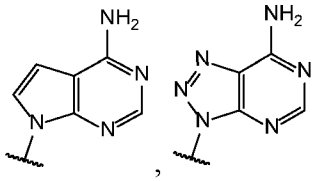
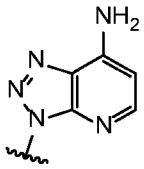
R^5 is selected from the group consisting of H, F, Cl, Br, I, OH, CN, NH_2, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

R^{6a} is selected from the group consisting of H, F, Cl, Br, I, OH, CN, N_3, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_2-C_6 haloalkenyl, C_2-C_6 haloalkynyl, -O-C_1-C_6 alkyl, -O-C_2-C_6 alkenyl, and -O-C_2-C_6 alkynyl;

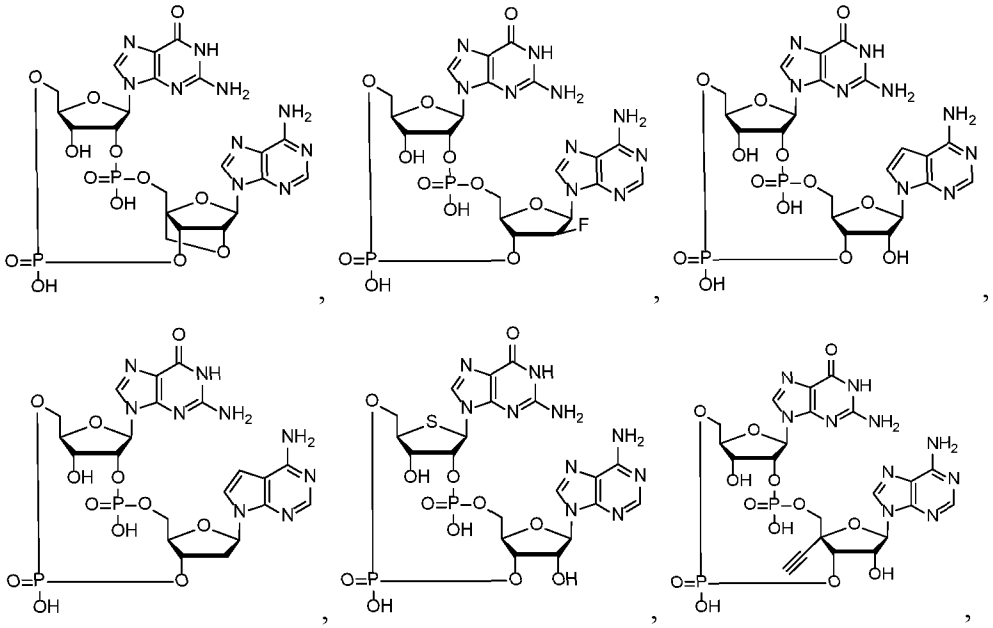
each R^9 is independently H; and

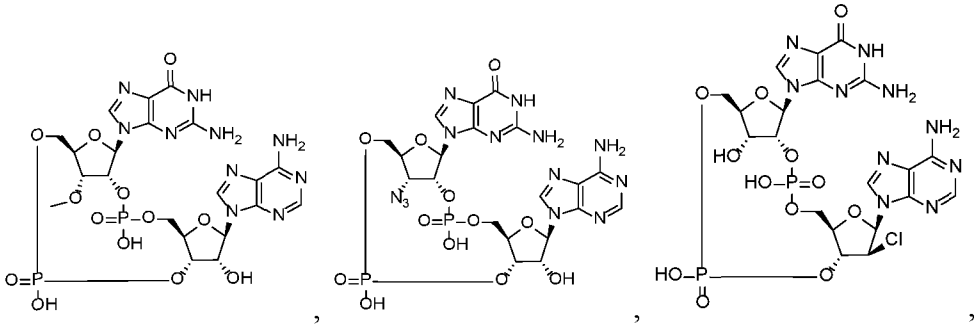
R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, such that where R^3 and R^{6a} are connected to form -O-C_1-C_6 alkylene, -O-C_2-C_6 alkenylene, or -O-C_2-C_6 alkynylene, said O is bound at the R^3 position.

20. The compound according to claim 19, or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof, wherein $Base^1$ and $Base^2$ are each independently selected from the group consisting of  .

21. The compound according to claim 15, wherein at least one of $Base^1$ and $Base^2$ are each independently selected from the group consisting of  , and  .

22. A compound selected from the group consisting of:




,

,

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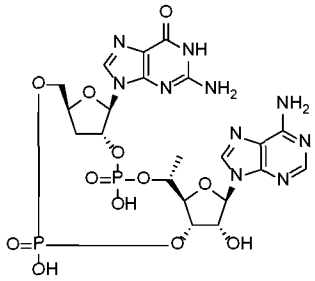
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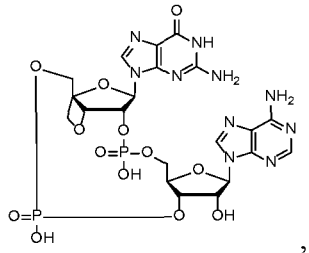
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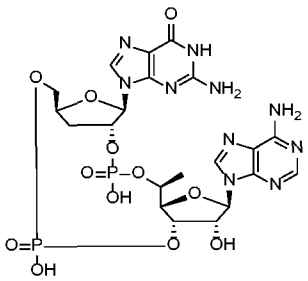
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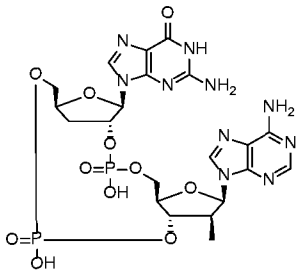
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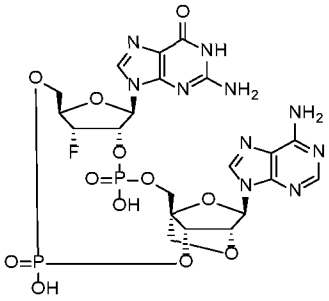
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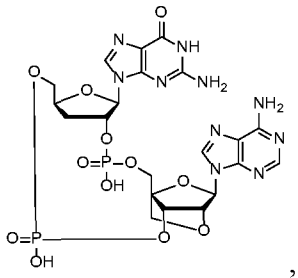
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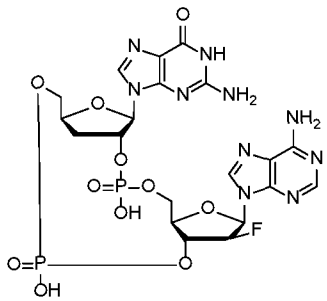
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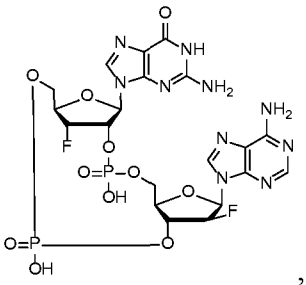
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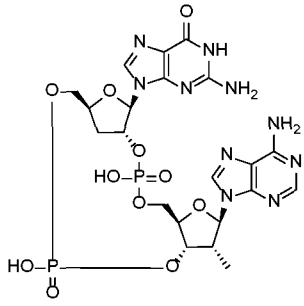
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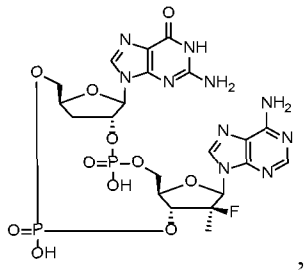
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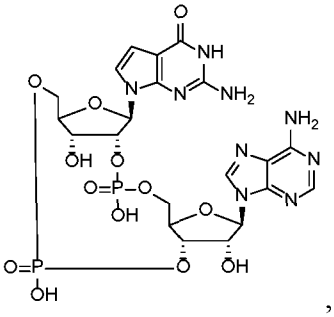
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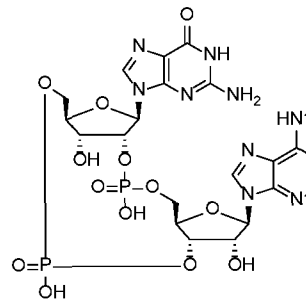
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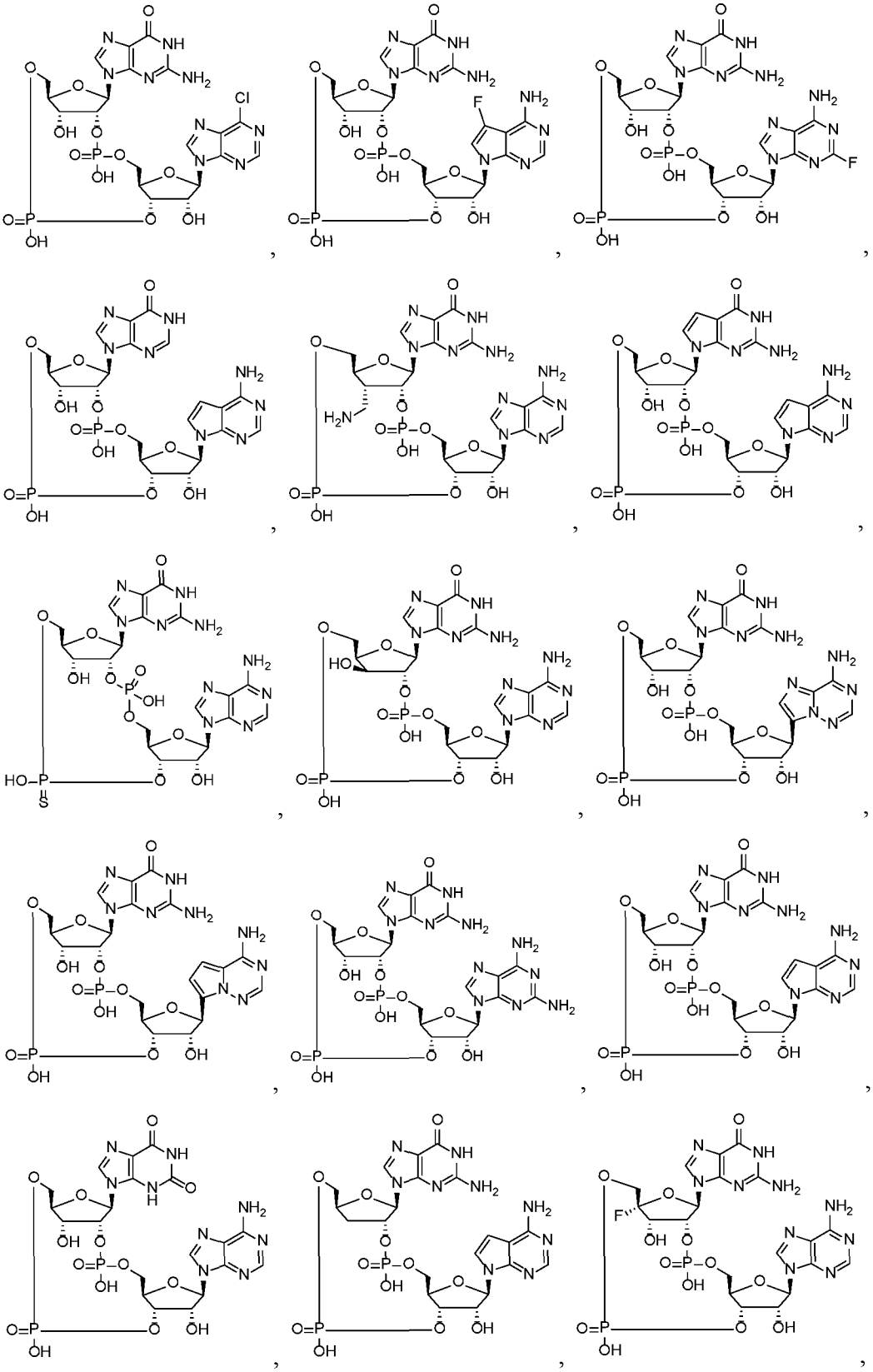

