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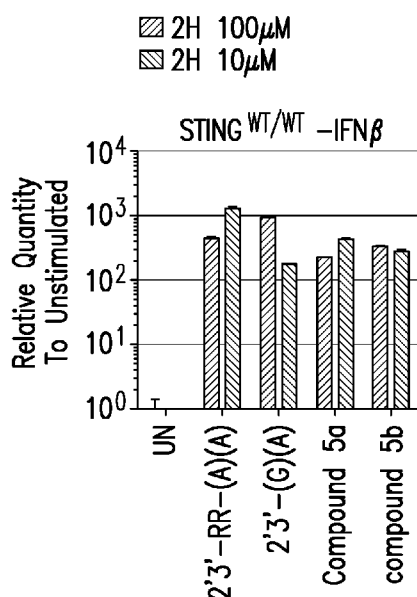


FIG. 1A

(57) Abstract: The present invention provides highly active locked nucleic acid cyclic -dinucleotide (LNA-CDN) immune stimulators that activate DCs via the cytoplasmic receptor known as STING (Stimulator of Interferon Genes). In particular, the LNA-CDNs of the present invention are provided in the form of a composition comprising one or more cyclic dinucleotides that induce human STING-dependent type I interferon production, wherein the cyclic dinucleotides present in the composition have at least one 2', 4' locked nucleic acids within the cyclic dinucleotide.

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**LOCKED NUCLEIC ACID CYCLIC DINUCLEOTIDE COMPOUNDS AND
USES THEREOF**

[0001] The present application claims priority to U.S. Provisional Patent Application 62/358,425, filed July 5, 2016, and U.S. Provisional Patent Application 62/362,907, filed July 15, 2016, each of which is hereby incorporated in its entirety including all tables, figures, and claims.

BACKGROUND OF THE INVENTION

[0002] The following discussion of the background of the invention is merely provided to aid the reader in understanding the invention and is not admitted to describe or constitute prior art to the present invention.

[0003] New insights into the mechanisms underlying immune-evasion, together with combination treatment regimens that potentiate the potency of therapeutic vaccination, either directly or indirectly, through combination with immune checkpoint inhibitors or other therapies, have served as a basis for the development of vaccines or immune modulators that can prime or boost an effective adaptive immune response. These modulators consist of tumor-specific CD4⁺ and CD8⁺ T cells specific for a targeted malignancy, resulting in an antitumor response and clinical benefit. How the innate immune system is engaged by targeted ligands shapes the development of an adaptive response and lends itself to the design of vaccines and immunomodulators (Reed et al., Trends Immunol., 30: 23-32, 2009; Dubensky and Reed, Semin. Immunol., 22: 155-61, 2010; Kastenmuller et al., J. Clin. Invest., 121: 1782–1796, 2011; Coffman et al., Immunity, 33: 492-503, 2010).

[0004] The cyclic-di-nucleotides (CDNs) cyclic-di-AMP (produced by *Listeria monocytogenes* and other bacteria) and its analogs cyclic-di-GMP and cyclic-GMP-AMP are recognized by the host cell as a pathogen associated molecular pattern (PAMP), which bind to the pathogen recognition receptor (PRR) known as Stimulator of INterferon Genes (STING). STING is an adaptor protein in the cytoplasm of host mammalian cells which activates the TANK binding kinase (TBK1)—IRF3 and the NF-κB signaling axis, resulting in the induction of IFN-β and other gene products that strongly activate innate immunity. It is now recognized that STING is a component of the host cytosolic

surveillance pathway (Vance et al., *Cell Host & Microbe*, 6:10-21, 2009), that senses infection with intracellular pathogens and in response induces the production of IFN- β , leading to the development of an adaptive protective pathogen-specific immune response consisting of both antigen-specific CD4⁺ and CD8⁺ T cells as well as pathogen-specific antibodies. Other publications related to cyclic dinucleotides include, for example: U.S. Patents 7,709,458 and 7,592,326; PCT Publication Nos. WO2007/054279, WO2014/093936, WO2014/179335, WO2014/189805, WO2015/185565, WO2016/096174, WO2016/145102, WO2017/027645, WO2017/027646, and WO2017/075477; and Yan et al., *Bioorg. Med. Chem Lett.* 18:5631-4, 2008.

[0005] An uncharacterized mouse gene with significant structural homology to the catalytic domain of human oligoadenylate synthase cyclic GMP-AMP synthase was reported to be the enzyme responsible for producing STING-binding CDNs in mammalian cells (Sun et al., *Science* 339(6121):786-91, 2013). Termed cyclic GMP-AMP Synthase (cGAS), this enzyme catalyzes the synthesis of cyclic GMP-AMP (cGAMP) from ATP and GTP in the presence of DNA. This cGAMP then functions as a second messenger that binds to and activates STING. These cGAS-produced CDNs differed structurally from the bacterially produced CDNs in that they possess an unusual phosphodiester linkage. Thus, while the bacterially produced CDNs contain a bis-3',5' linkage between the two nucleotides, mammalian CDNs contained one 2',5' linkage and one 3',5' linkage, or a so-called "mixed linkage" (ML) or non-canonical CDNs. These 2',5'-3',5' molecules bind STING with nM affinity, some 300-fold better than bacterial c-di-GMP.

[0006] Human STING (hSTING) also has known polymorphisms, including alleles encoding histidine at position 232, which are refractory to bis-3',5' (canonical) CDNs, but not 2',5'-3',5' (non-canonical, mixed linkage) CDNs (Diner et al., *Cell Reports* 3, 1355-61, 2013; Jin et al., *Genes and Immunity*, 12: 263-9, 2011). Single nucleotide polymorphisms in the hSTING gene have been reported to affect the responsiveness to bacterial-derived canonical CDNs (Diner et al., 2013; Gao et al., *Cell* 154, 748-762, 2013; Conlon et. al., *J. Immunol.* 190: 5216-5225, 2013). Five haplotypes of hSTING have been reported (WT, REF, HAQ, AQ and Q alleles), which vary at amino acid positions 71, 230, 232 and 293 (Jin et al., 2011; Yi et al., *PLOS One* 8: e77846, 2013). Cells expressing hSTING reportedly respond poorly to stimulation with bacterial CDNs

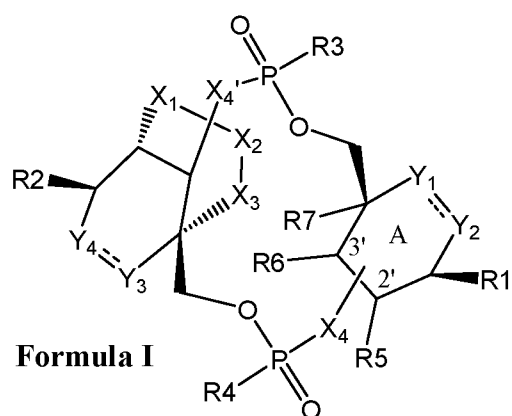
cGAMP, c-di-AMP and c-di-GMP having bis-(3', 5') linkages, but are responsive to the endogenously produced cGAS product, ML cGAMP (Diner et al., 2013). Thus, it has been suggested that the 2',5'-3',5' molecules represent much more potent physiological ligands in terms of hSTING targeting (Zhang et al., Mol. Cell. 51:226-35, 2013; Xiao and Fitzgerald, Mol. Cell 51:135-39, 2013).

[0007] There are therapeutic applications for compounds that target hSTING as either an agonist (e.g. increasing the tumor-specific CD4⁺ and CD8⁺ T cells specific for a targeted malignancy, or improving a response to vaccination) or as an antagonist (e.g. decreasing a type I interferon response associated with autoimmune disease). Thus there is a desire for improved compounds for the modulation of hSTING.

SUMMARY OF THE INVENTION

[0008] It is an object of the present invention to provide compositions and methods which modulate immune responses to diseases. It is a further object of the invention to provide compositions and methods which provide locked nucleic acid cyclic dinucleotide analogs that exhibit improved characteristics when employed for modulation of mammalian, and preferably human, STING. It is yet a further object of the invention to provide locked nucleic acid cyclic dinucleotide analogs that activate hSTING, and compositions and methods thereof for the treatment of cancer.

[0009] In a first aspect, the present invention provides a locked nucleic acid cyclic dinucleotide ("LNA-CDN") compound of Formula I:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof,

wherein:

R1 and R2 are independently a pyrimidine or purine nucleic acid base or analog or derivative thereof;

R3 and R4 are independently -OH, -OR^a, -SH or -SR^a;

Y₁ is -O-, -S- or -CRR- and Y₂ is absent, wherein each R is independently -H or -C₁₋₆alkyl, or the two R together with the carbon to which they are attached form a 3-6 membered heterocycloalkyl or C₃₋₆cycloalkyl spirocyclic ring; or

Y₁ is -O-, -CH₂- or -CH= and Y₂ is -CH₂- or =CH-, selected to provide -Y₁-Y₂- as -O-CH₂-, -CH₂-CH₂- or -CH=CH-;

Y₃ is -O-, -S- or -CRR- and Y₄ is absent, wherein each R is independently -H or -C₁₋₆alkyl, or the two R together with the carbon to which they are attached form a 3-6 membered heterocycloalkyl or C₃₋₆cycloalkyl spirocyclic ring; or

Y₃ is -O-, -CH₂- or -CH= and Y₄ is -CH₂- or =CH-, selected to provide -Y₃-Y₄- as -O-CH₂-, -CH₂-CH₂- or -CH=CH-;

X₁, X₂, and X₃ are all present, X₁ and X₂ are -C(R^bR^c)-, -N(R^d), -O- or -S(=O)₂-, and X₃ is -C(R^bR^c)- or -O-, selected to provide -X₁-X₂-X₃- as -C(R^bR^c)-C(R^bR^c)-C(R^bR^c)-, -C(R^bR^c)-N(R^d)-C(R^bR^c)-, -C(R^bR^c)-O-C(R^bR^c)-, -C(R^bR^c)-C(R^bR^c)-O-, -O-C(R^bR^c)-C(R^bR^c)-, -O-N(R^d)-C(R^bR^c)-, -S(=O)₂-N(R^d)-C(R^bR^c)-, -N(R^d)-C(R^bR^c)-C(R^bR^c)-, -N(R^d)-O-C(R^bR^c)- or -N(R^d)-S(=O)₂-C(R^bR^c)-; or

X₂ is absent, X₁ and X₃ are both present, X₁ is -C(R^bR^c)-, -N(R^d) or -O- and X₃ is -C(R^bR^c)- or -S(=O)₂-, selected to provide -X₁-X₃- as -C(R^bR^c)-C(R^bR^c)-, -O-C(R^bR^c)-, -N(R^d)-C(R^bR^c)- or -N(R^d)-S(=O)₂-;

X₄' is -O- or -NH-;

X₄ is -O- or -NH- bonded to either the 2' or the 3' carbon atom of the indicated ring A;

wherein:

when X₄ is -O- or -NH- bonded to the 2' carbon atom of ring A:

R₅ is absent, R₆ is selected from the group consisting of -H, -OH, halogen and -OR^e, and R₇ is -H;

when X₄ is -O- or -NH- bonded to the 3' carbon atom of ring A:

R₆ is absent, R₅ is selected from the group consisting of -H, -OH, halogen and -OR^e, and R₇ is -H; or

R6 is absent, and R5 and R7 join to form -X₅-X₆-X₇-, resulting in a fused ring system with X₅ bonded to the 2' carbon atom of ring A and X₇ bonded to the carbon atom of ring A to which R7 is attached,

wherein:

X₅, X₆, and X₇ are all present, X₅ and X₆ are -C(R^bR^c)-, -N(R^d), -O- or -S(=O)₂-, and X₇ is -C(R^bR^c)- or -O-, selected to provide -X₅-X₆-X₇- as -C(R^bR^c)-C(R^bR^c)-C(R^bR^c)-, -C(R^bR^c)-N(R^d)-C(R^bR^c)-, -C(R^bR^c)-O-C(R^bR^c)-, -C(R^bR^c)-C(R^bR^c)-O-, -O-C(R^bR^c)-C(R^bR^c)-, -O-N(R^d)-C(R^bR^c)-, -S(=O)₂-N(R^d)-C(R^bR^c)-, -N(R^d)-C(R^bR^c)-C(R^bR^c)-, -N(R^d)-O-C(R^bR^c)- or -N(R^d)-S(=O)₂-C(R^bR^c)-; or

X₆ is absent, X₅ and X₇ are both present, X₅ is -C(R^bR^c)-, -N(R^d) or -O- and X₇ is -C(R^bR^c)- or -S(=O)₂-, selected to provide -X₅-X₇- as -C(R^bR^c)-C(R^bR^c)-, -O-C(R^bR^c)-, -N(R^d)-C(R^bR^c)- or -N(R^d)-S(=O)₂-;

R^a at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl and -C(=O)R^f; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^m, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -Rⁿ;

R^b and R^c, at each occurrence, are independently selected from the group consisting of -H, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R' and -L₁-R^g; or R^b and R^c together on a carbon are =O, =CR^hR^h, =N-ORⁱ, =N-Rⁱ, or =N-NRⁱRⁱ; or R^b and R^c together with the carbon to which they are attached form a C₃₋₆cycloalkyl or 3 to 6 membered heterocycloalkyl spirocyclic ring; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^m, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -Rⁿ;

R^d at each occurrence is independently selected from the group consisting of -H, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R'' and -L₂-R^j; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^m, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -Rⁿ; or R^d is a suitable nitrogen protecting group.

- R^e is selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl and $-C(=O)R^f$; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^n$; or R^e is a suitable oxygen protecting group;
- R^f at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^n$;
- R' at each occurrence is selected from the group consisting of $-CN$, $-NO_2$, $-OH$, $-SH$, $-NH_2$, $-C(=W)-WH$, $-C(=W)-NH_2$, $-C(=W)-NH-WH$, $-C=N-OH$, $-C(=NH)-NH_2$, $-W-C(=W)-WH$, $-W-C(=W)-NH_2$, $-S(=O)_2-NH_2$, $-S(=O)_2-NH-W-$, $-NH-NH_2$, $-NH-WH$, $-NH-C(=W)-WH$, $-NH-C(=W)-NH_2$, $-NH-S(=O)_2-NH_2$, and $-NH-C(=NH)-NH_2$;
- R'' at each occurrence is selected from the group consisting of $-OH$, $-SH$, $-NH_2$, $-C(=W)-WH$, $-C(=W)-NH_2$, $-C(=NH)-NH_2$, and $-S(=O)_2-NH_2$;
- L_1 is selected from the group consisting of $-O-$, $-S-$, $-S(=O)-$, $-S(=O)_2-$, $-C(=W)-$, $-C(=W)-W-$, $-C(=W)-NR^k-$, $-C(=W)-NR^k-W-$, $-C=N-O-$, $-C(=NR^k)NR^k-$, $-C(NR^kR^k)=N-$, $-W-C(=W)-$, $-W-C(=W)-W-$, $-W-C(=W)-NR^k-$, $-S(=O)_2-NR^k-$, $-S(=O)_2-NR^k-W-$, $-NR^k-$, $-N=CR^h-$, $-NR^k-W-$, $-NR^k-NR^k-$, $-NR^k-C(=NR^k)NR^k-$, $-NR^k-C(NR^kR^k)=N-$, $-NR^k-C(=W)-$, $-NR^k-C(=W)-W-$, $-NR^k-C(=W)-NR^k-$, $-NR^k-S(=O)_2-NR^k-$ and $-NR^k-S(=O)_2-$;
- L_2 is selected from the group consisting of $-O-$, $-S-$, $-NR^k-$, $-C(=W)-$, $-C(=W)-W-$, $-C(=W)-NR^k-$, $-C(=NR^k)NR^k-$, $-C(NR^kR^k)=N-$, $-S(=O)_2-NR^k-$, and $-S(=O)_2-$;
- R^g and R^j , at each occurrence, are independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^n$;
- R^h at each occurrence is independently selected from the group consisting of $-H$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl;

wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^n$;

R^i and R^k , at each occurrence, are independently selected from the group consisting of $-H$, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^n$;

R^m at each occurrence is independently selected from the group consisting of $=O$, halogen, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-R'$ and $-L_3-R^o$, when substituted on an available carbon atom, and carbocyclyl, heterocyclyl, aryl, heteroaryl, $-R''$ and $-L_4-R^p$, when substituted on an available nitrogen atom; wherein carbocyclyl, heterocyclyl, aryl and heteroaryl are optionally independently substituted with one or more $-R^n$.

R^n at each occurrence is independently selected from the group consisting of $=O$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-R'$ and $-L_3-R^o$, when substituted on an available carbon atom, and alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-R''$ and $-L_4-R^p$, when substituted on an available nitrogen atom;

L_3 is selected from the group consisting of $-O-$, $-S-$, $-S(=O)-$, $-S(=O)_2-$, $-C(=W)-$, $-C(=W)-W-$, $-C(=W)-NR^q-$, $-C(=W)-NR^q-W-$, $-C=N-O-$, $-C(=NR^q)NR^q-$, $-C(NR^qR^q)=N-$, $-W-C(=W)-$, $-W-C(=W)-W-$, $-W-C(=W)-NR^q-$, $-S(=O)_2-NR^q-$, $-S(=O)_2-NR^q-W-$, $-NR^q-$, $-N=CR^r-$, $-NR^q-W-$, $-NR^q-NR^q-$, $-NR^q-C(=NR^q)NR^q-$, $-NR^q-C(NR^qR^q)=N-$, $-NR^q-C(=W)-$, $-NR^q-C(=W)-W-$, $-NR^q-C(=W)-NR^q-$, $-NR^q-S(=O)_2-NR^q-$, and $-NR^q-S(=O)_2-$;

L_4 is selected from the group consisting of $-O-$, $-S-$, $-NR^q-$, $-C(=W)-$, $-C(=W)-W-$, $-C(=W)-NR^q-$, $-C(=NR^q)NR^q-$, $-C(NR^qR^q)=N-$, $-S(=O)_2-NR^q-$, and $-S(=O)_2-$;

each W is independently O or S ;

R^o and R^p , at each occurrence, are independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently substituted with one or more $-R^s$; carbocyclyl or heterocyclyl are optionally

independently substituted with one or more $-R^t$; and aryl or heteroaryl are optionally independently substituted with one or more $-R^u$;

R^q at each occurrence is independently selected from the group consisting of $-H$, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently substituted with one or more $-R^s$; carbocyclyl or heterocyclyl are optionally independently substituted with one or more $-R^t$; and aryl or heteroaryl are optionally independently substituted with one or more $-R^u$;

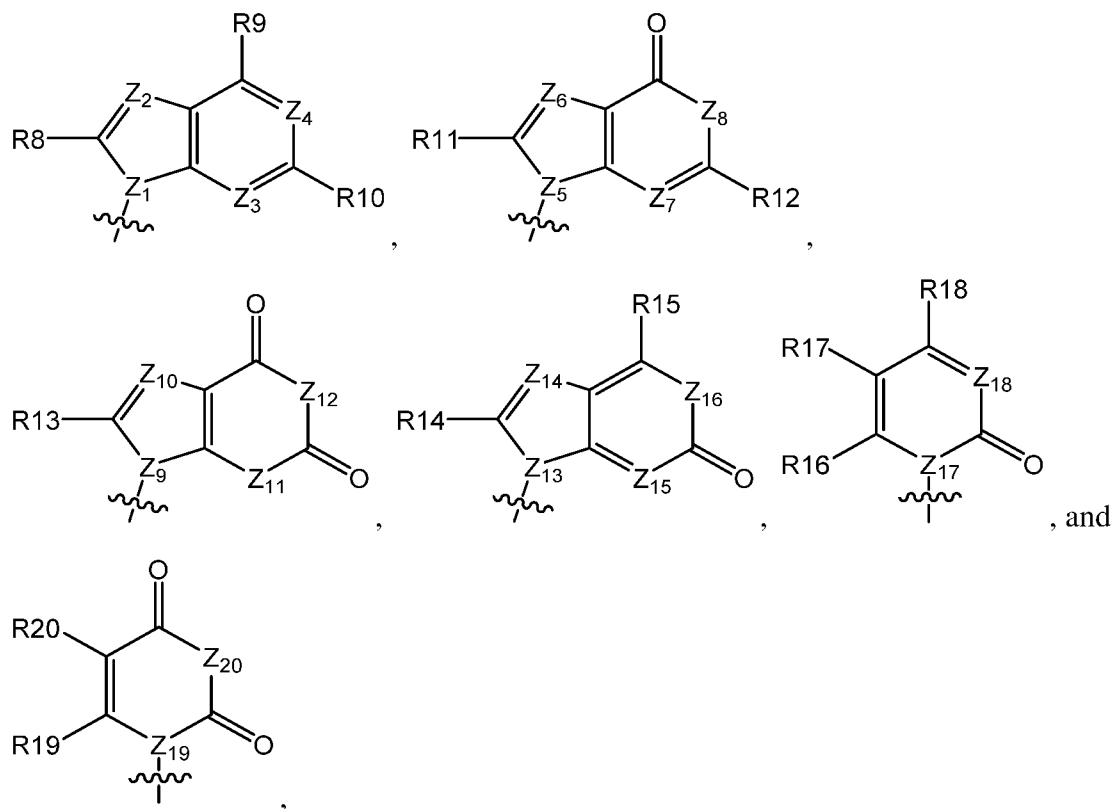
R^r at each occurrence is independently selected from the group consisting of $-H$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently substituted with one or more $-R^s$; carbocyclyl or heterocyclyl are optionally independently substituted with one or more $-R^t$; and aryl or heteroaryl are optionally independently substituted with one or more $-R^u$;

R^s at each occurrence is independently selected from the group consisting of halogen, $=O$, $-OH$, $-SH$, $-NH_2$, $-CN$, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{1-6} alkylamino, C_{1-6} dialkylamino, and N-linked-heterocycloalkyl; wherein C_{1-6} alkyl, alone or as part of C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{1-6} alkylamino, or C_{1-6} dialkylamino, is optionally substituted with one or more substituents independently selected from the group consisting of $-OH$, $-SH$, $-NH_2$, halogen, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} alkylthio, C_{1-6} haloalkylthio, C_{1-6} alkylsulfinyl, C_{1-6} haloalkylsulfinyl, C_{1-6} alkylsulfonyl, C_{1-6} haloalkylsulfonyl, C_{1-6} alkylamino, C_{1-6} dialkylamino and N-linked-heterocycloalkyl;

R^t at each occurrence is independently selected from the group consisting of halogen, $=O$, $-OH$, $-SH$, $-NH_2$, $-CN$, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{1-6} alkylamino, C_{1-6} dialkylamino and N-linked-heterocycloalkyl; wherein C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} alkyl, alone or as part of C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{1-6} alkylamino, or C_{1-6} dialkylamino, is optionally substituted with one or more substituents independently selected from the group consisting of $-OH$, $-SH$, $-NH_2$, halogen, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} alkylthio, C_{1-6} haloalkylthio, C_{1-6} alkylsulfinyl, C_{1-6} haloalkylsulfinyl, C_{1-6} alkylsulfonyl, C_{1-6} haloalkylsulfonyl, C_{1-6} alkylamino, C_{1-6} dialkylamino and N-linked-heterocycloalkyl; and

R^u at each occurrence is independently selected from the group consisting of halogen, -OH, -SH, -NH₂, -CN, -NO₂, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino, and N-linked-heterocycloalkyl; wherein C₂₋₆alkenyl, C₂₋₆alkynyl, and C₁₋₆alkyl, alone or as part of C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, or C₁₋₆dialkylamino, is optionally substituted with one or more substituents independently selected from the group consisting of -OH, -SH, -NH₂, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylthio, C₁₋₆haloalkylthio, C₁₋₆alkylsulfinyl, C₁₋₆haloalkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆haloalkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl.