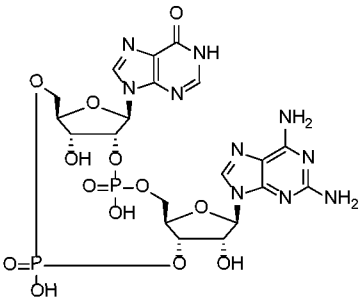
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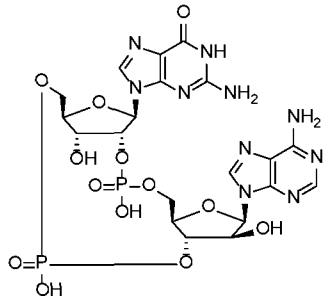
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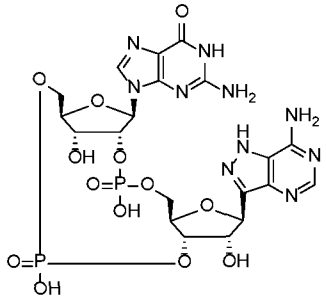
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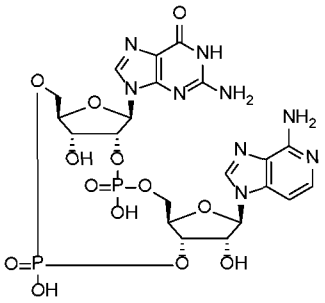
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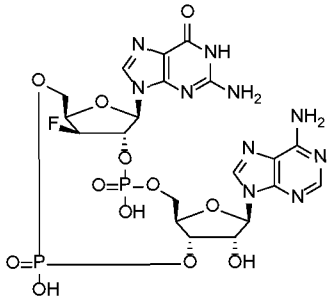
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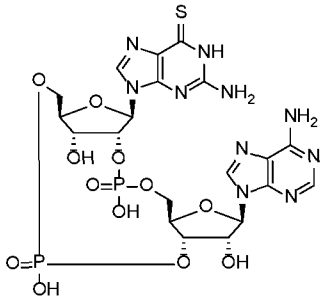
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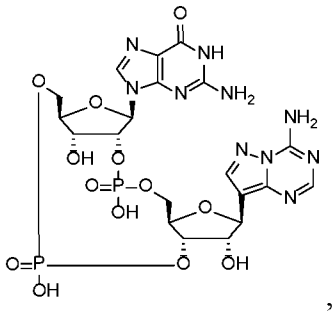
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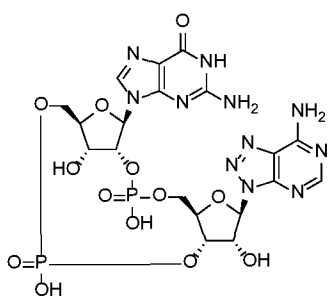
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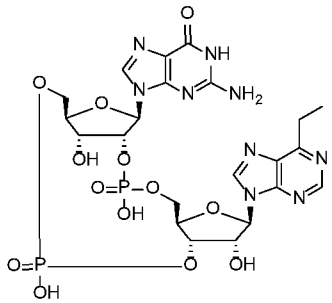
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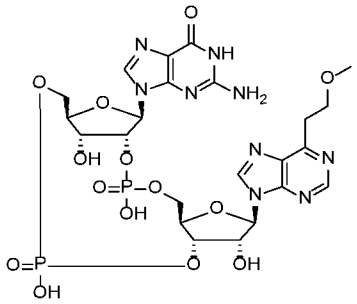
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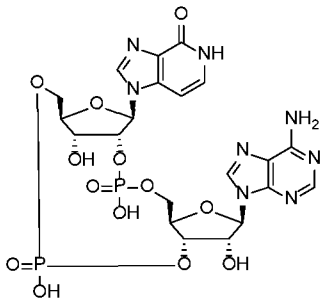
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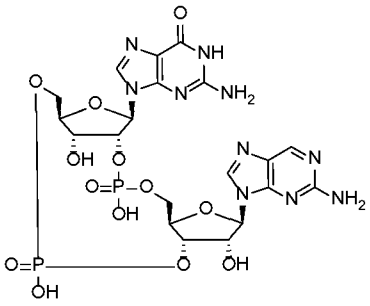
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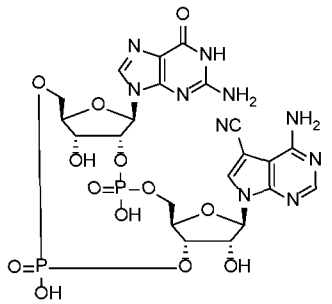
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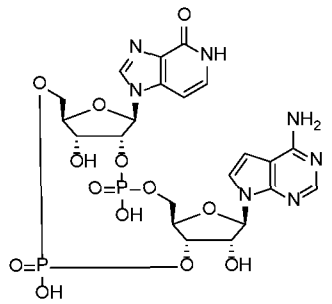
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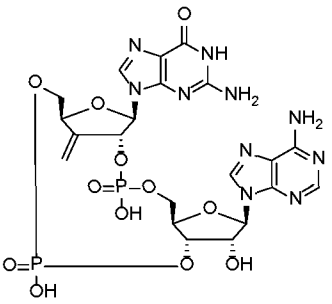
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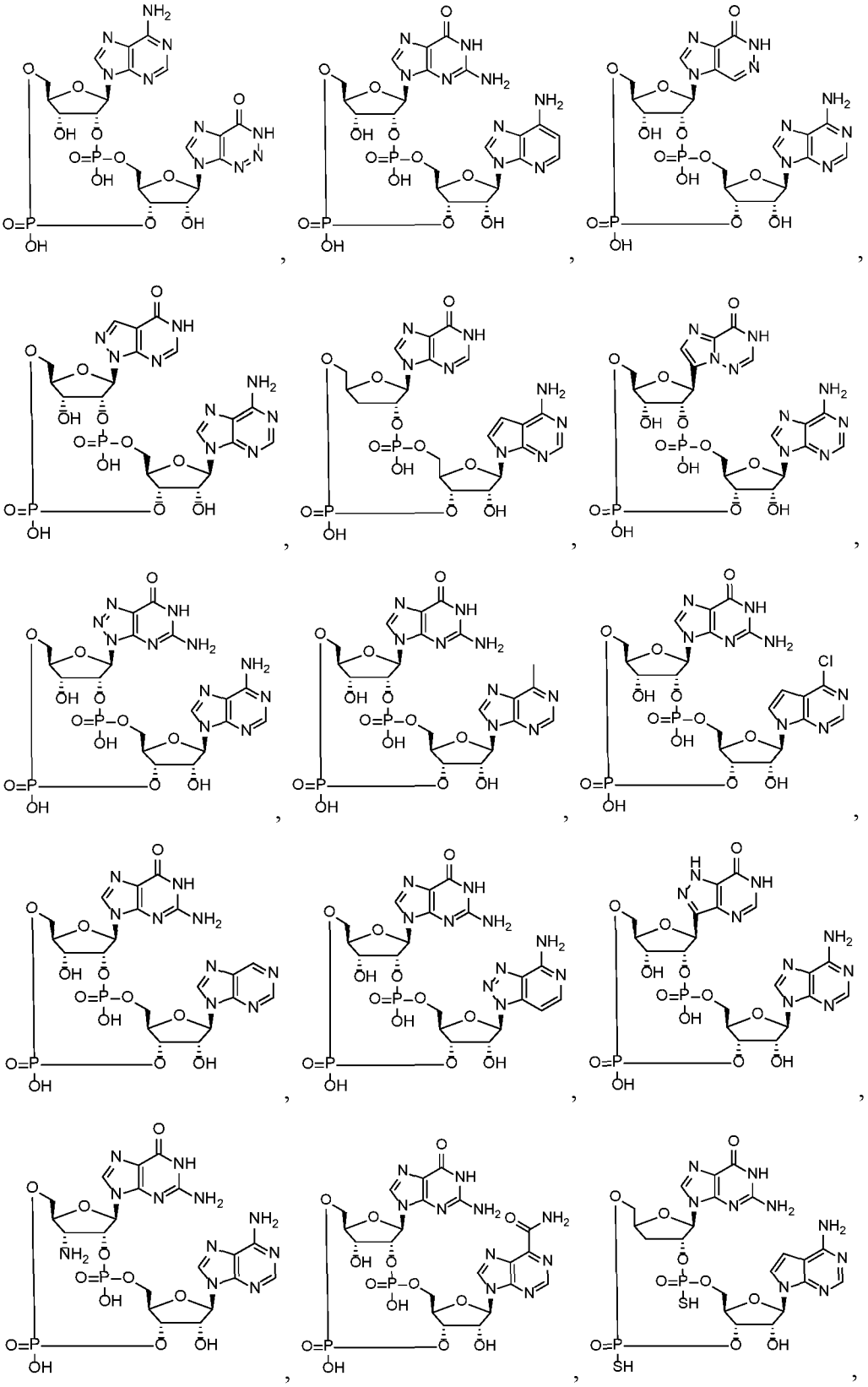

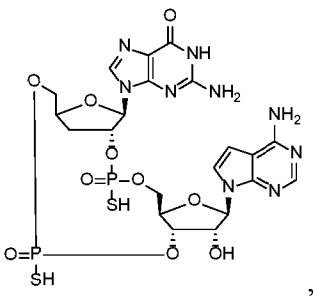
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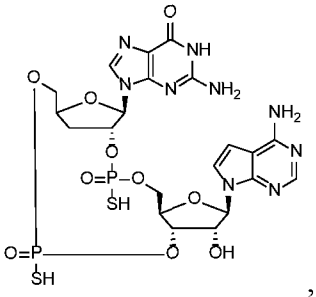
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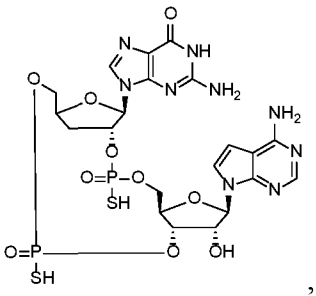
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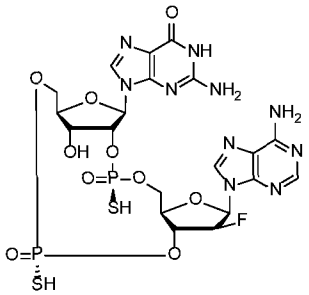
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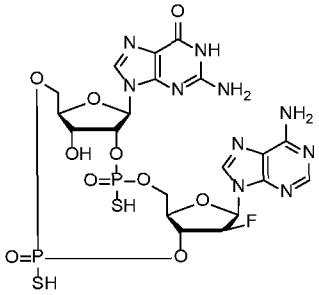
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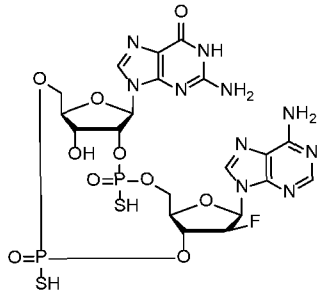
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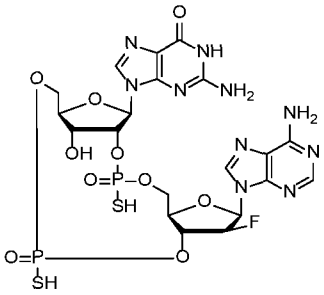
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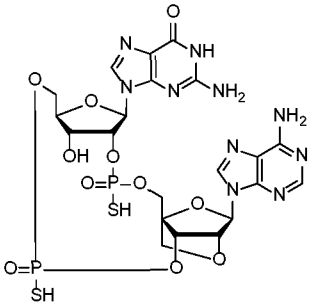
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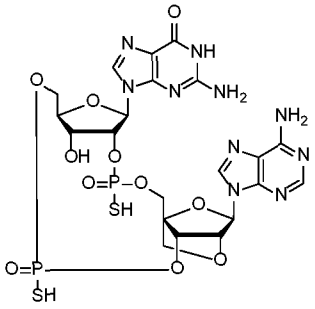
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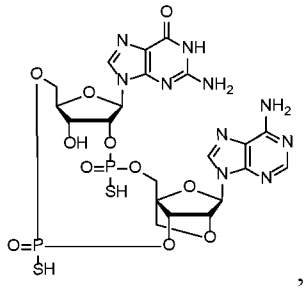
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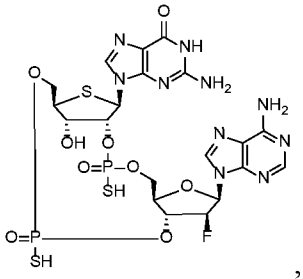
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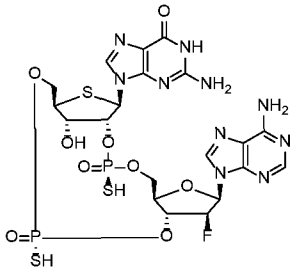
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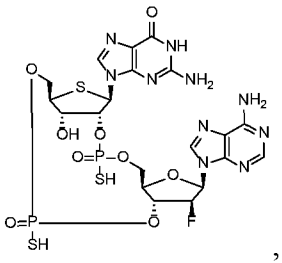
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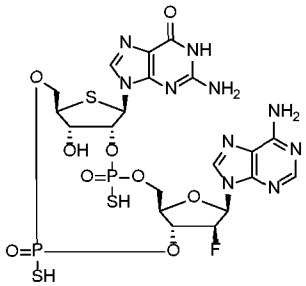
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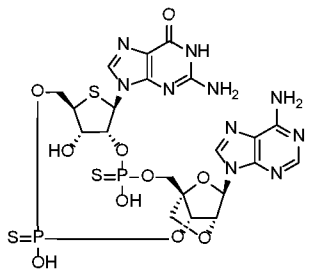
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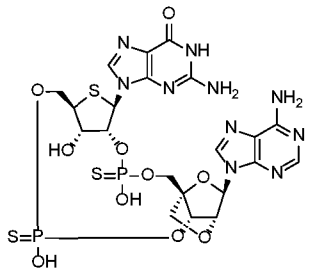