[0010] In a first embodiment of the first aspect, R1 and R2 are independently selected from the group consisting of:



wherein:

 indicates the bond of R1 or R2 to the ring as shown in Formula I;

Z₁, Z₅, Z₉, Z₁₃, Z₁₇ and Z₁₉ are independently $\text{—}\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{—}$ or $\text{—}\overset{\text{O}}{\underset{\text{O}}{\text{N}}}\text{—}$;

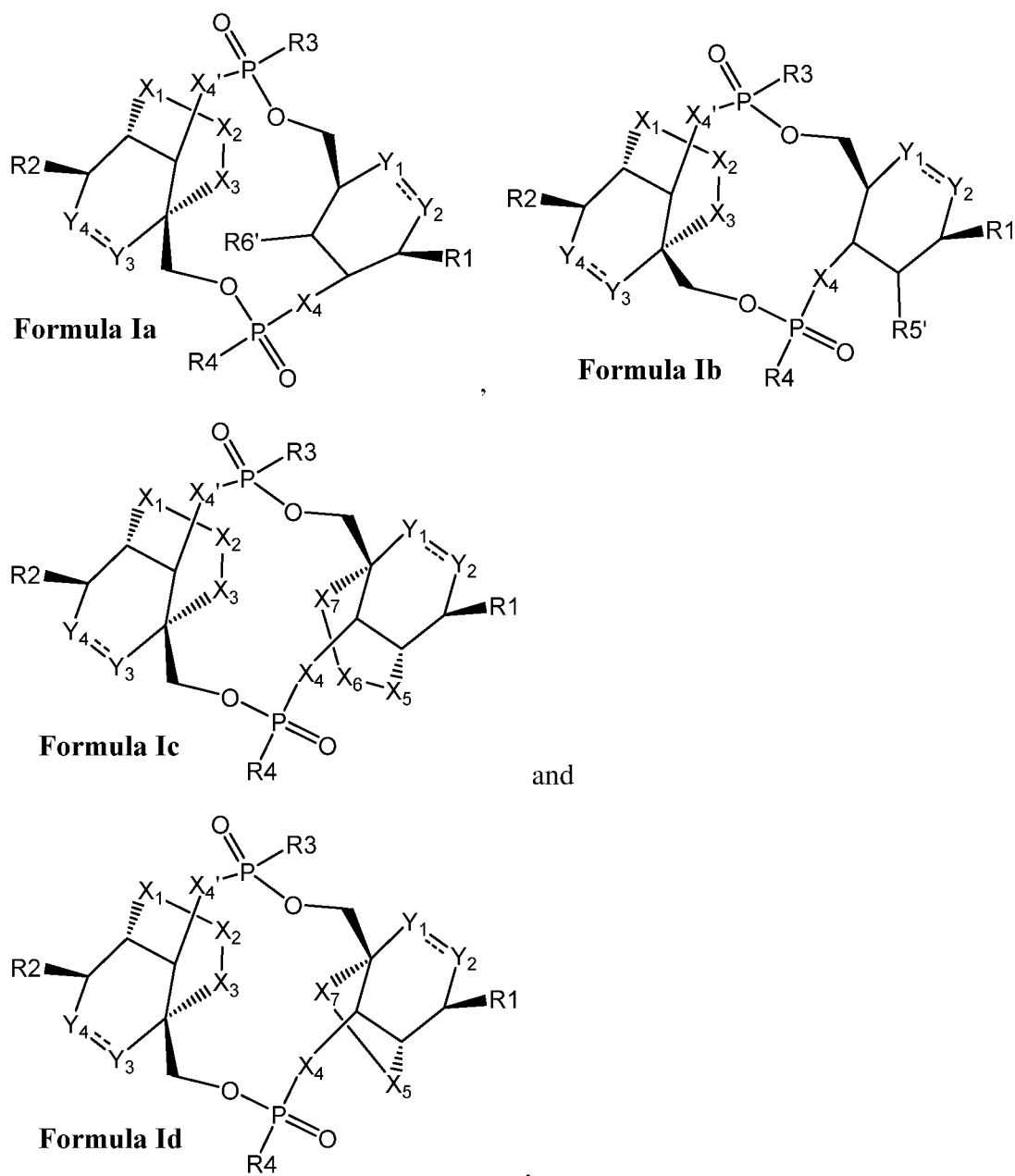
Z₈, Z₁₁, Z₁₂, Z₁₆ and Z₂₀ are independently $\text{—}\text{CR}^v\text{R}^w\text{—}$, $\text{—}\text{O—}$ or $\text{—}\text{NR}^x\text{—}$;

$Z_2, Z_3, Z_4, Z_6, Z_7, Z_{10}, Z_{14}, Z_{15}$ and Z_{18} are independently $=CR^v-$ or $=N-$;
 R^v and R^w , at each occurrence, are independently selected from the group consisting of
 $-H$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl,
heteroaryl, $-R'$ and $-L_1-R^g$; or R^v and R^w together are $=O$, $=CR^hR^h$, $=N-OR^i$, $=N-R^i$, or
 $=N-NR^iR^i$; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently
substituted with one or more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are
optionally independently substituted with one or more $-R^n$;
each R^x is independently selected from the group consisting of $-H$, alkyl, alkenyl, alkynyl,
heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-R''$ and $-L_2-R^j$; wherein alkyl,
alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or
more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally
independently substituted with one or more $-R^n$; and
 $R_8, R_9, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}$ and R_{20} are independently
selected from the group consisting of $-H$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl,
carbocyclyl, heterocyclyl, aryl, heteroaryl, $-R'$ and $-L_1-R^g$; wherein alkyl, alkenyl,
alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^m$,
and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently
substituted with one or more $-R^n$,
wherein $R', R'', R^g, R^h, R^i, R^j, R^m, R^n, L_1$ and L_2 are as defined for Formula I, and R^g is
 R^g or a suitable nitrogen protecting group when L_1 is $-NH-$.

[0011] In a second embodiment of the first aspect and first embodiment thereof, when R_5
or R_6 are selected from the group consisting of $-H$, $-OH$, halogen and $-OR^e$, R_5 or R_6 are
selected from the group consisting of $-H$, $-OH$ optionally substituted with a suitable
oxygen protecting group, halogen, $-O-C_{1-14}alkyl$ and $-O-(C=O)-C_{1-14}alkyl$. In some
embodiments, when R_5 or R_6 are selected from the group consisting of $-H$, $-OH$, halogen
and $-OR^e$, R_5 and R_6 are selected from the group consisting of $-H$, $-OH$, $-F$ and
 $-O-(C=O)-C_{1-14}alkyl$. In some embodiments, when R_5 or R_6 are selected from the group
consisting of $-H$, $-OH$, halogen and $-OR^e$, R_5 and R_6 are $-H$, $-OH$ or $-F$, in some
embodiments $-OH$ or $-F$.

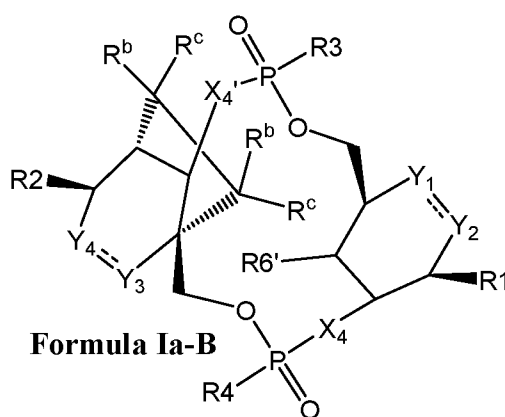
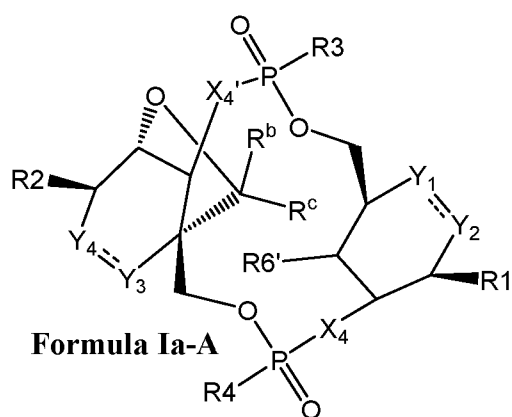
[0012] In a third embodiment of the first aspect and any of the above embodiments
thereof, the compound of Formula I is selected from the group consisting of a compound of
Formula Ia, a compound of Formula Ib, a compound of Formula Ic and a compound of

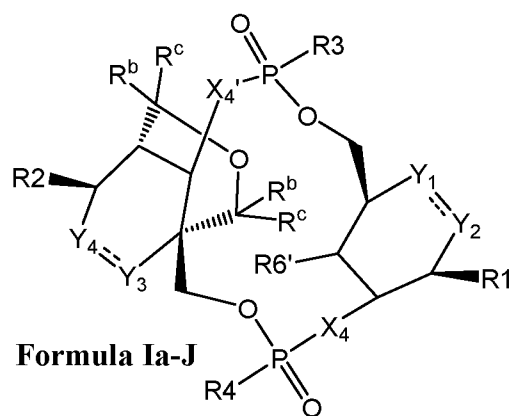
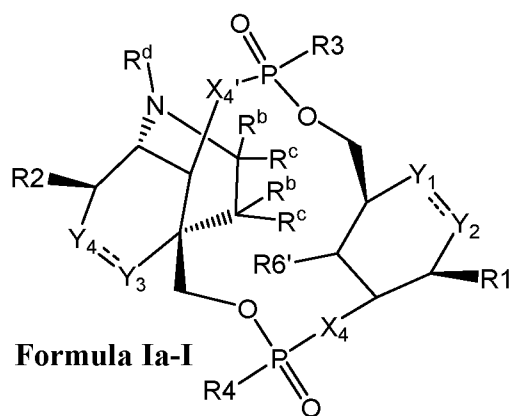
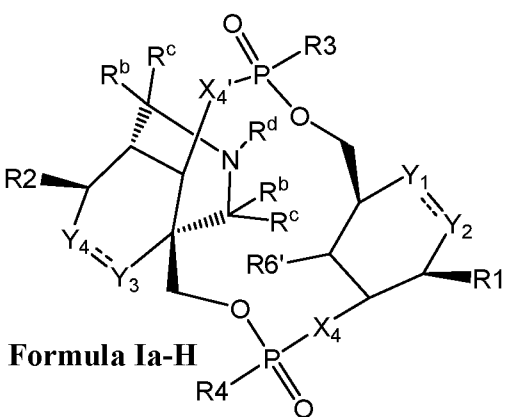
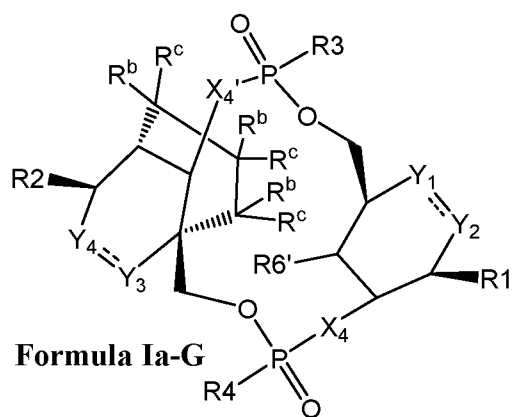
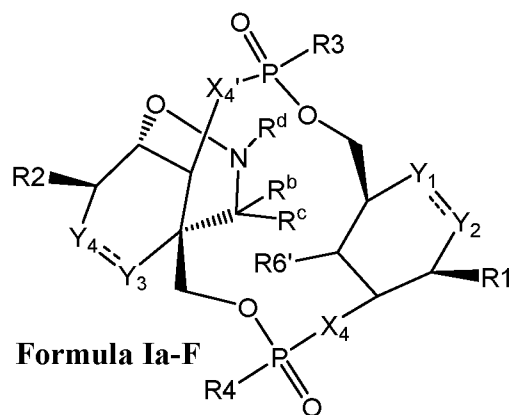
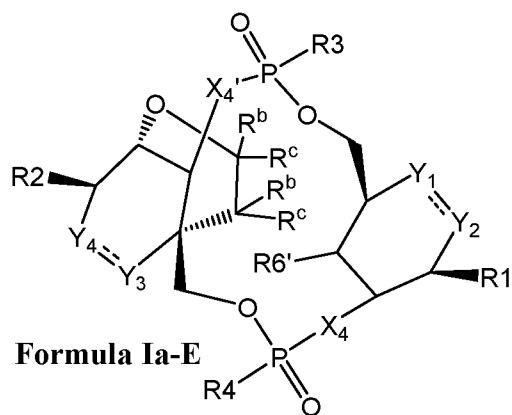
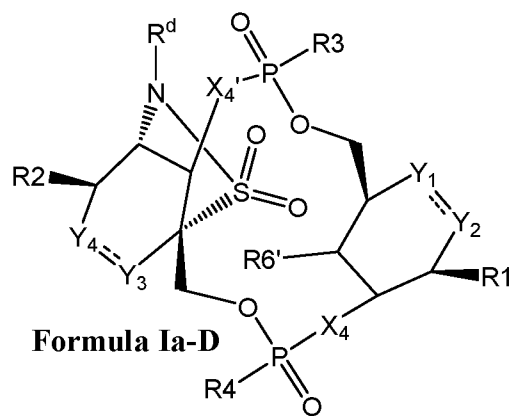
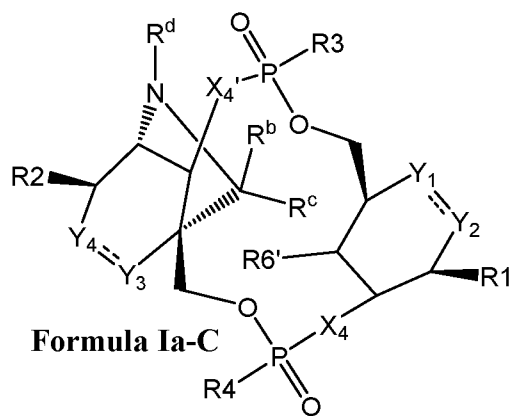
Formula Id:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R5' and R6' are selected from the group consisting of -H, -OH, halogen and -OR^e, and R1, R2, R3, R4, R^e, Y1, Y2, Y3, Y4, X1, X2, X3, X4, X4', X5, X6 and X7 are as defined for Formula I. In some embodiments, R5' and R6' are selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl. In some embodiments, R5' and R6' are selected from the group consisting of -H, -OH, -F and -O-(C=O)-C₁₋₁₄alkyl. In some embodiments, R5' and R6' are -H, -OH or -F. In some embodiments, R5' and R6' are -OH or -F.

[0013] In a fourth embodiment of the first aspect and any of the above embodiments thereof, the compound of Formula I is selected from the group consisting of a compound of Formula Ia-A, a compound of Formula Ia-B, a compound of Formula Ia-C, a compound of Formula Ia-D, a compound of Formula Ia-E, a compound of Formula Ia-F, a compound of Formula Ia-G, a compound of Formula Ia-H, a compound of Formula Ia-I, a compound of Formula Ia-J, a compound of Formula Ia-K, a compound of Formula Ia-L, a compound of Formula Ia-M, a compound of Formula Ia-N, a compound of Formula Ib-A, a compound of Formula Ib-B, a compound of Formula Ib-C, a compound of Formula Ib-D, a compound of Formula Ib-E, a compound of Formula Ib-F, a compound of Formula Ib-G, a compound of Formula Ib-H, a compound of Formula Ib-I, a compound of Formula Ib-J, a compound of Formula Ib-K, a compound of Formula Ib-L, a compound of Formula Ib-M, a compound of Formula Ib-N, a compound of Formula Ic-A, a compound of Formula Ic-B, a compound of Formula Ic-C, a compound of Formula Ic-D, a compound of Formula Ic-E, a compound of Formula Ic-F, a compound of Formula Ic-G, a compound of Formula Ic-H, a compound of Formula Ic-I, a compound of Formula Ic-J, a compound of Formula Ic-K, a compound of Formula Ic-L, a compound of Formula Ic-M, a compound of Formula Ic-N, a compound of Formula Id-A, a compound of Formula Id-B, a compound of Formula Id-C, a compound of Formula Id-D, a compound of Formula Id-E, a compound of Formula Id-F, a compound of Formula Id-G, a compound of Formula Id-H, a compound of Formula Id-I, a compound of Formula Id-J, a compound of Formula Id-K, a compound of Formula Id-L, a compound of Formula Id-M and a compound of Formula Id-N:





Formula Ia-K

Formula Ia-L

The chemical structure of Formula Ia-M is a macrocycle consisting of two fused six-membered rings. The left ring contains a nitrogen atom (N) with a substituent R^d and a carbon atom bonded to R² and Y₄. The right ring contains a carbon atom bonded to R¹ and Y₂. The two rings are connected by a bridge containing an oxygen atom (O) and a carbon atom bonded to R^b and R^c. Two phosphonate groups are attached to the macrocycle via phosphorus atoms (P). The top phosphorus atom is bonded to R³ and X₄' and is connected to the left ring via an oxygen atom (O). The bottom phosphorus atom is bonded to R⁴ and X₄ and is connected to the right ring via an oxygen atom (O). The substituents Y₁, Y₂, Y₃, and Y₄ are part of the ring system, with Y₁ and Y₂ connected by a double bond, and Y₃ and Y₄ connected by a double bond. The phosphorus atoms are also bonded to oxygen atoms (O).

Formula Ia-M

Formula Ia-N

The chemical structure of Formula Ib-A is a macrocycle consisting of two fused six-membered rings. The left ring has a dashed bond to an oxygen atom at the top, a wedge bond to a substituent R² on the left, and a dashed bond to a substituent Y₄ at the bottom. The right ring has a wedge bond to a substituent Y₁ at the top, a dashed bond to a substituent Y₂ on the right, and a wedge bond to a substituent R¹ at the bottom. The two rings are connected by two phosphate groups. The top phosphate group is bonded to the left ring at X₄' and to the right ring at X₄. It has a double bond to an oxygen atom at the top and single bonds to substituents R³ and R^b. The bottom phosphate group is bonded to the left ring at X₄ and to the right ring at X₄. It has a double bond to an oxygen atom at the bottom and single bonds to substituents R⁴ and R^c. The substituents Y₃ and Y₄ are connected by a double bond, and Y₁ and Y₂ are connected by a dashed bond.