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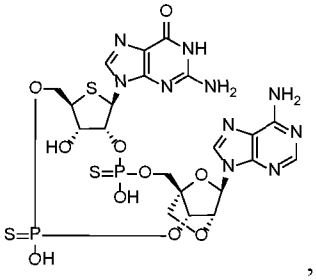
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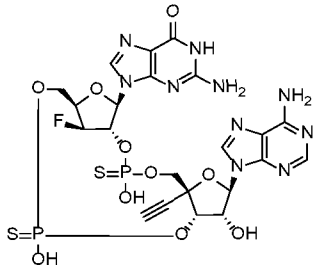
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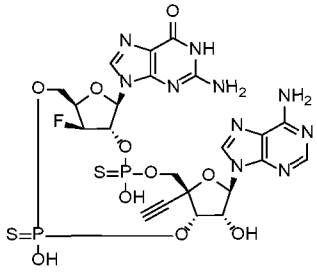
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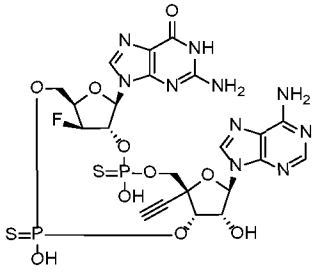
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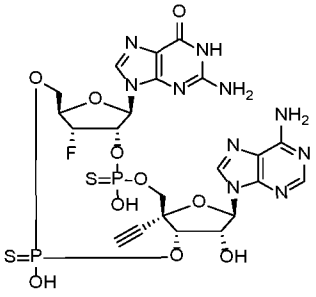
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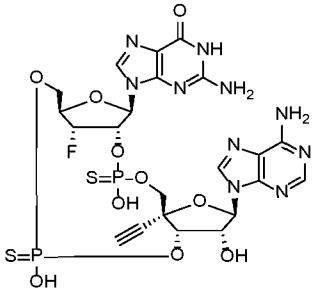
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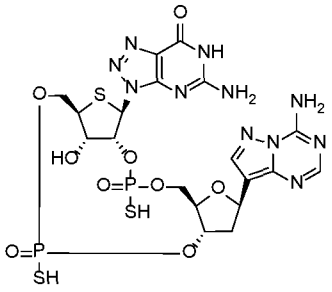
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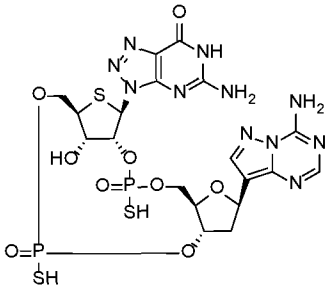
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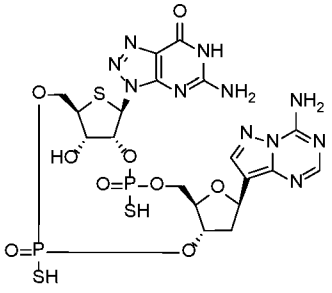
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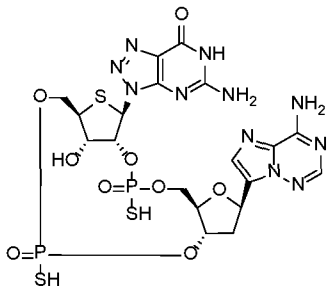
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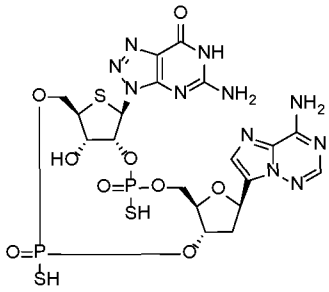
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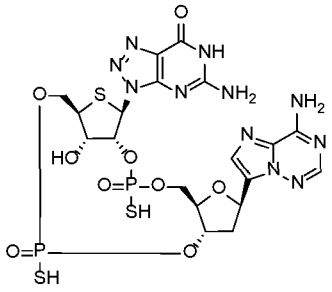
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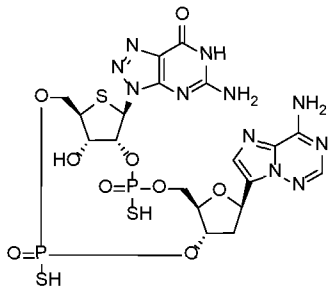
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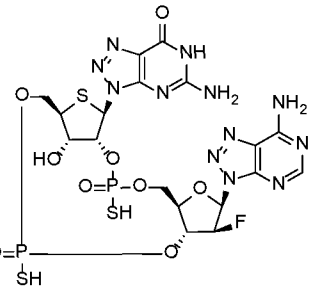
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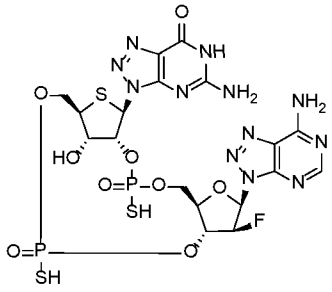
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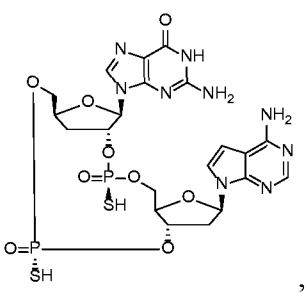
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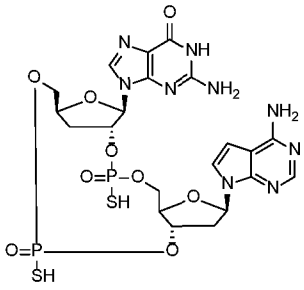
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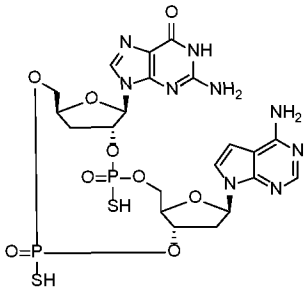
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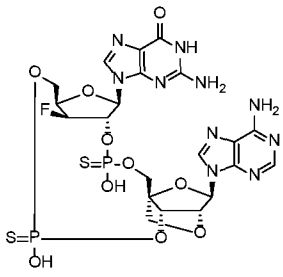
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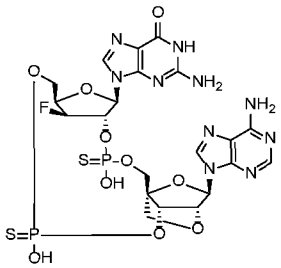
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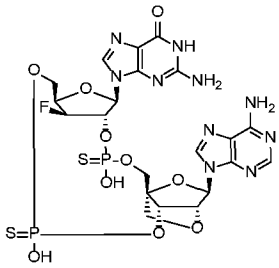
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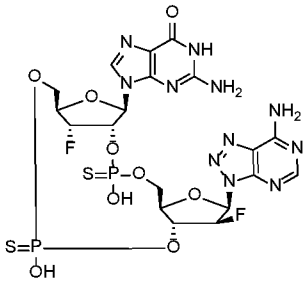
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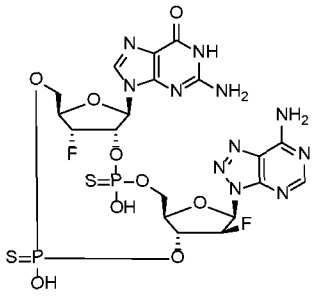
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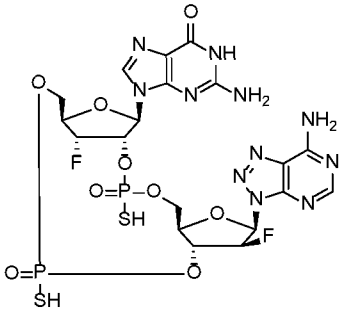
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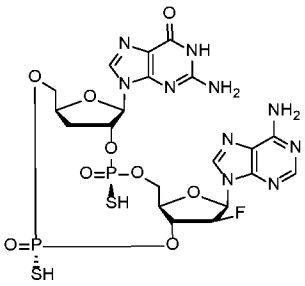
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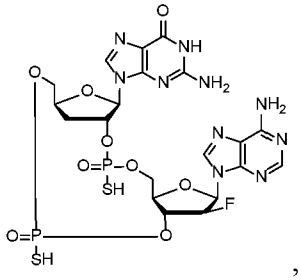
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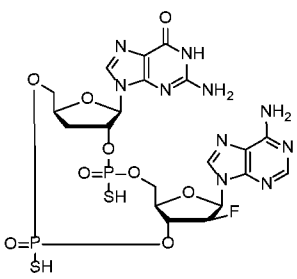
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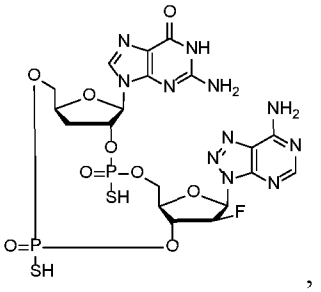
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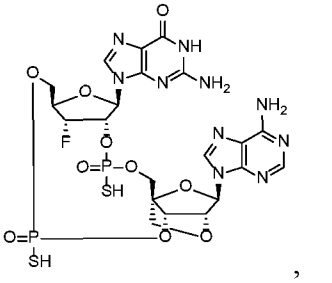
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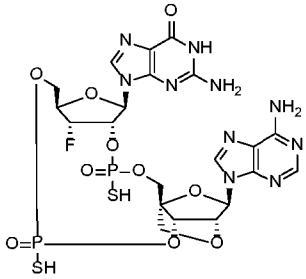
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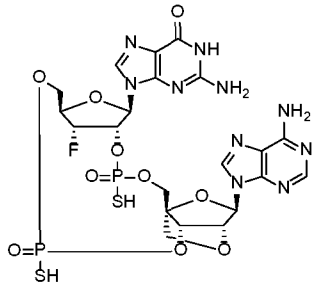
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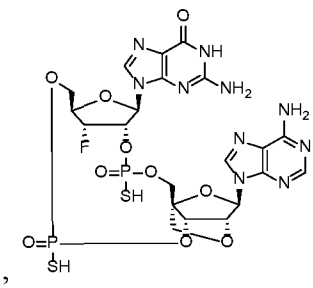
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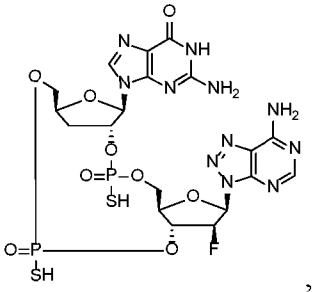
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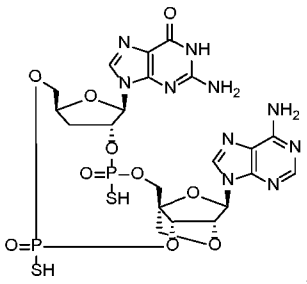
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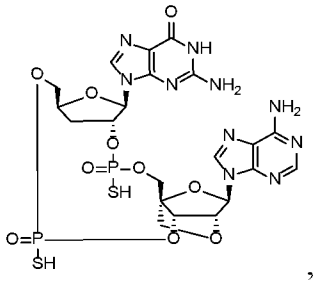
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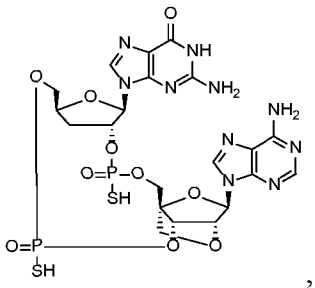
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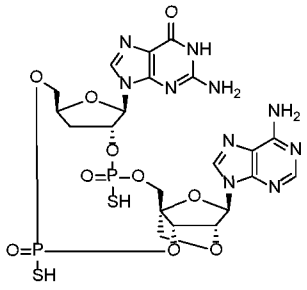
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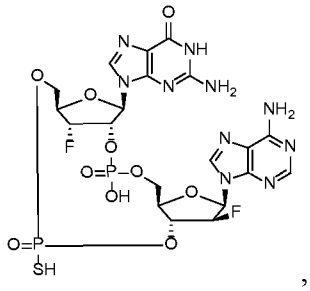
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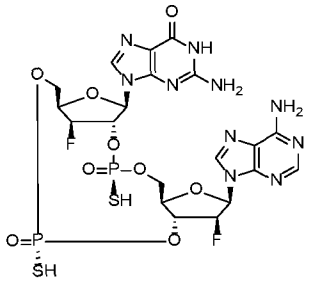
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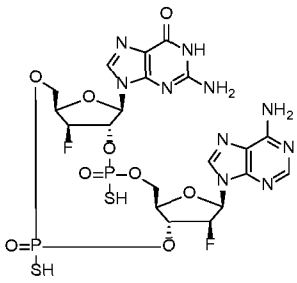
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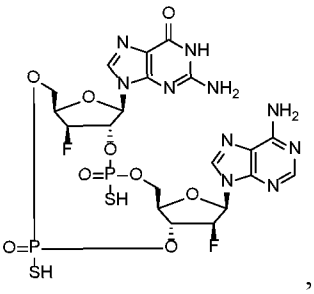
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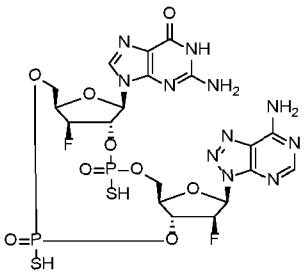
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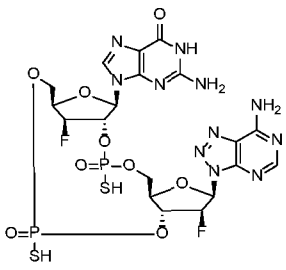
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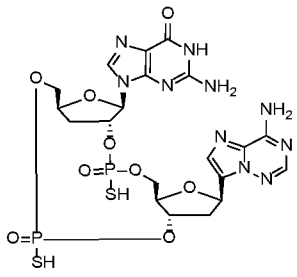
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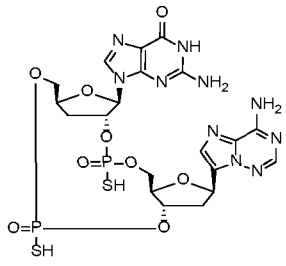
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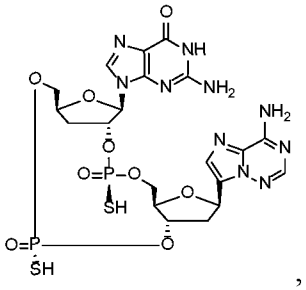
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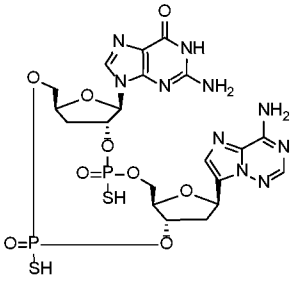
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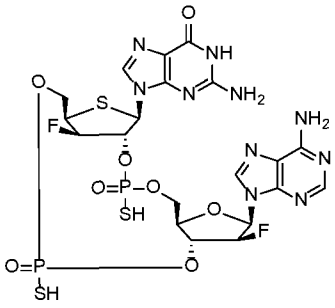
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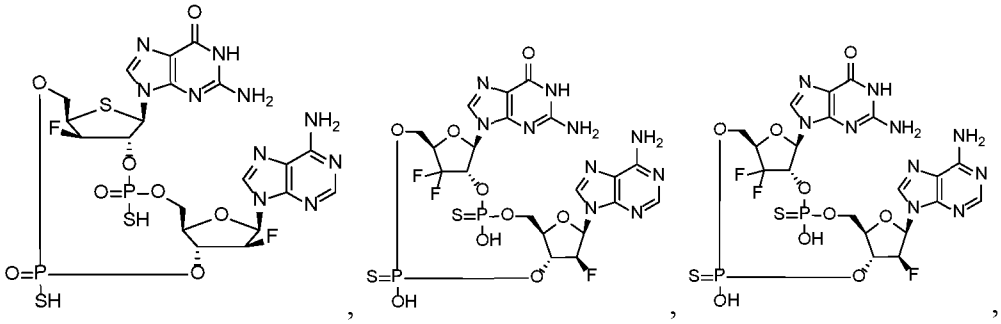
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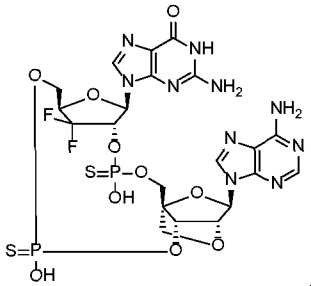
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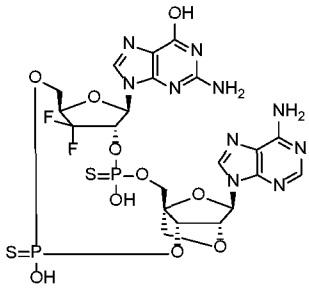
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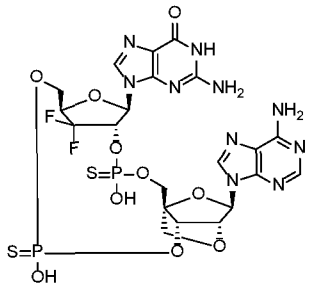
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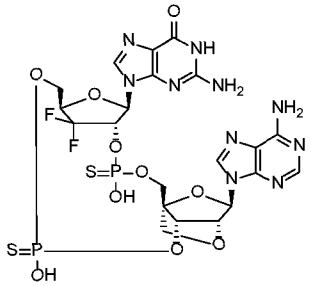
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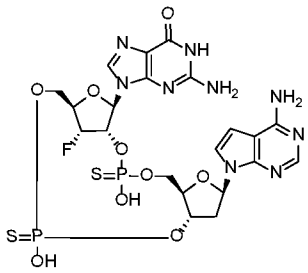
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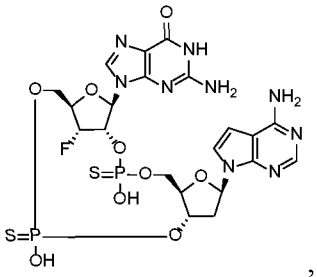
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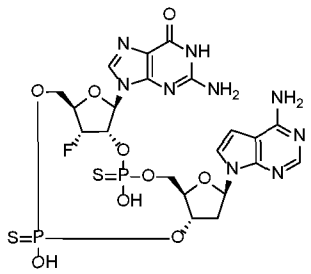
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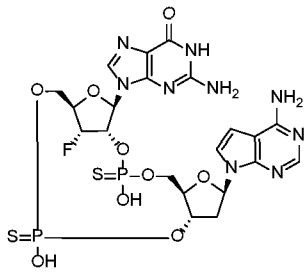
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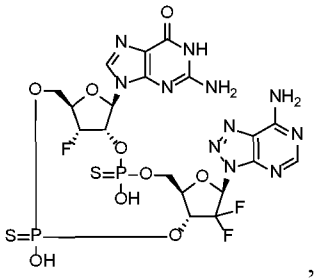
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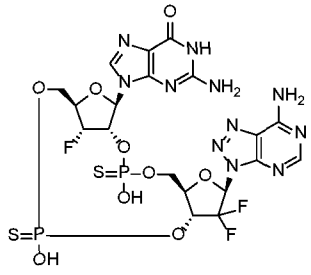
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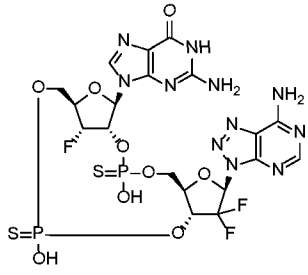
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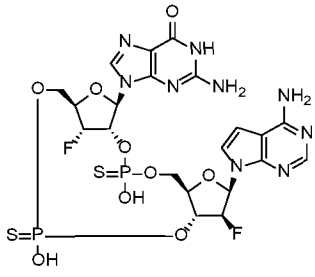