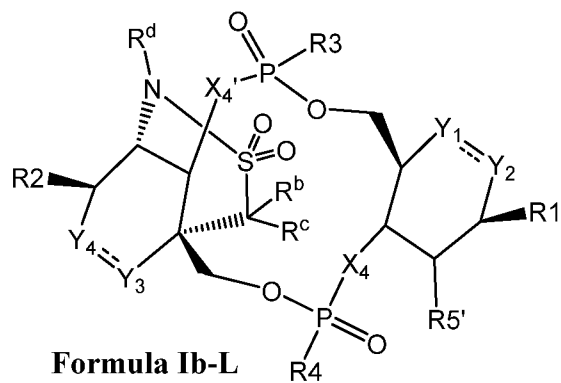
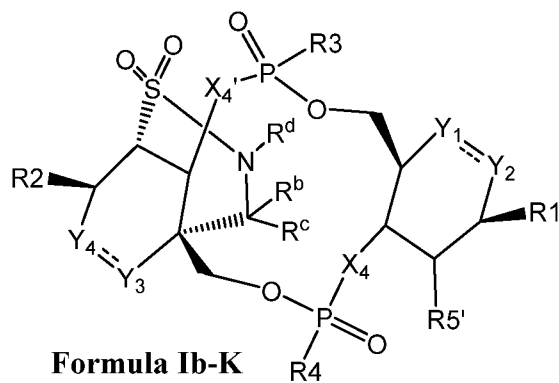
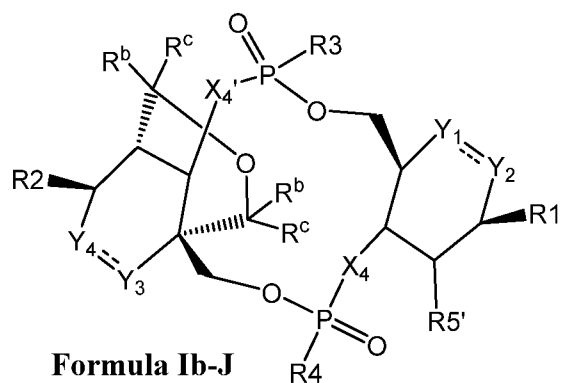
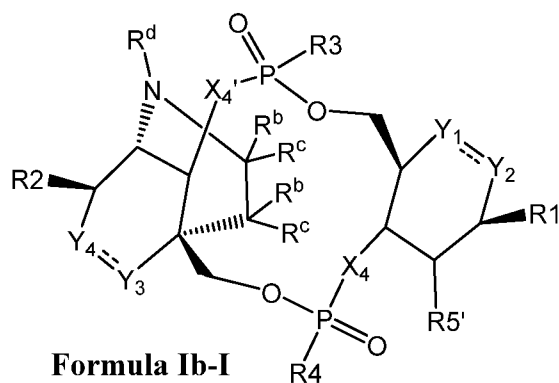
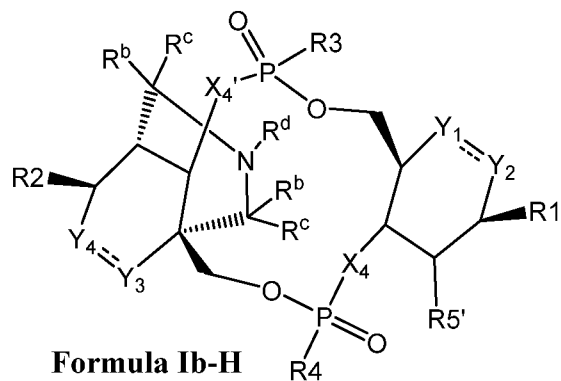
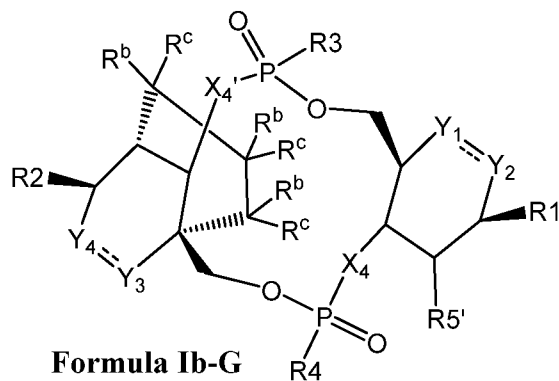
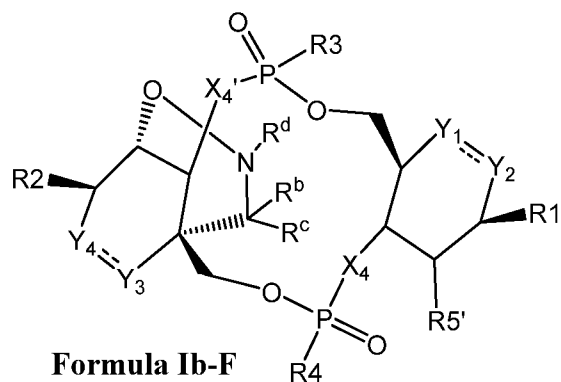
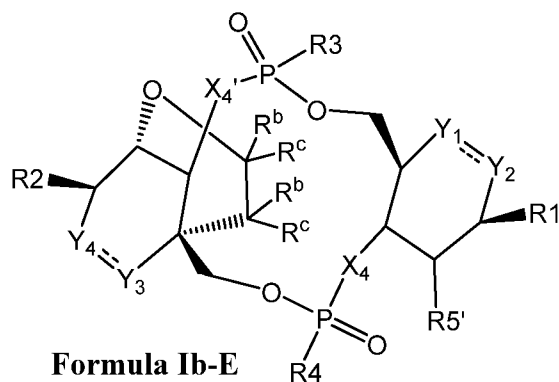
Formula Ib-A

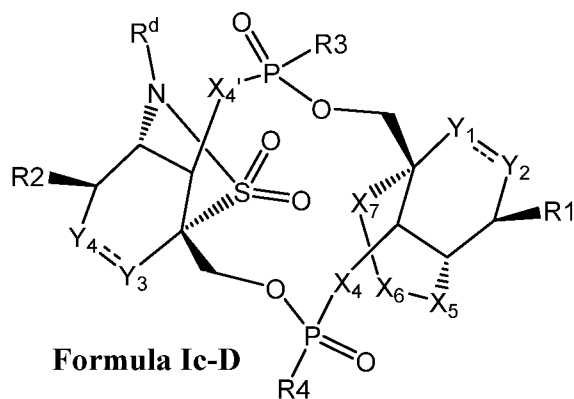
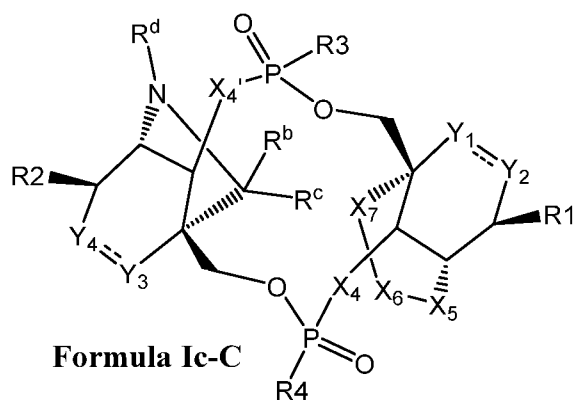
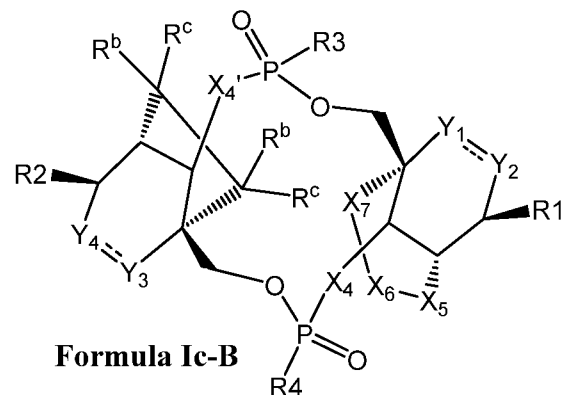
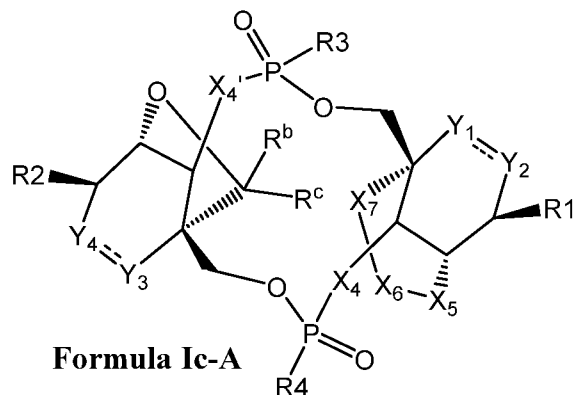
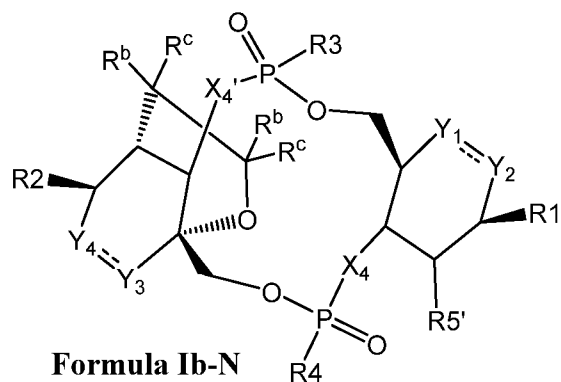
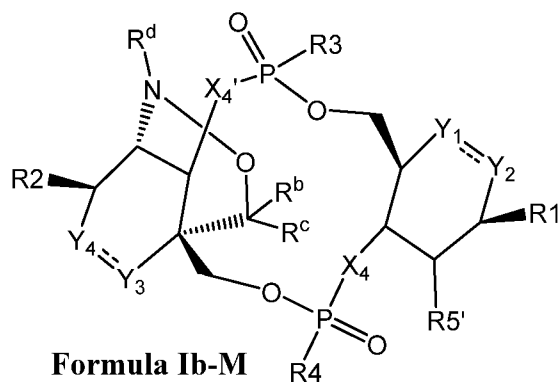
Formula Ib-B

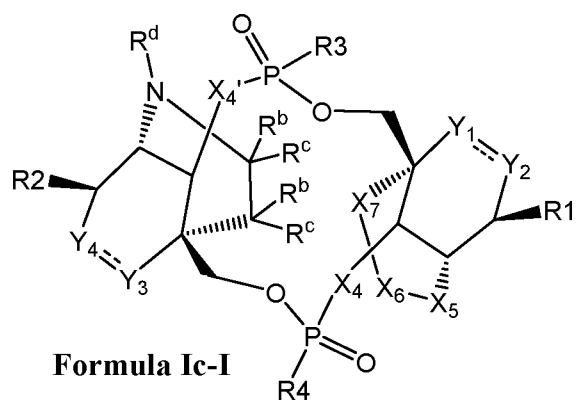
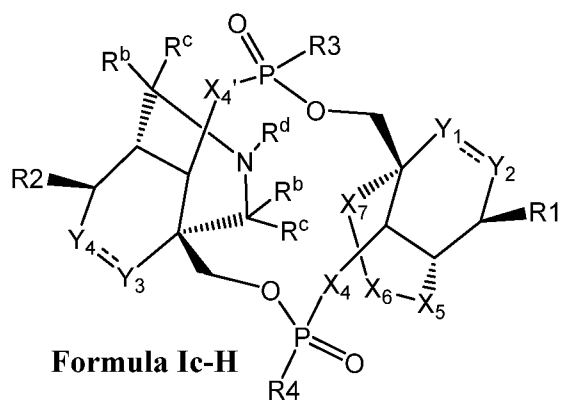
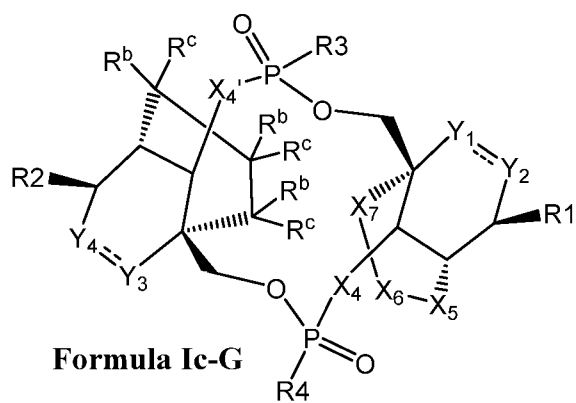
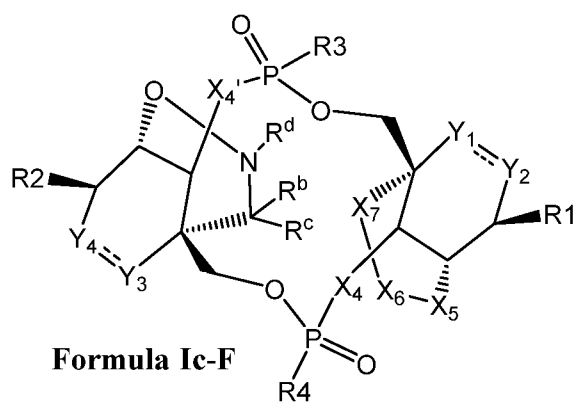
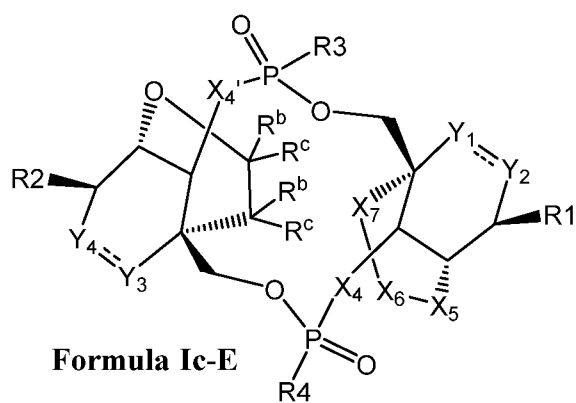
Formula Ib-C