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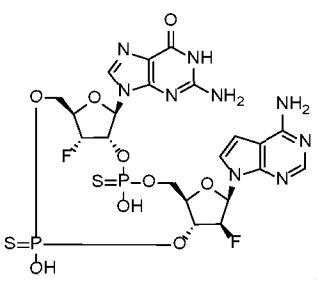
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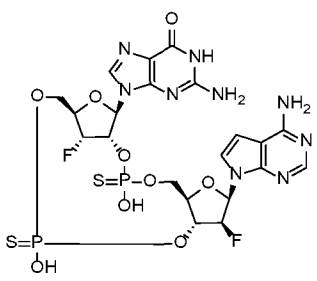
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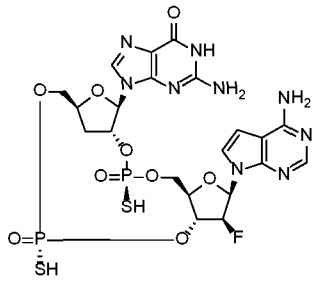
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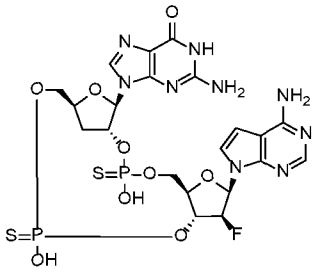
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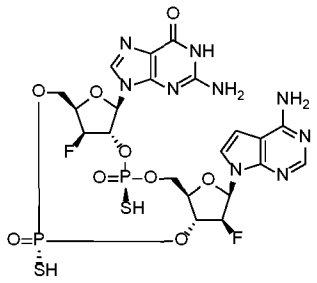
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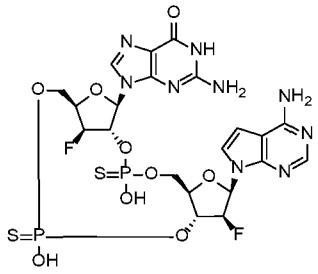
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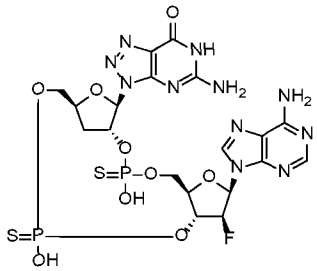
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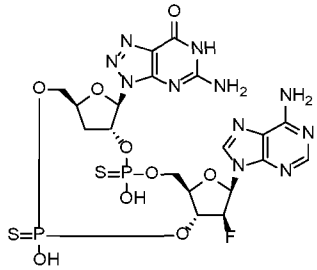
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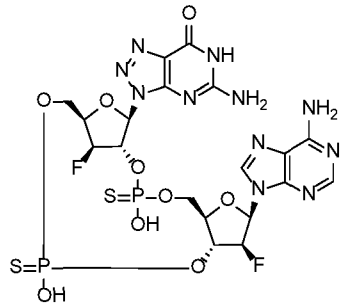
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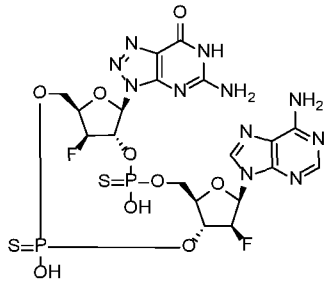
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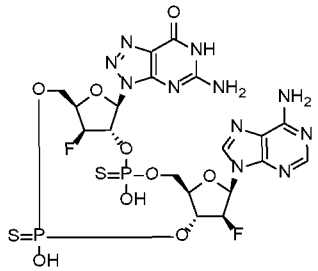
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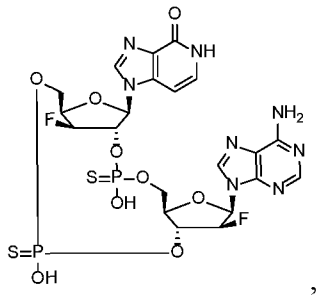
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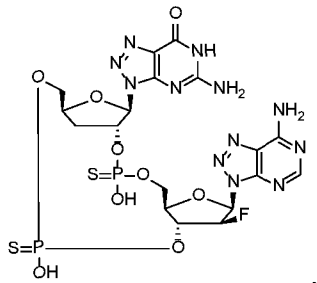
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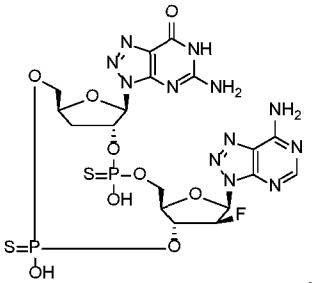
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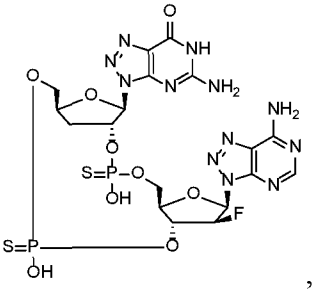
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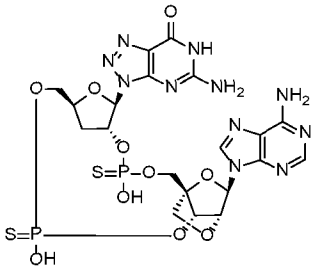
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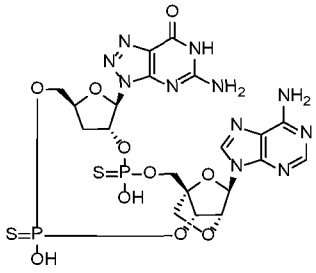
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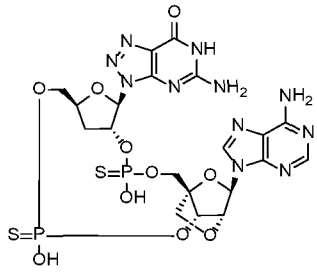
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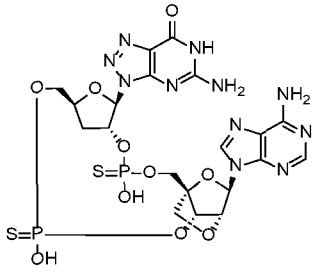
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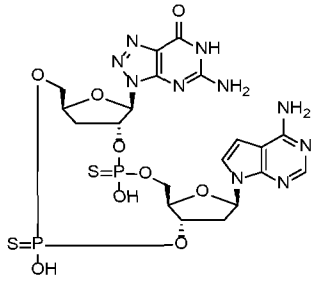
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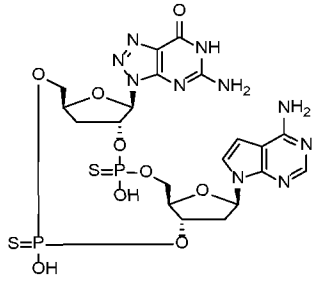
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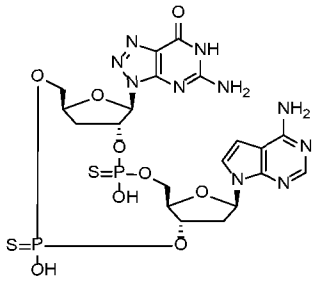
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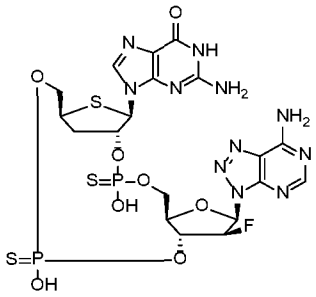
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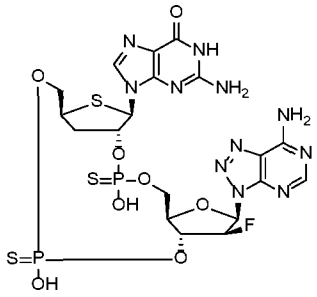
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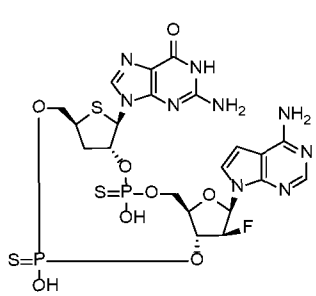
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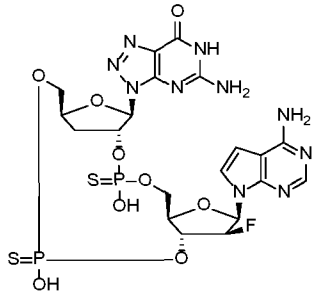
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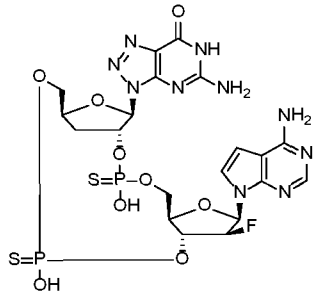
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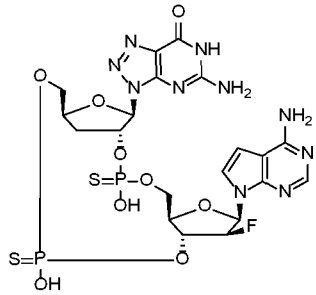
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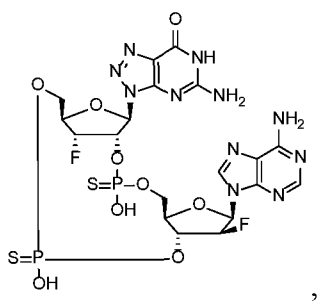
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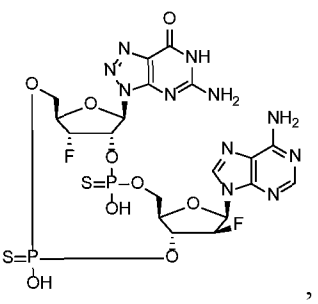
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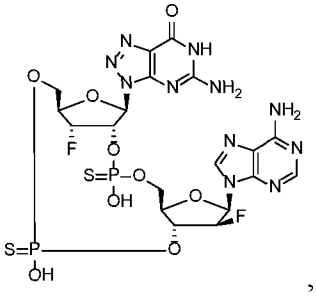
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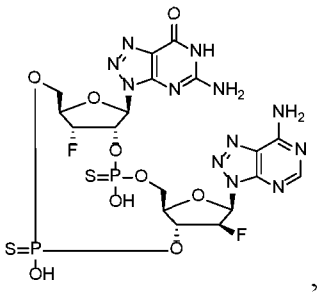
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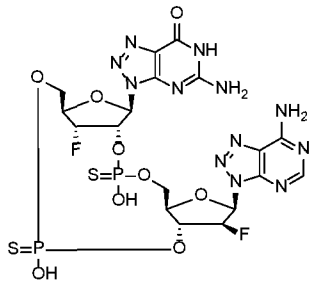
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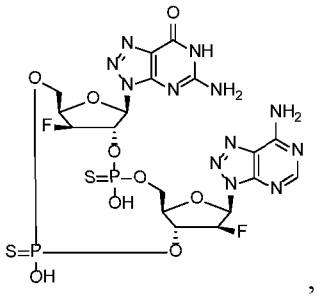
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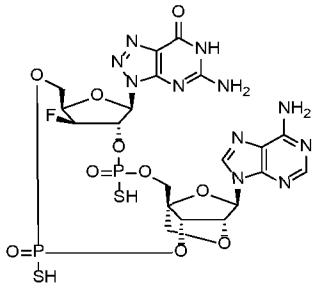
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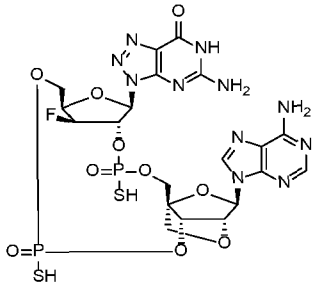
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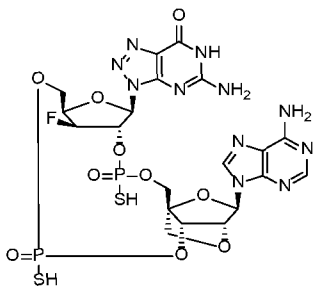
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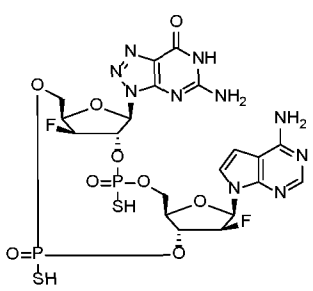
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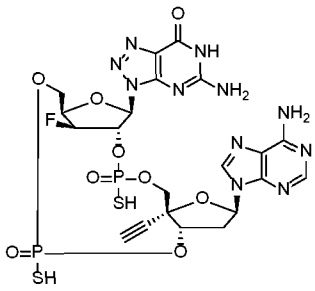
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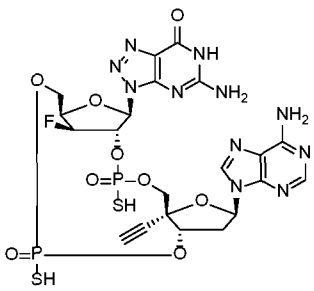
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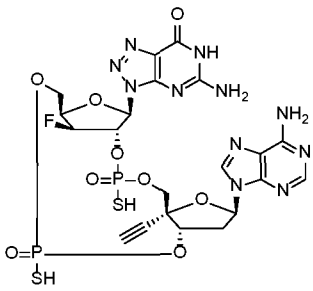
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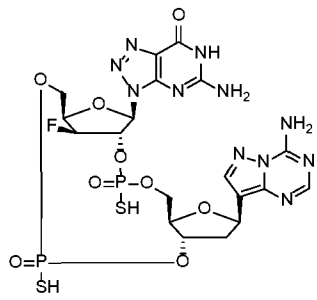
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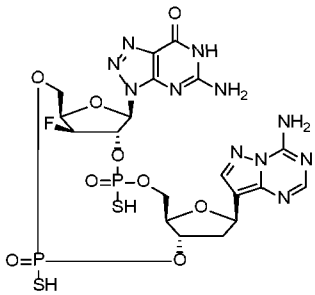
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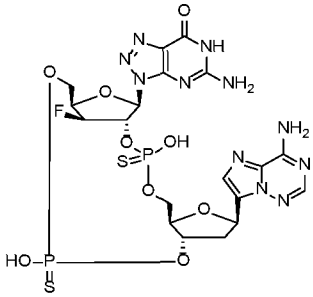
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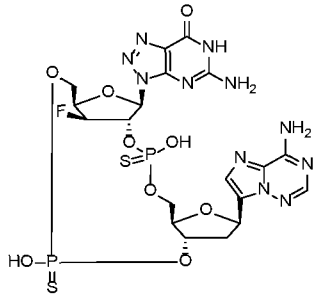
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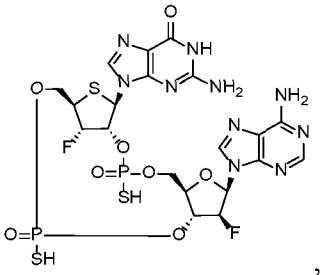
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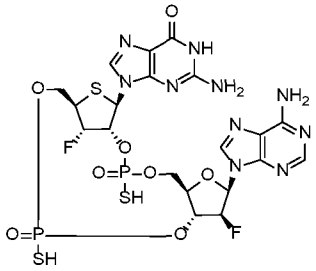
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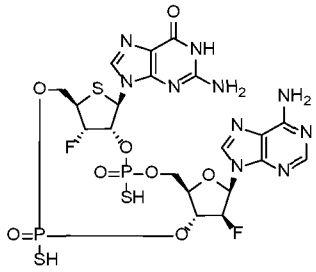
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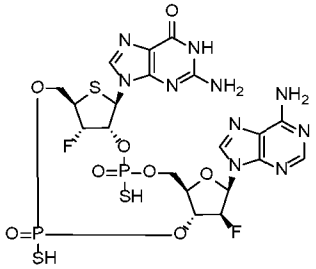
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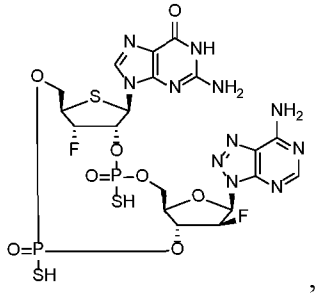
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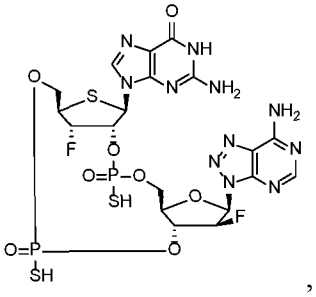
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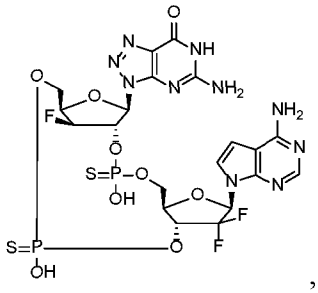
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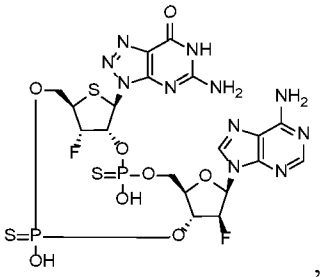
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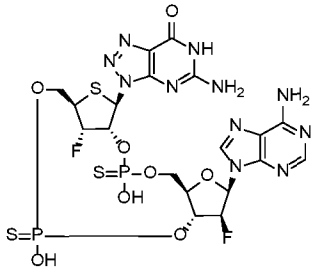
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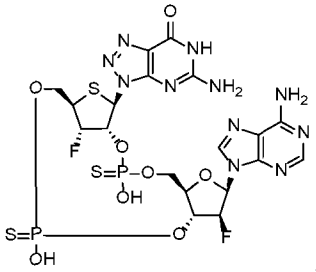
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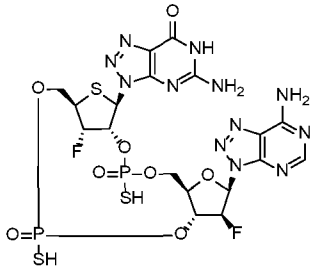
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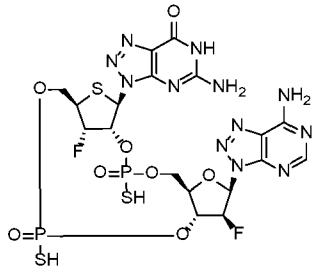
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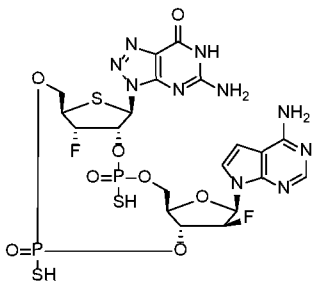
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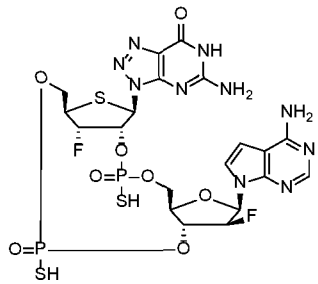
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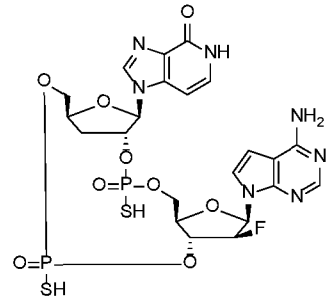
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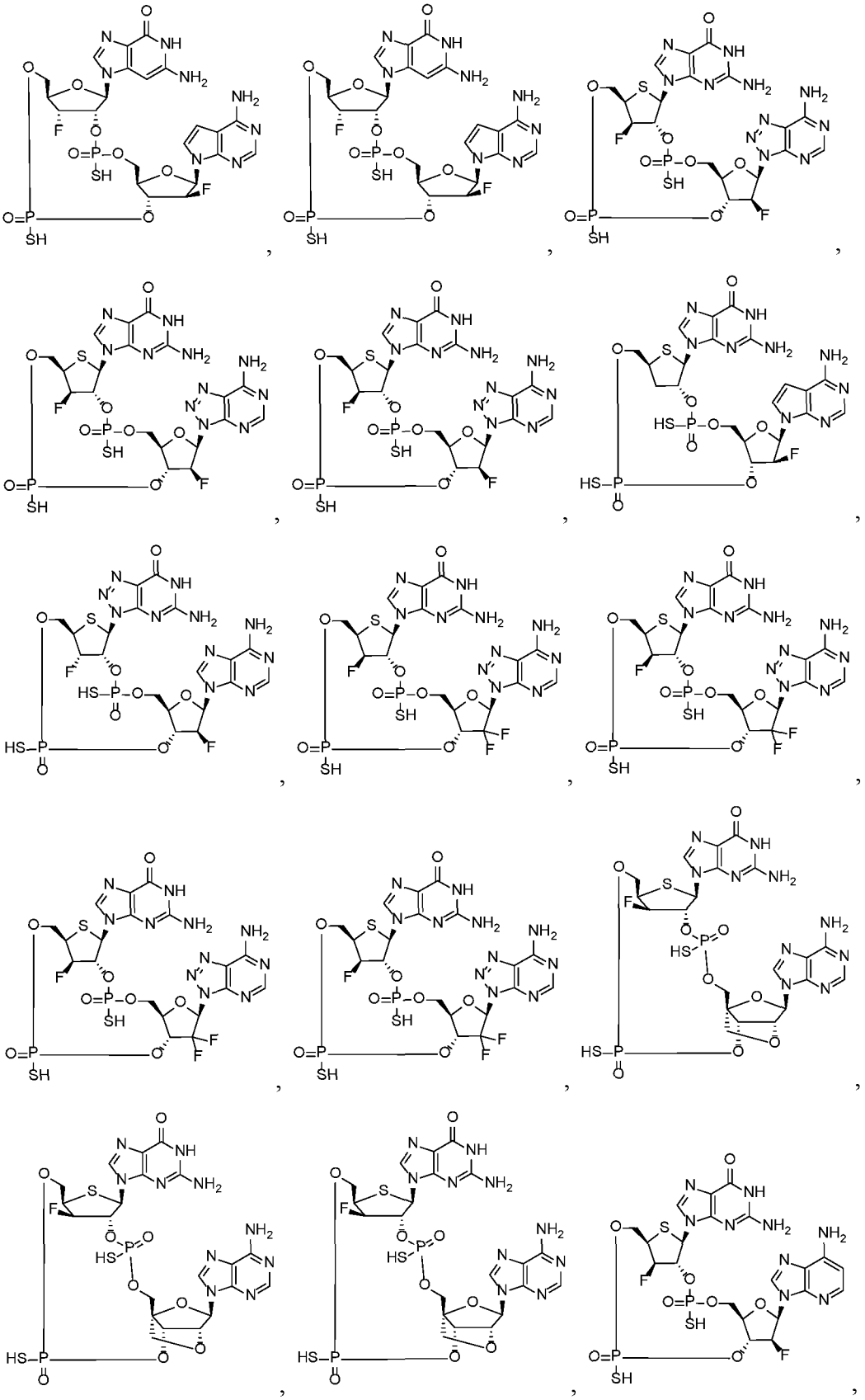

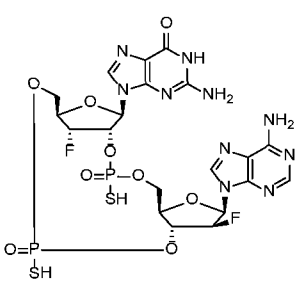
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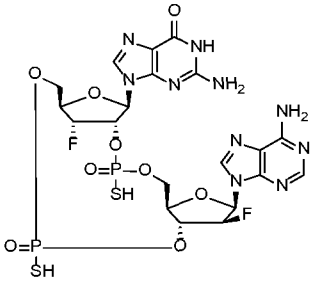
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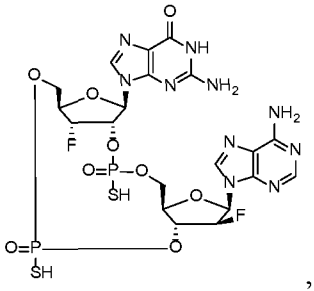
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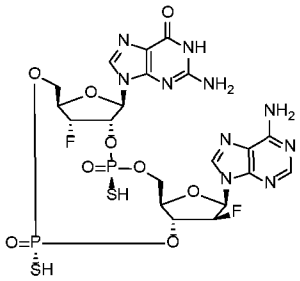
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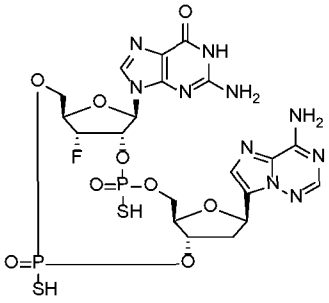
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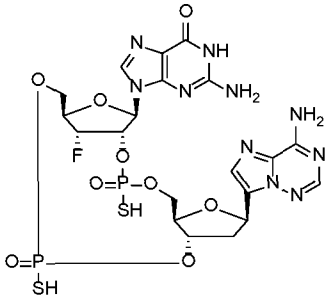
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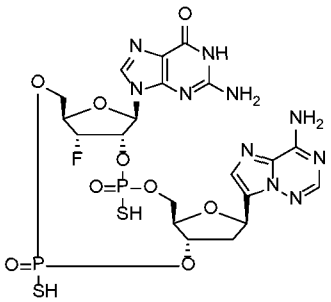
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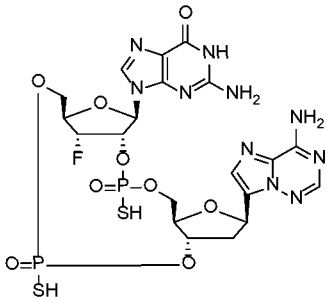
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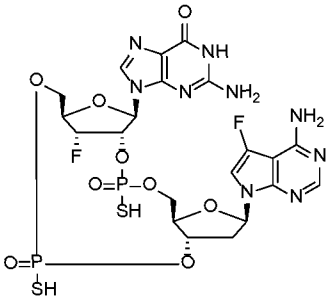
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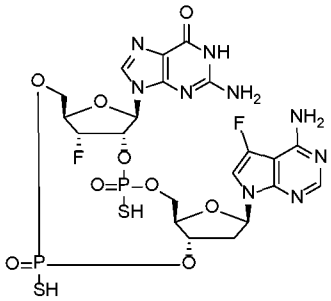
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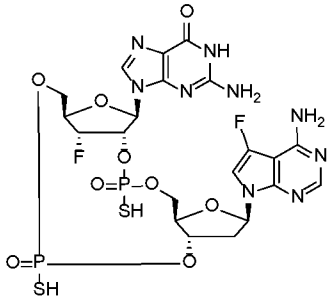
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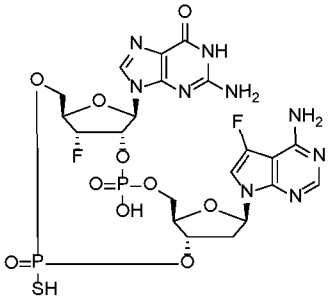
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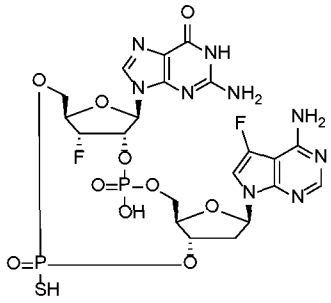
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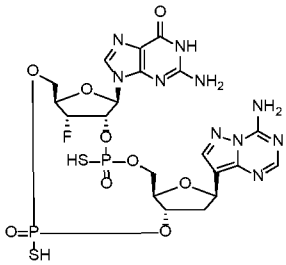
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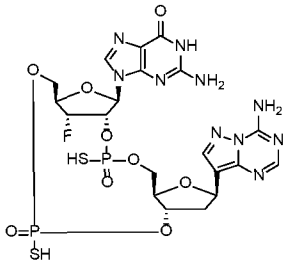
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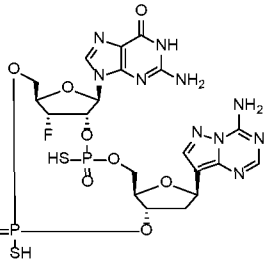