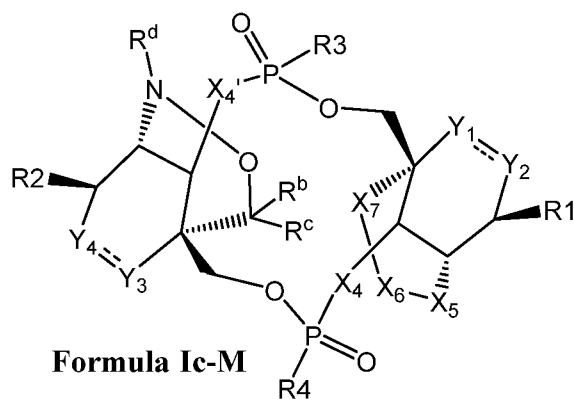
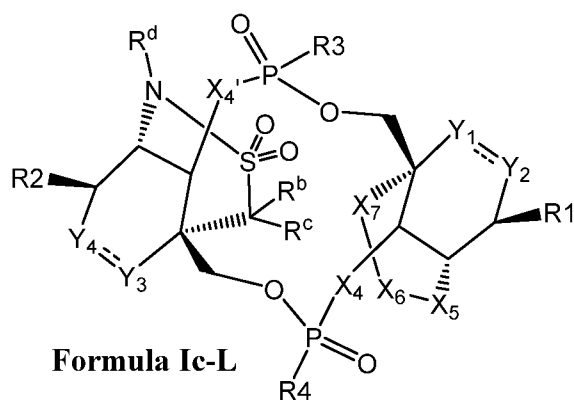
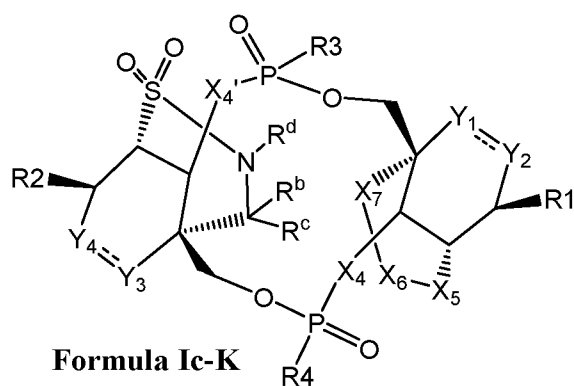
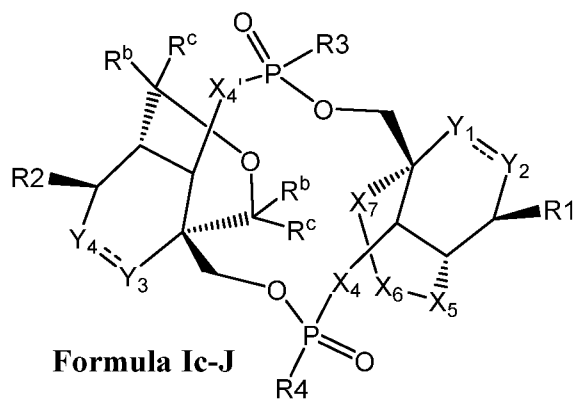
The structure shows a bicyclic system with two phosphate groups. The left ring is a cyclohexane derivative with substituents R², Y₄, Y₃, and a nitrogen atom bonded to R^d. The right ring is a cyclohexane derivative with substituents Y₁, Y₂, R¹, and R^{5'}. Two phosphate groups are attached via oxygen atoms to the rings, with phosphorus atoms bonded to R³, R^b, R^c, and R⁴. Stereochemistry is indicated with wedges and dashes.

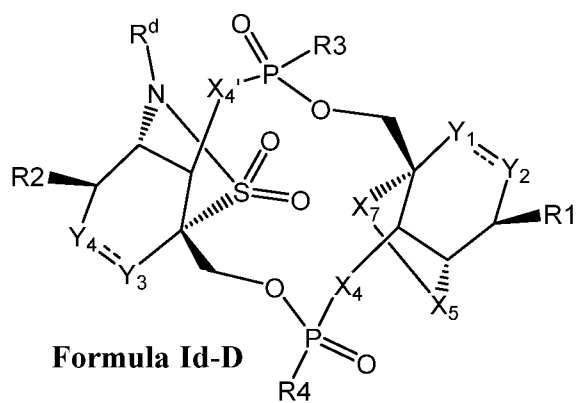
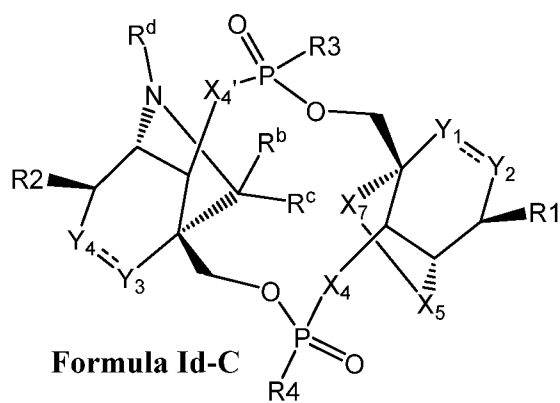
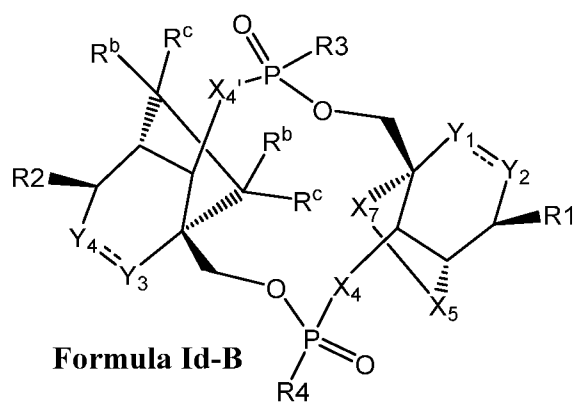
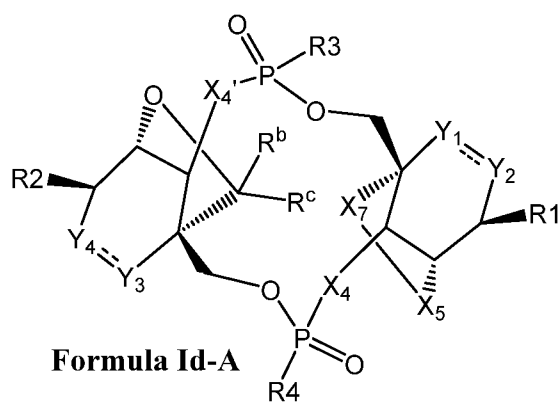
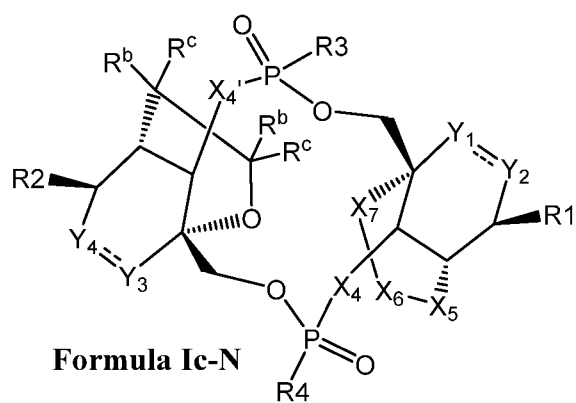
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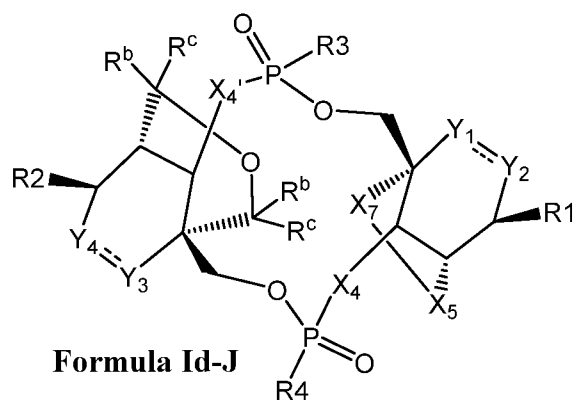
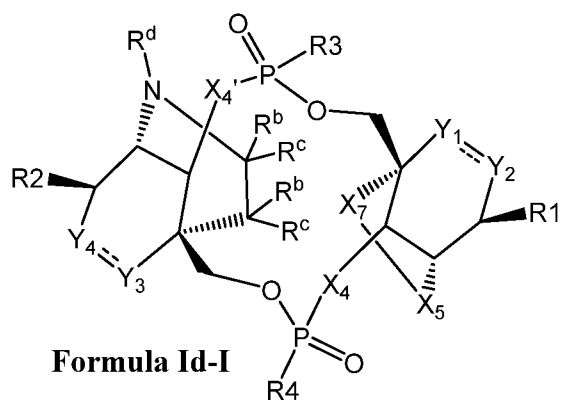
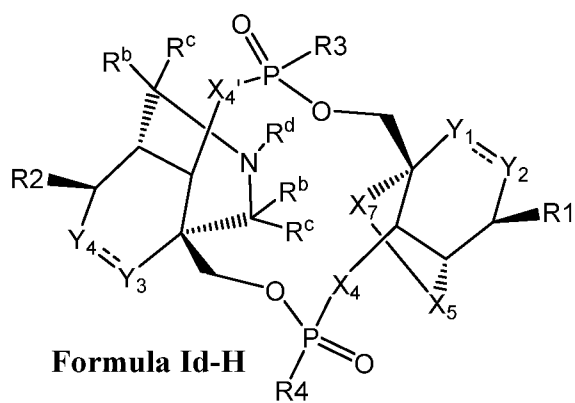
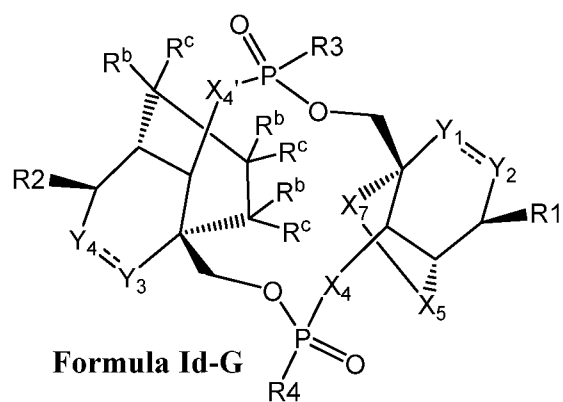
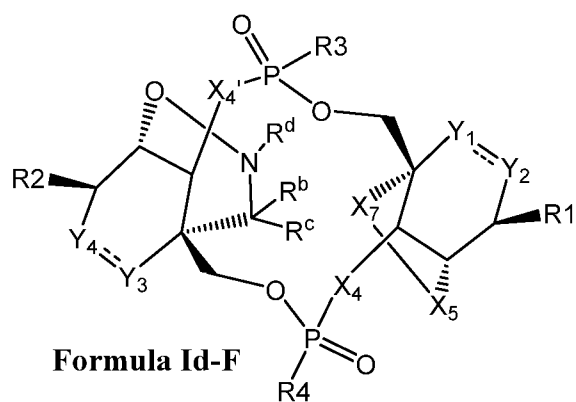
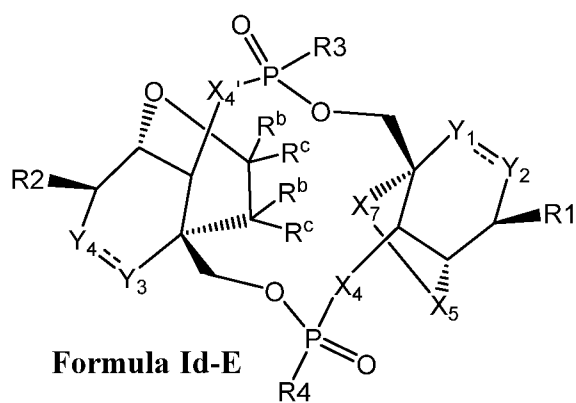