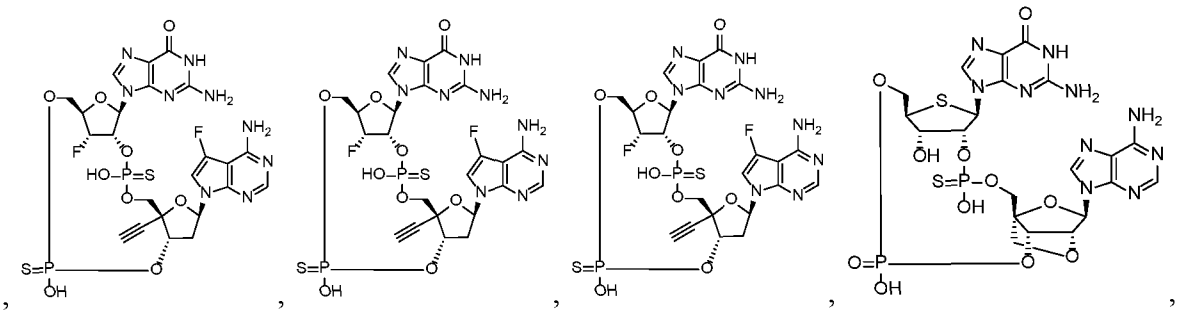

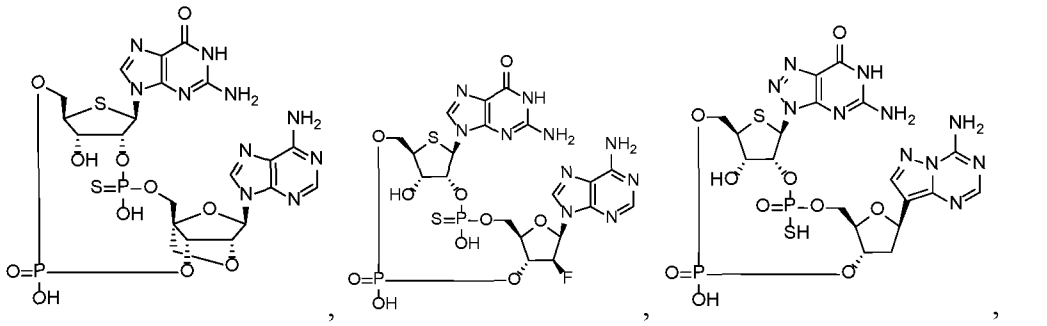

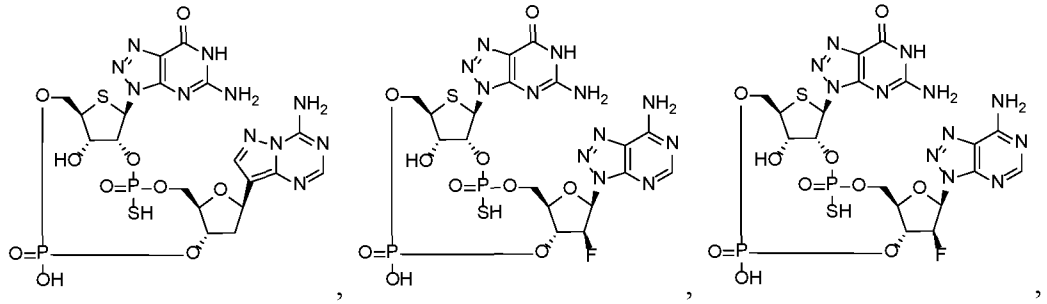

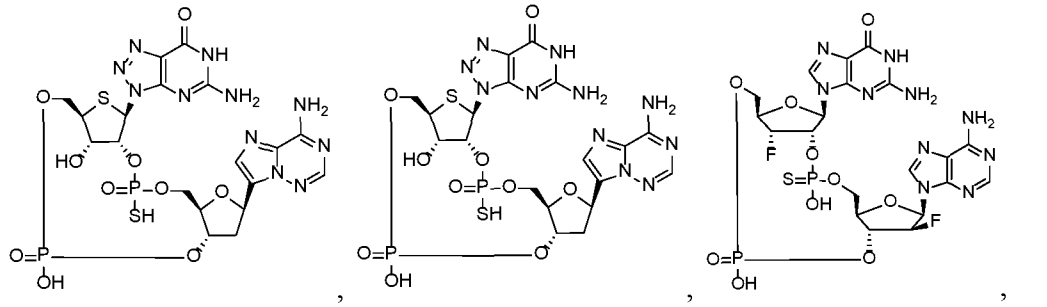

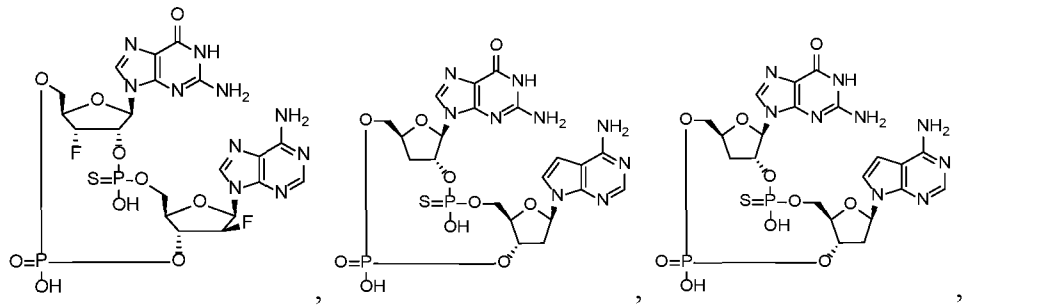

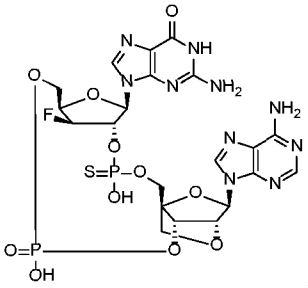
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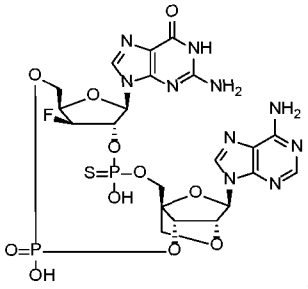
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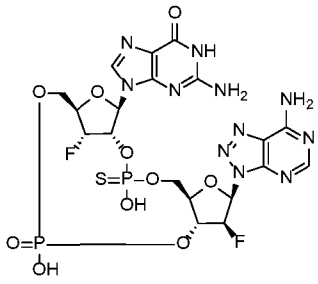
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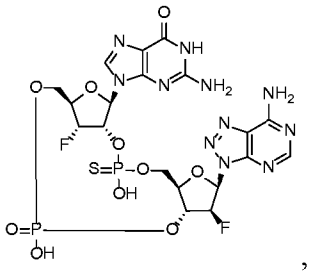
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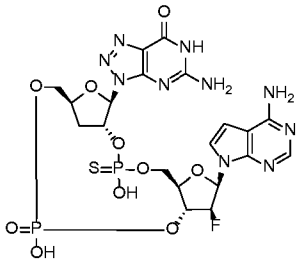
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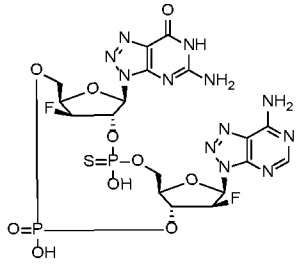
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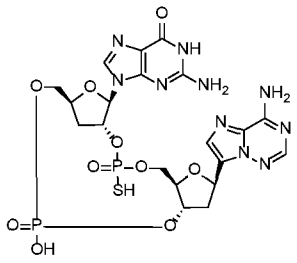
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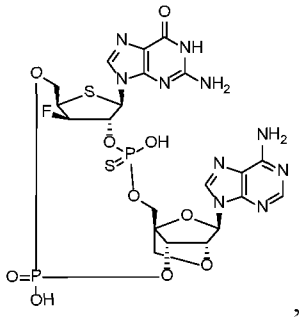
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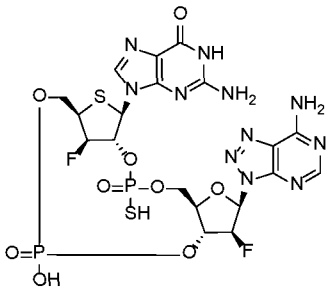
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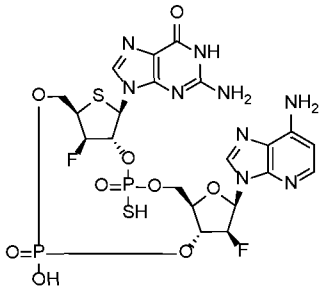
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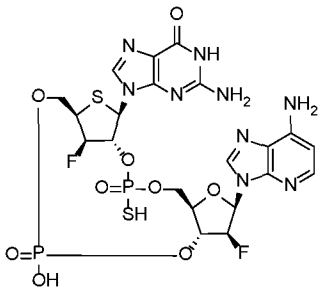
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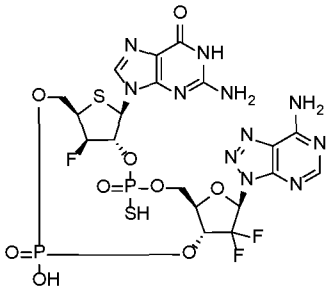
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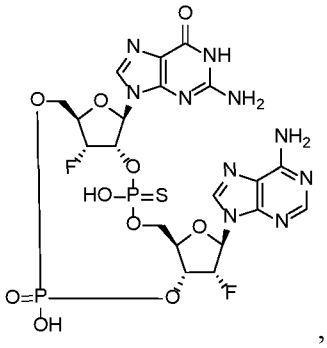
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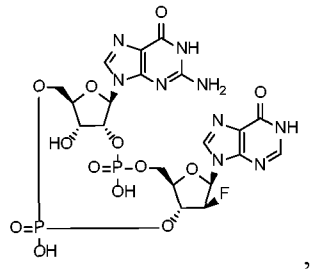
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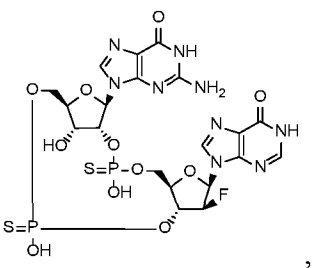
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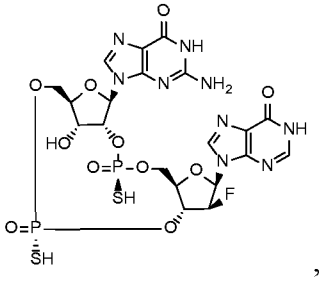
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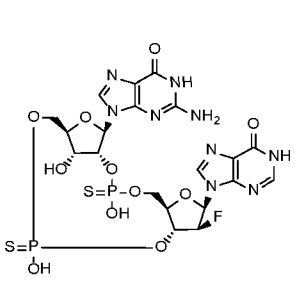
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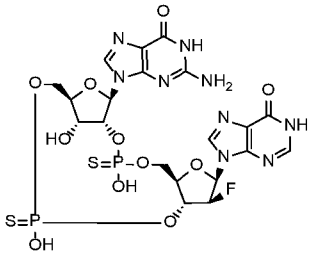
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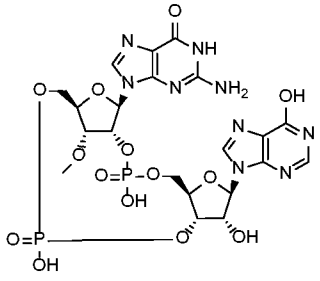
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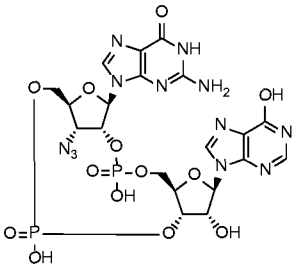
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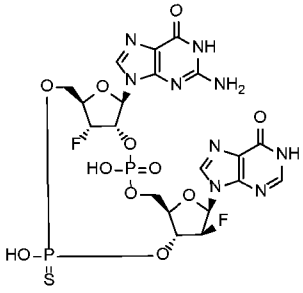
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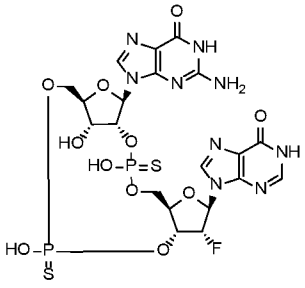
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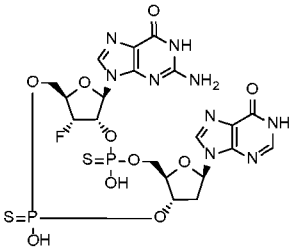
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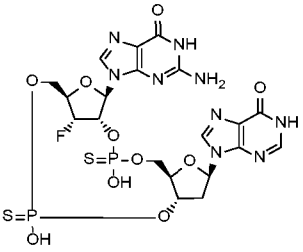
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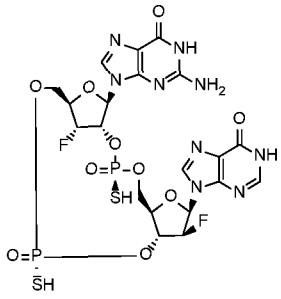
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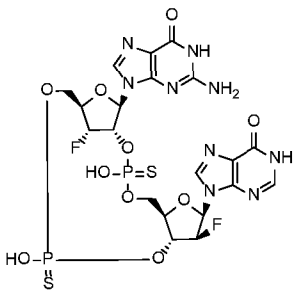
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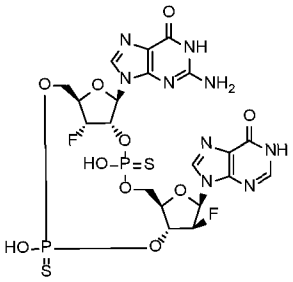
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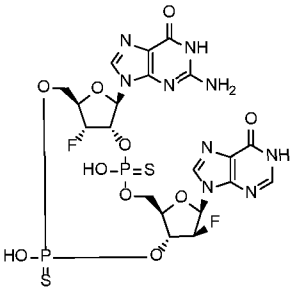
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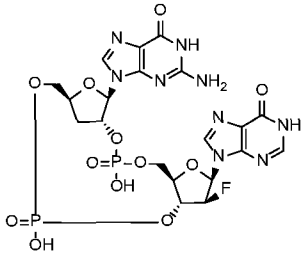
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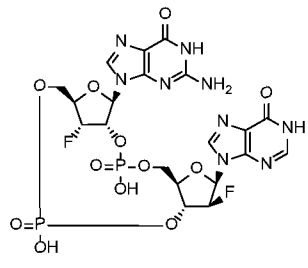
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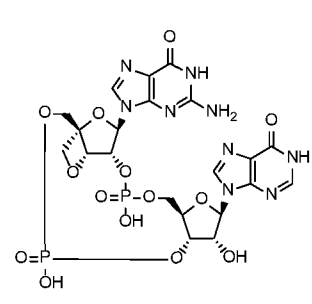
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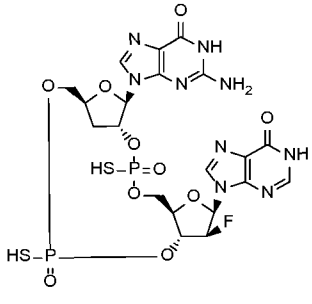
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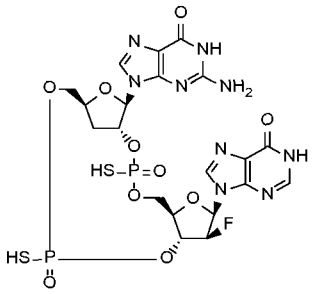
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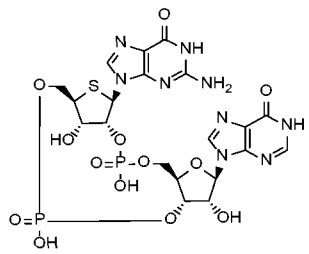
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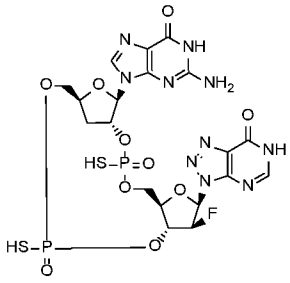
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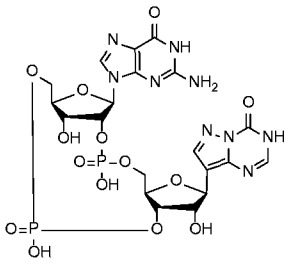
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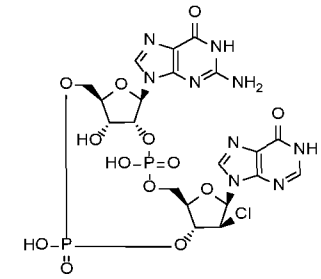
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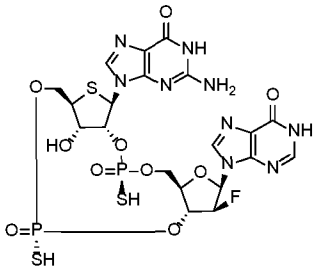
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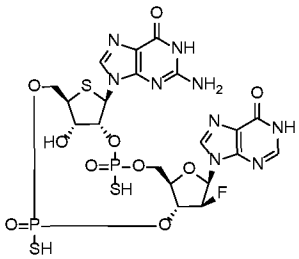
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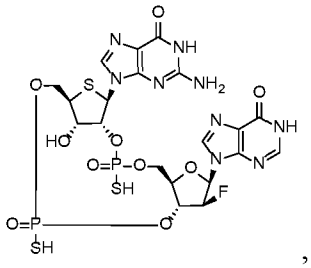
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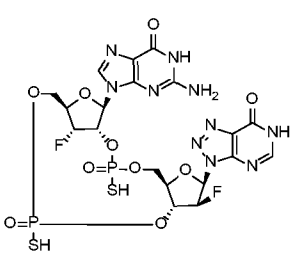
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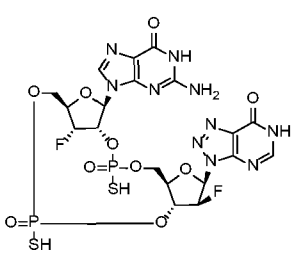
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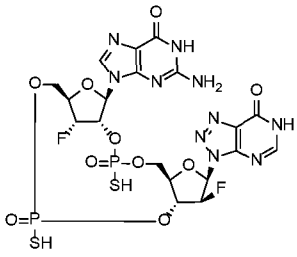
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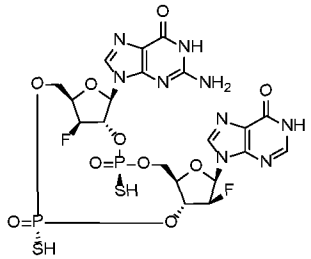
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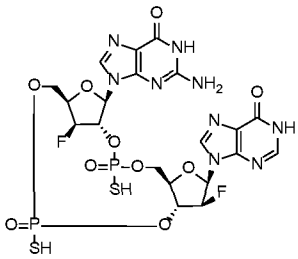
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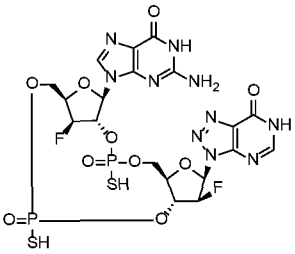
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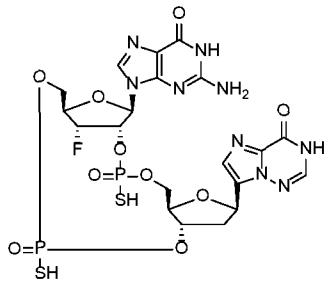
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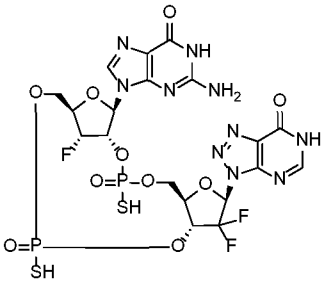
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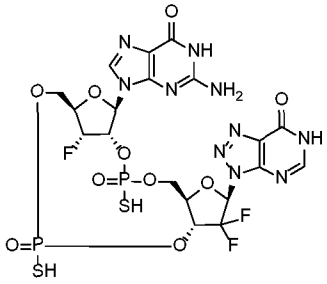
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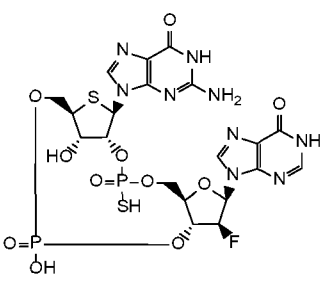
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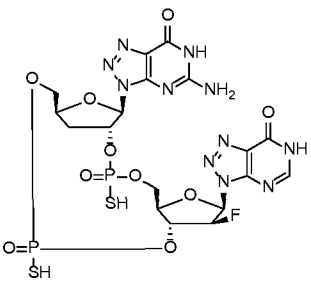
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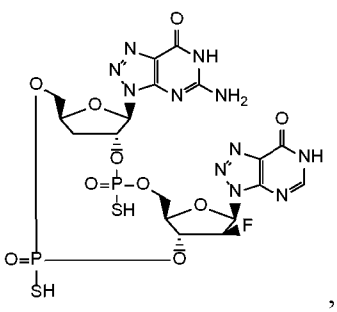
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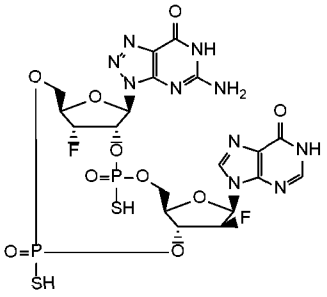
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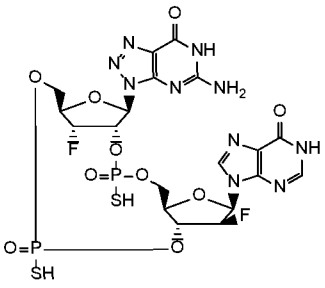
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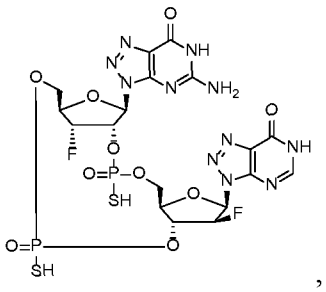
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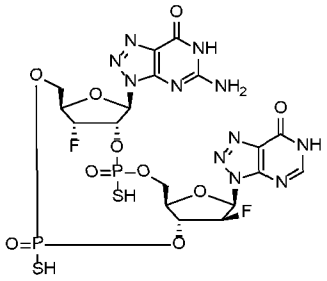
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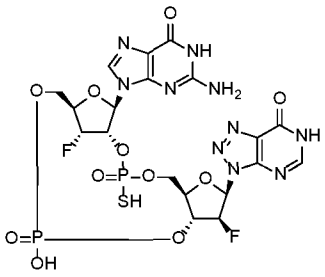
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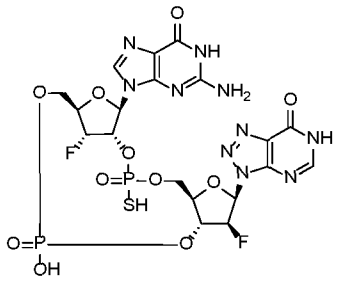
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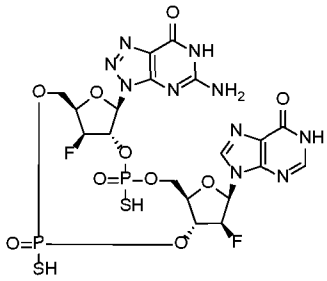
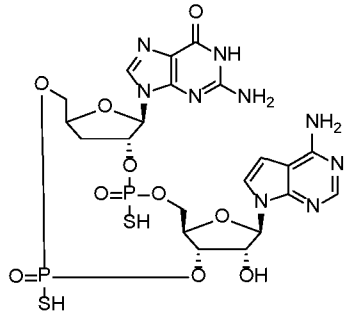

, and pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof.