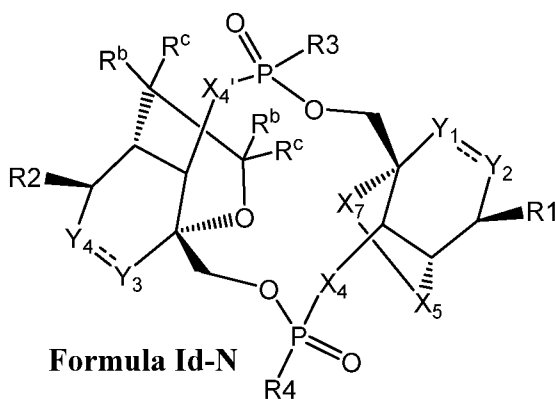
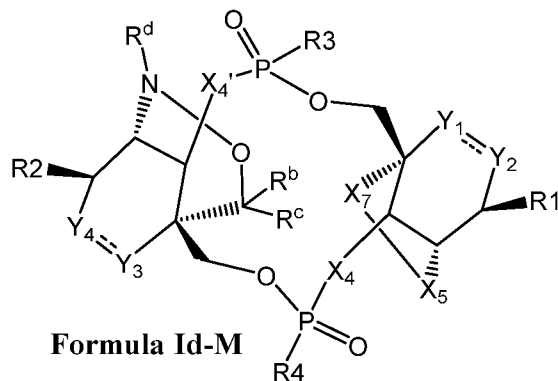
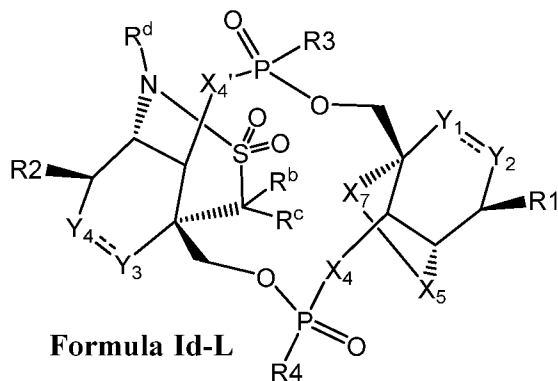
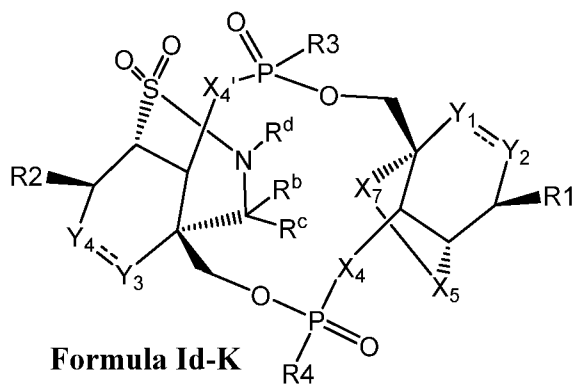










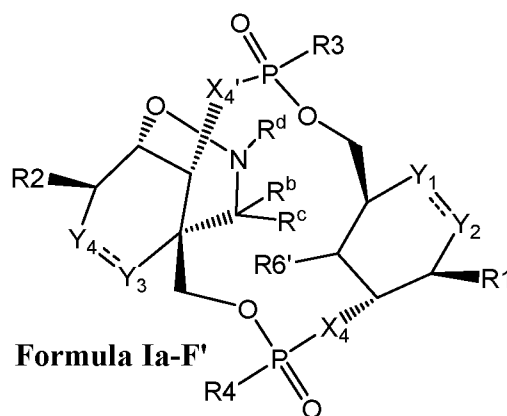
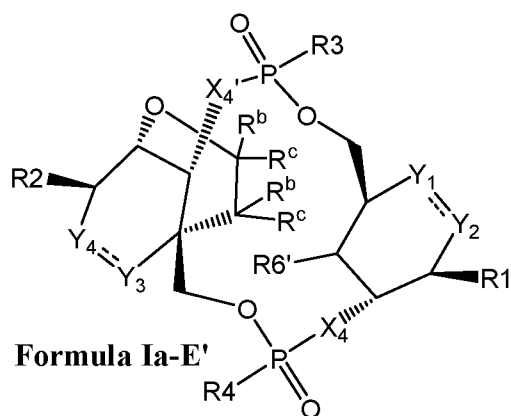
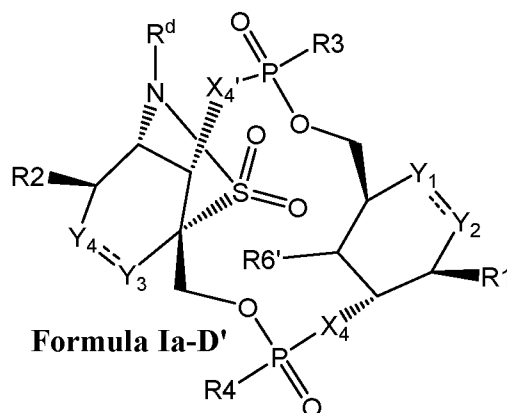
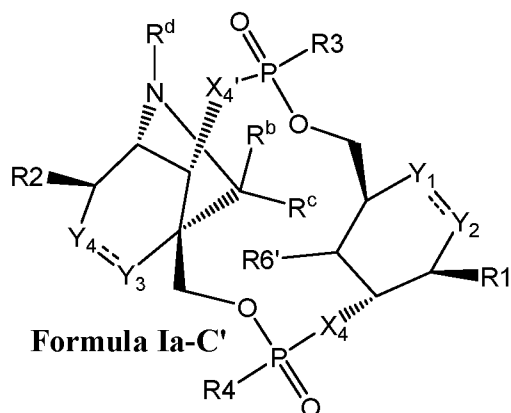
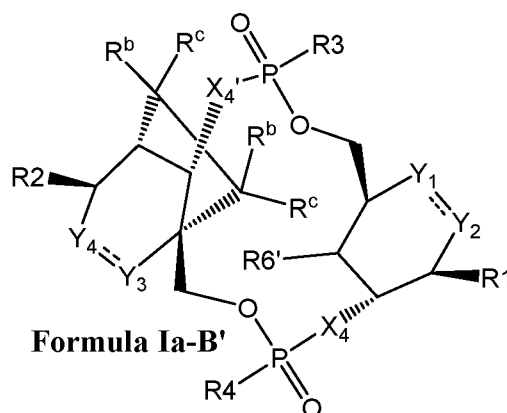
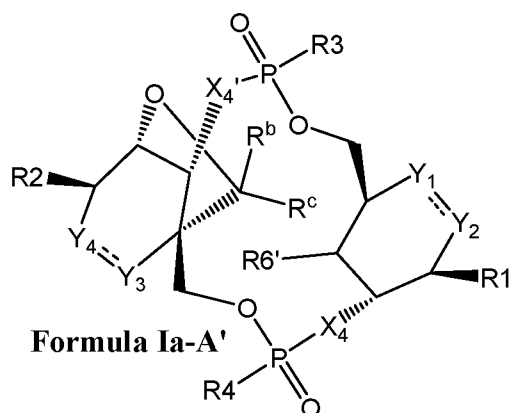