23. The compound according to claim 22, wherein the compound is selected from the group consisting of: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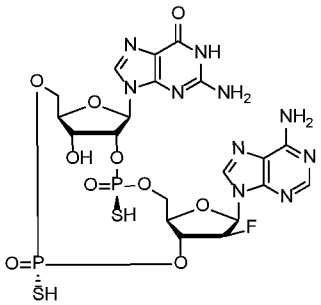
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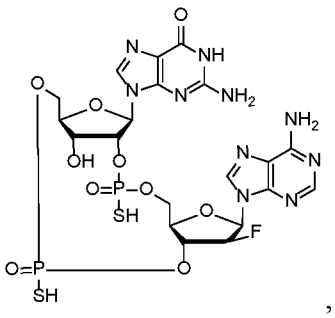
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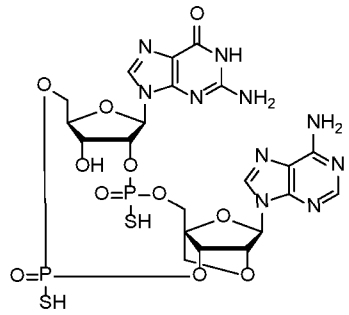
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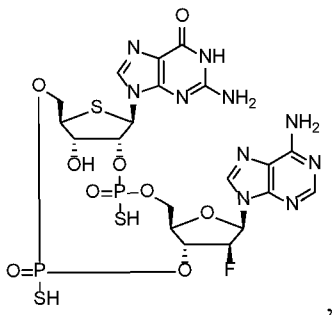
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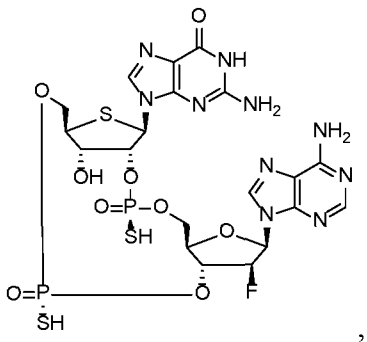
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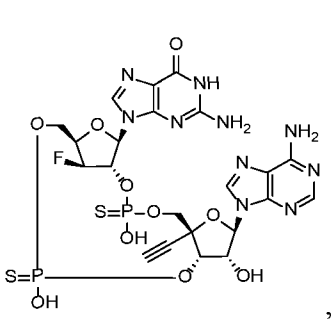
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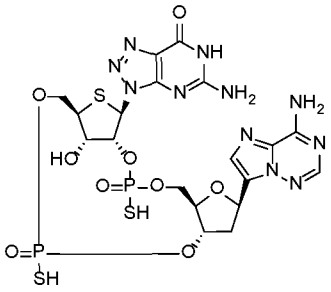
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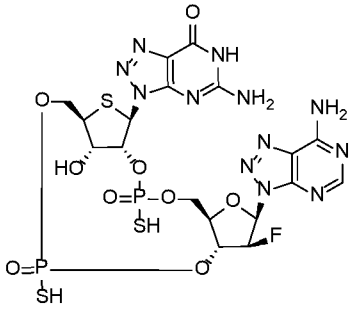
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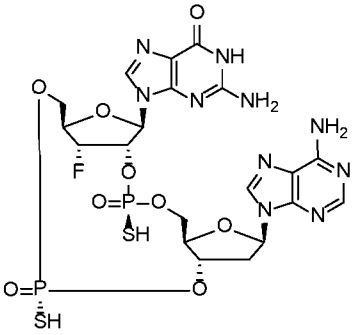
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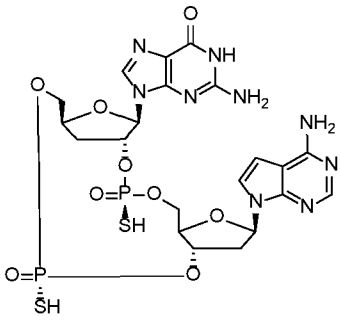
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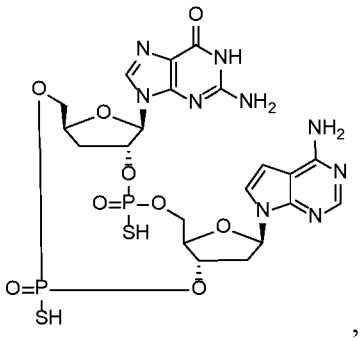
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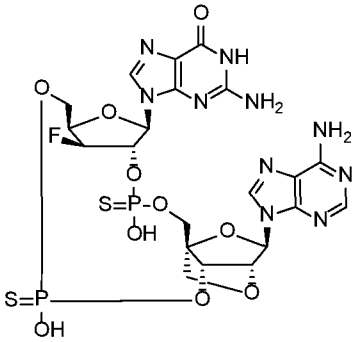
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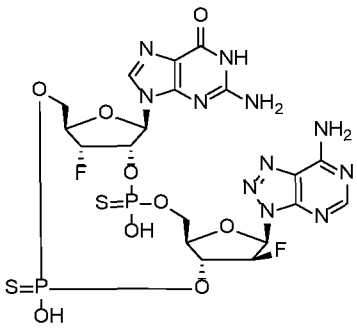
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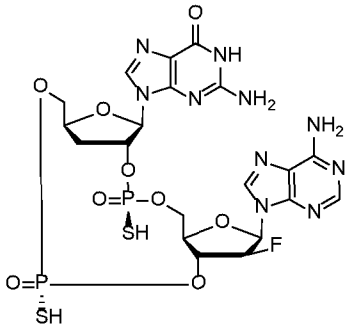
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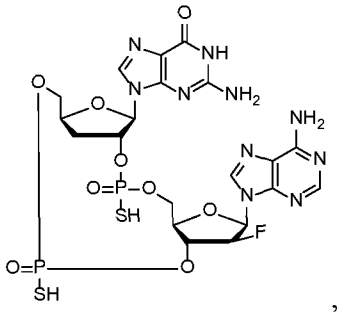
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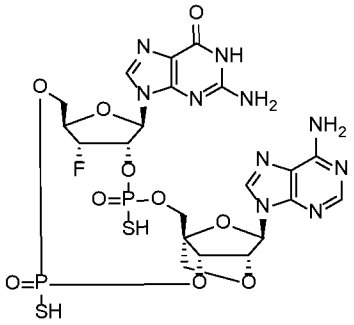
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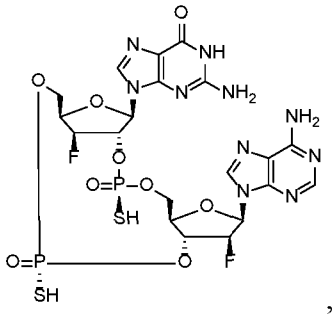
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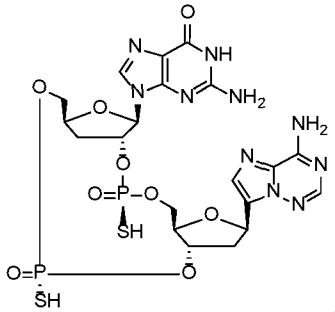
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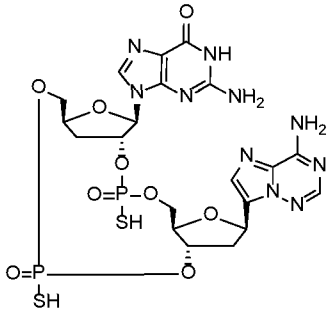
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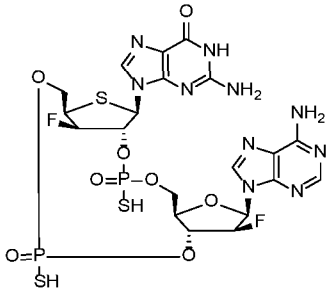
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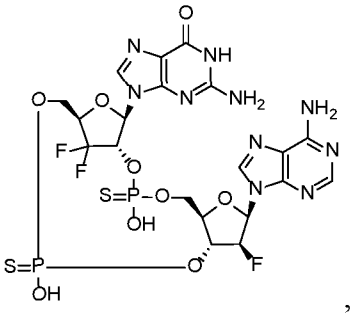
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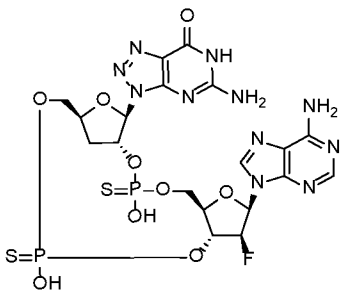
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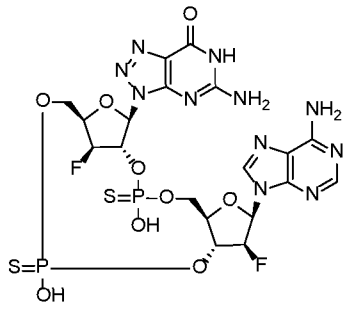
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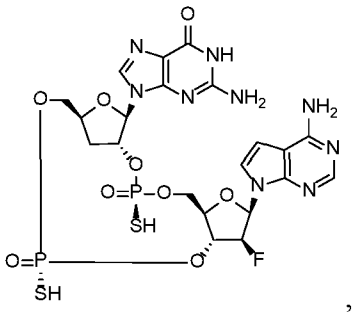
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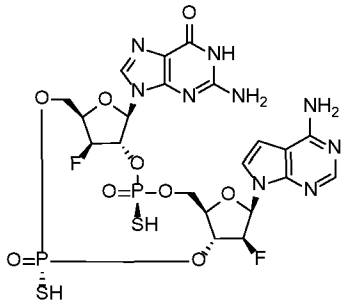
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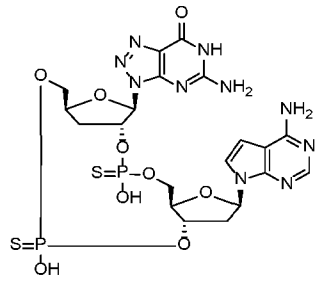
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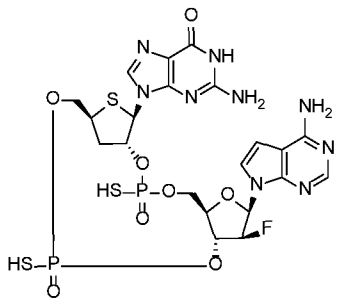
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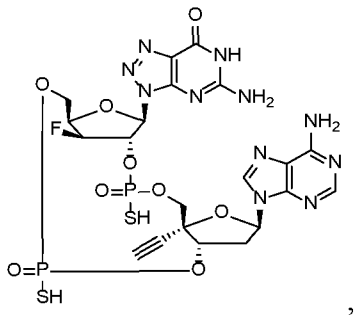
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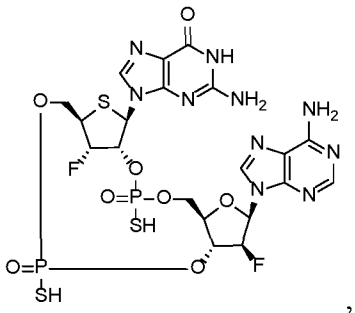
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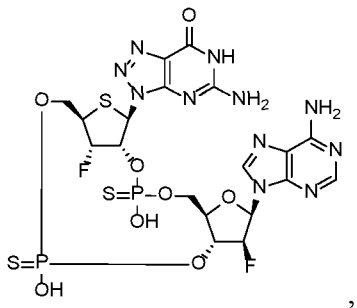
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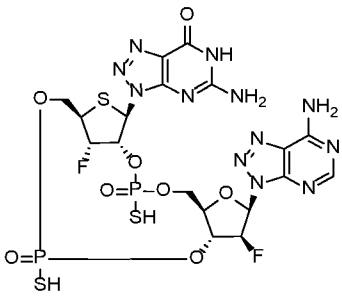
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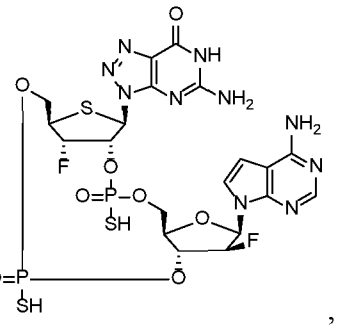
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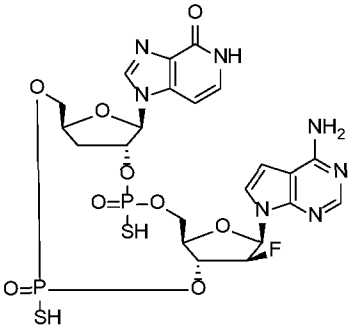
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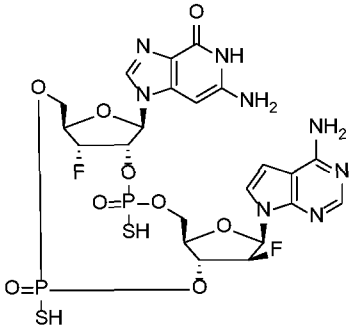
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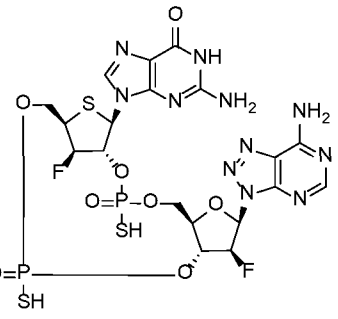
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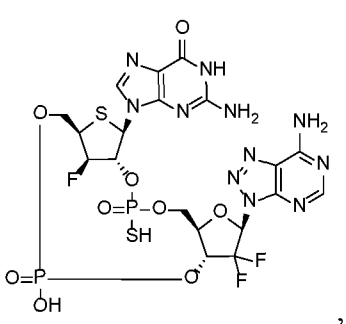
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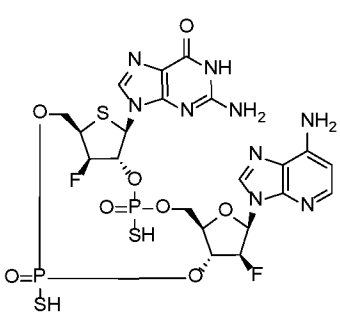
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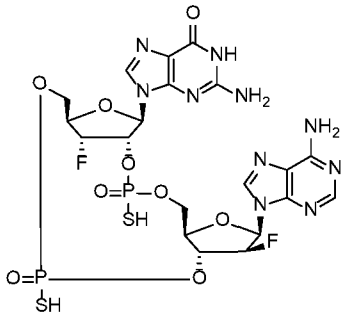
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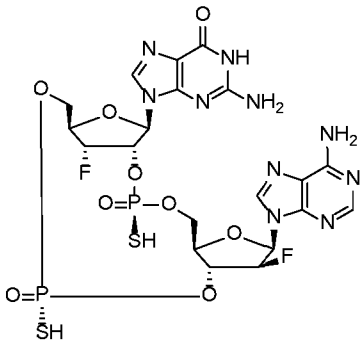
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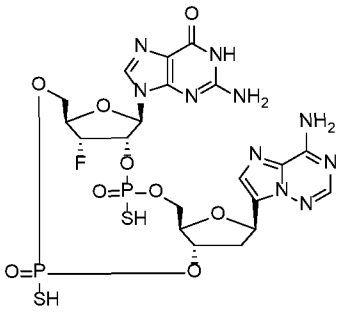
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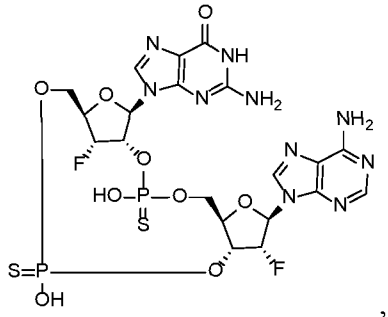
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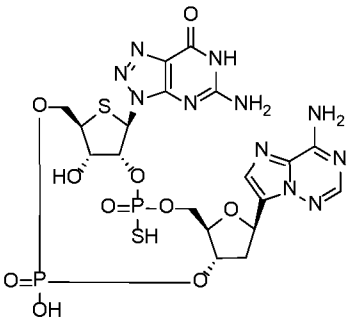
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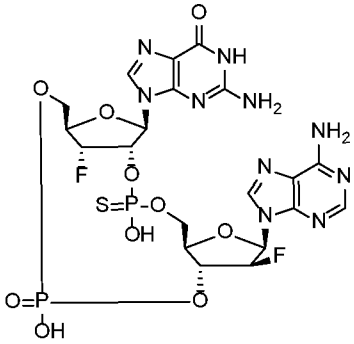
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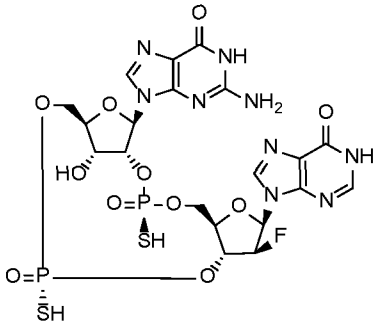
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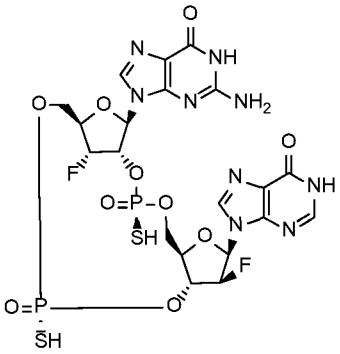
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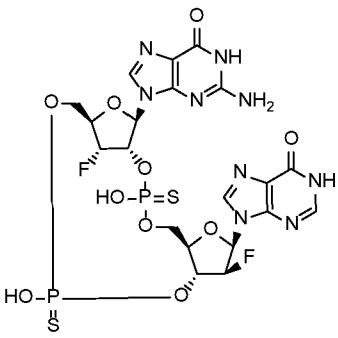
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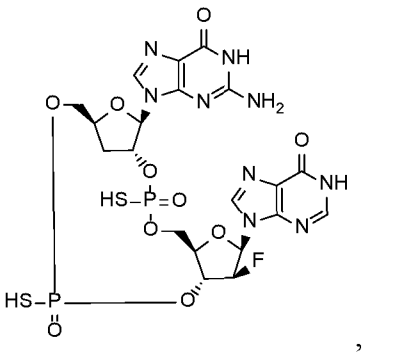
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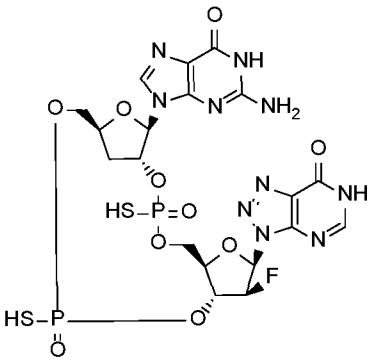
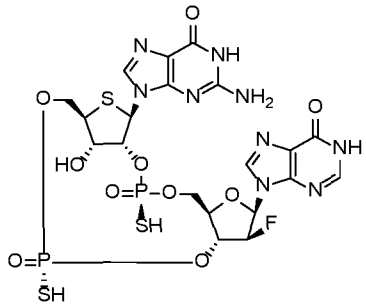
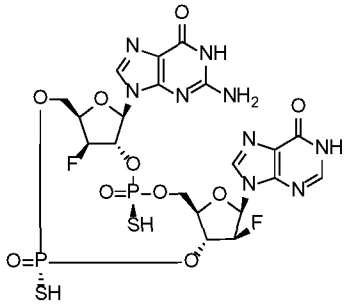
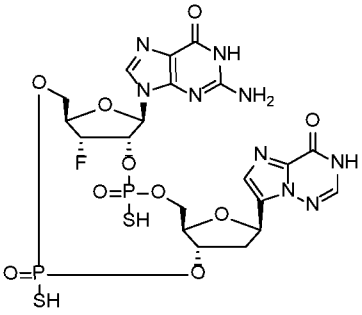
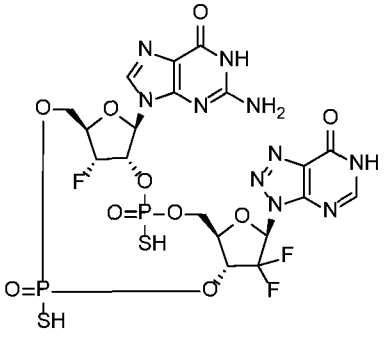
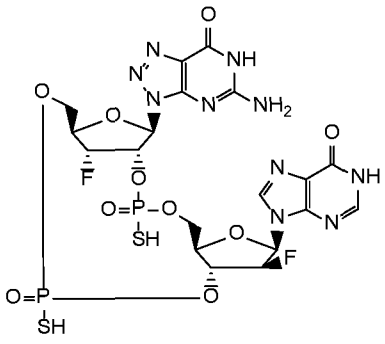
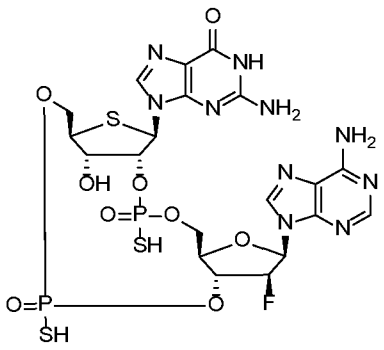
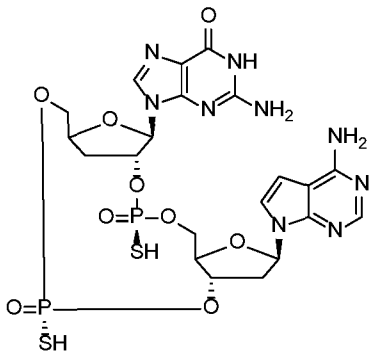
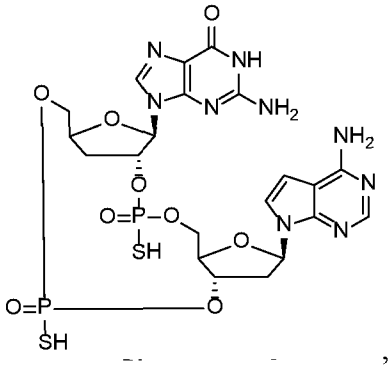
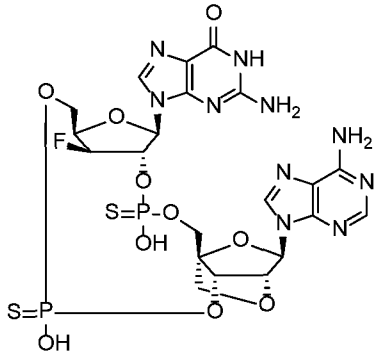
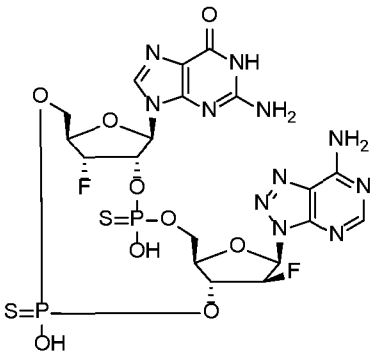
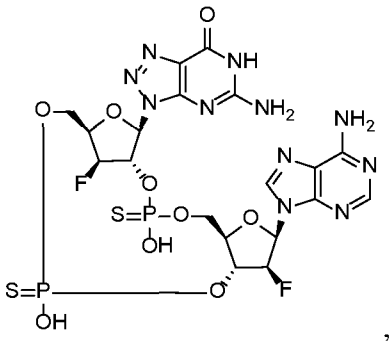
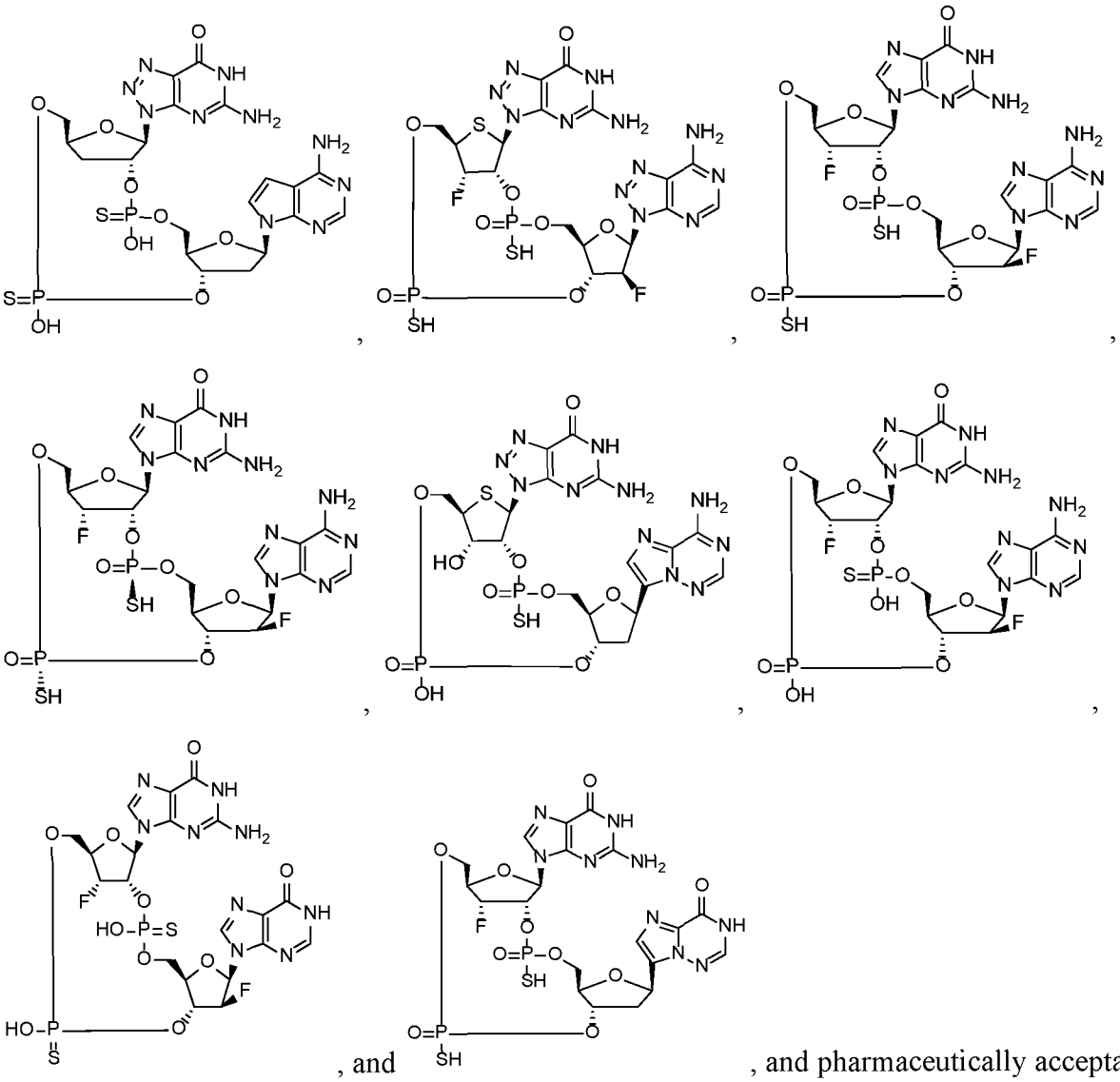







and pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof.

24. The compound according to claim 22, wherein the compound is selected from the group consisting of:



, and pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof.

25. A pharmaceutical composition, said pharmaceutical composition comprising:

(a) a compound according to any one of claims 11 to 24 or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof; and

(b) a pharmaceutically acceptable carrier.

26. A method of inducing an immune response in a subject, said method comprising administering a therapeutically effective amount of a compound according to any one of claims 11 to 24 to the subject.

27. A method of inducing an immune response in a subject, said method comprising administering a therapeutically effective amount of a pharmaceutical composition according to claim 25 to the subject.

28. A method of inducing a STING-dependent type I interferon production in a subject, said method comprising administering a therapeutically effective amount of a compound according to any one of claims 11 to 24 to the subject.

29. A method of inducing a STING-dependent type I interferon production in a subject, said method comprising administering a therapeutically effective amount of a pharmaceutical composition according to claim 25 to the subject.

30. A method of treating a cell proliferation disorder in a subject, said method comprising administering a therapeutically effective amount of a compound according to any one of claims 11 to 24 to the subject.

31. The method of claim 30, wherein the cell proliferation disorder is cancer.

32. A method of treating a cell proliferation disorder in a subject, said method comprising administering a therapeutically effective amount of a pharmaceutical composition according to claim 25 to the subject.

33. The method of claim 32, wherein the cell proliferation disorder is cancer.

SEQUENCE LISTING

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Ala Val Ser Gln Arg Leu Tyr Ile Leu Leu Pro Leu Asp Cys Gly Val
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Pro Asp Asn Leu Ser Met Ala Asp Pro Asn Ile Arg Phe Leu Asp Lys
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Leu Pro Gln Gln Thr Ala Asp Arg Ala Gly Ile Lys Asp Arg Val Tyr
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Ser Asn Ser Ile Tyr Glu Leu Leu Glu Asn Gly Gln Arg Ala Gly Thr
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Cys Val Leu Glu Tyr Ala Thr Pro Leu Gln Thr Leu Phe Ala Met Ser
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Gln Tyr Ser Gln Ala Gly Phe Ser Arg Glu Asp Arg Leu Glu Gln Ala
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Lys Leu Phe Cys Gln Thr Leu Glu Asp Ile Leu Ala Asp Ala Pro Glu
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Ser Gln Asn Asn Cys Arg Leu Ile Ala Tyr Gln Glu Pro Ala Asp Asp
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Ser Ser Phe Ser Leu Ser Gln Glu Val Leu Arg His Leu Arg Gln Glu
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Glu Lys Pro Leu Pro Leu Arg Thr Asp Phe Ser Gly Gly Gly Leu Asn
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