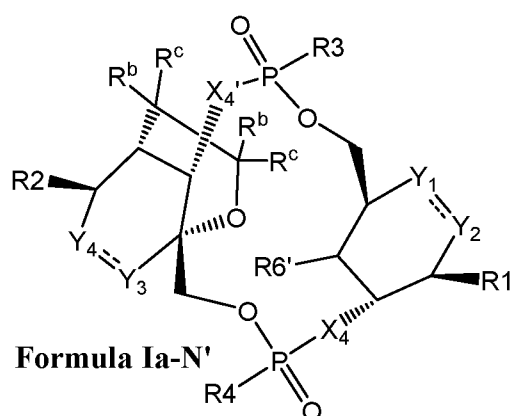
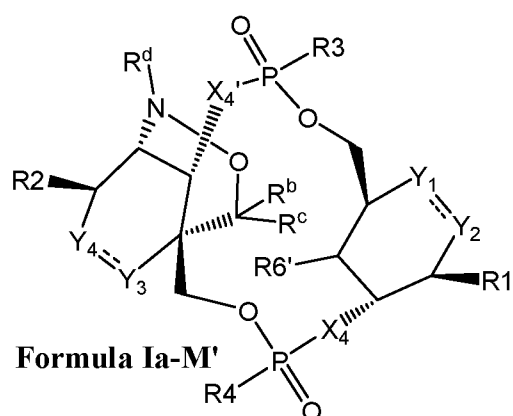
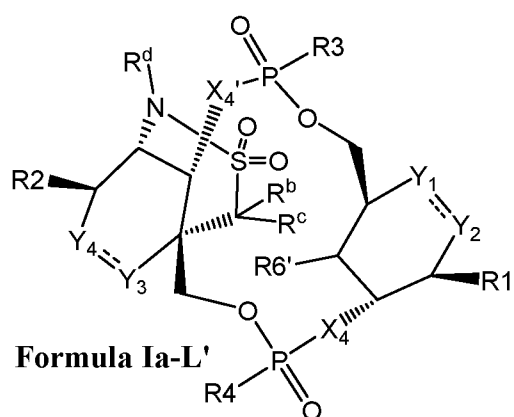
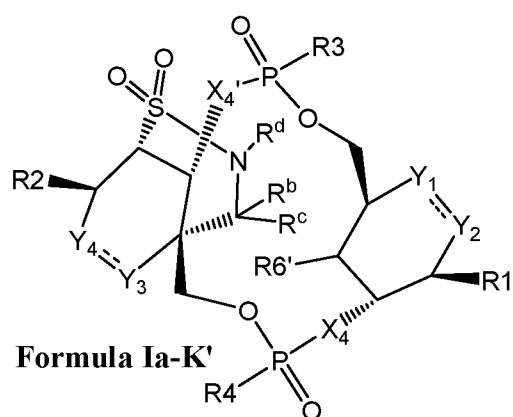
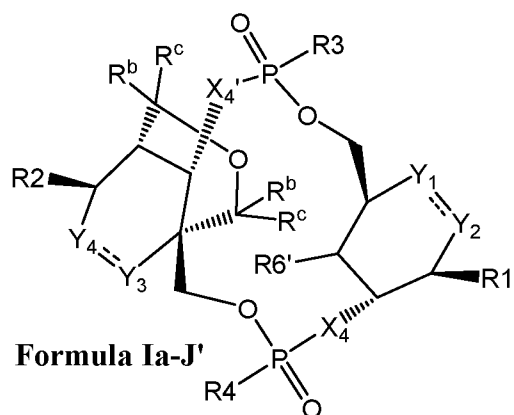
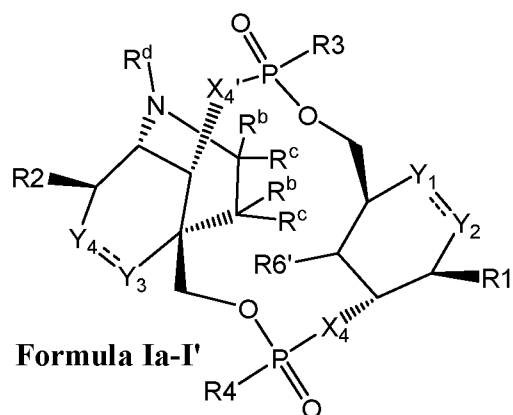
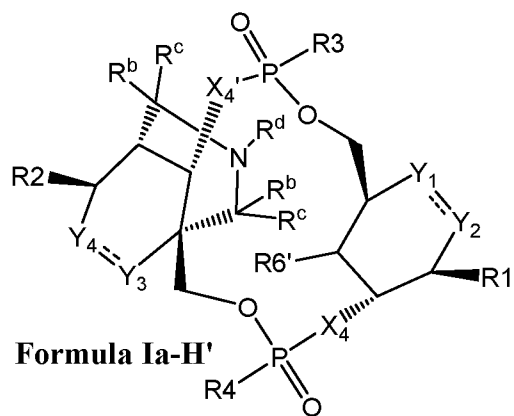
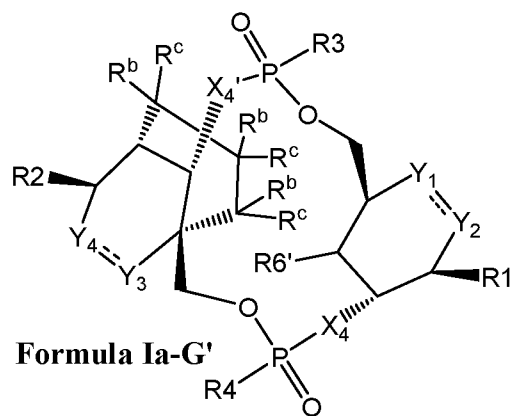
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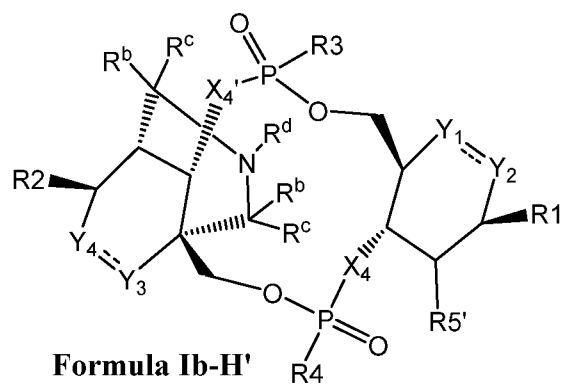
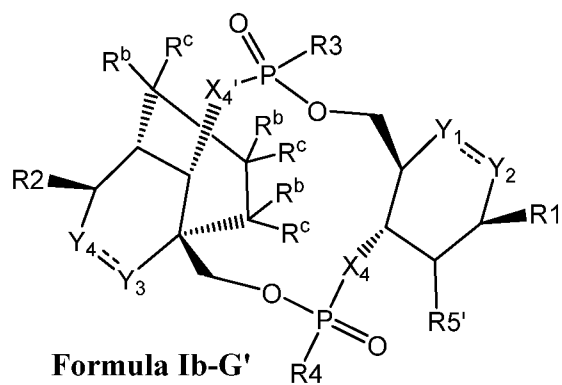
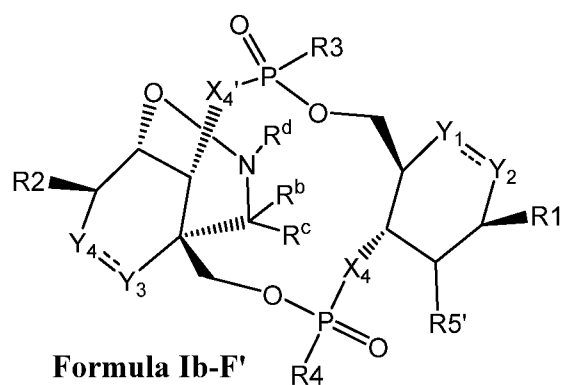
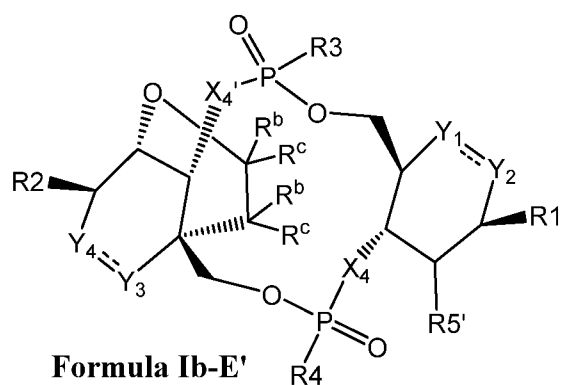
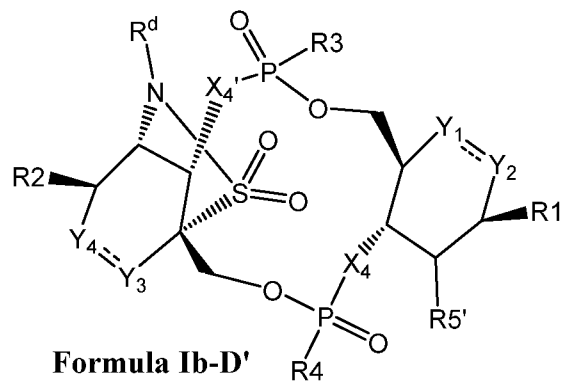
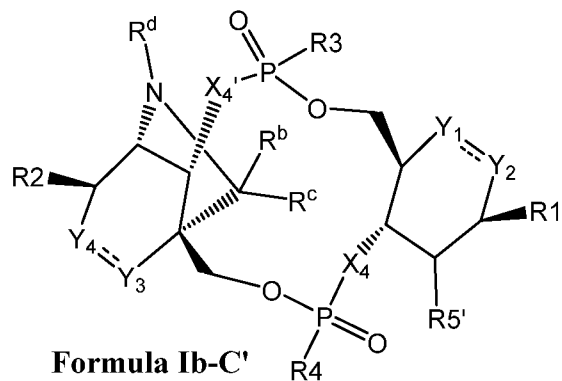
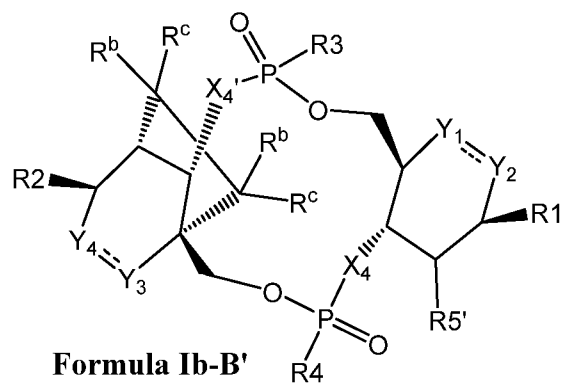
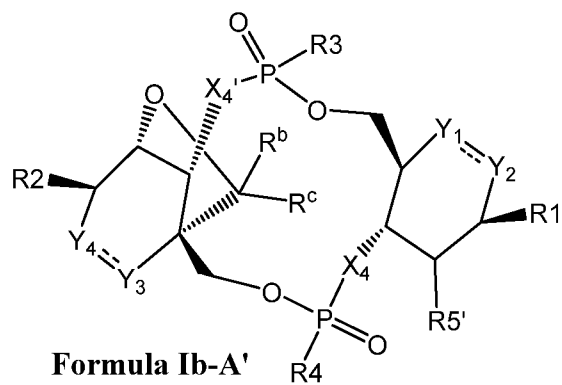
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R5' and R6' are selected from the group consisting of -H, -OH, halogen and -OR^e, and R1, R2, R3, R4, R^b, R^c, R^d, R^e, Y1, Y2, Y3, Y4, X1, X2, X3, X4, X4', X5, X6 and X7 are as defined for Formula I. In some embodiments, R5' and R6' are selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl. In some embodiments, R5' and R6' are selected from the group consisting of -H, -OH, -F and -O-(C=O)-C₁₋₁₄alkyl. In some embodiments, R5' and R6' are -H, -OH or -F. In some embodiments, R5' and R6' are -OH or -F.

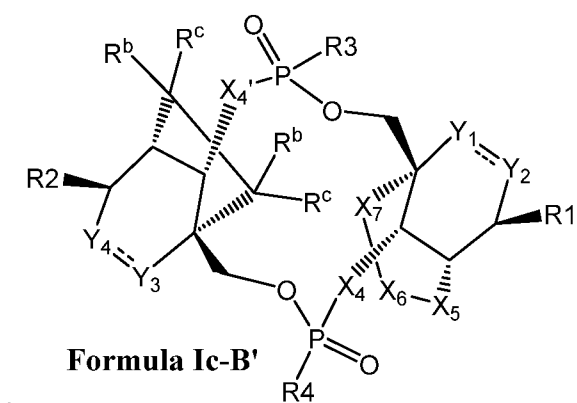
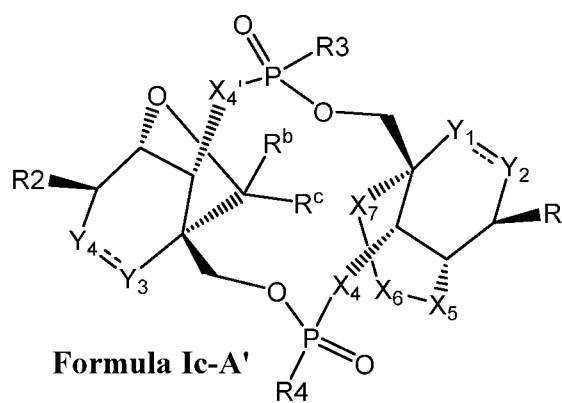
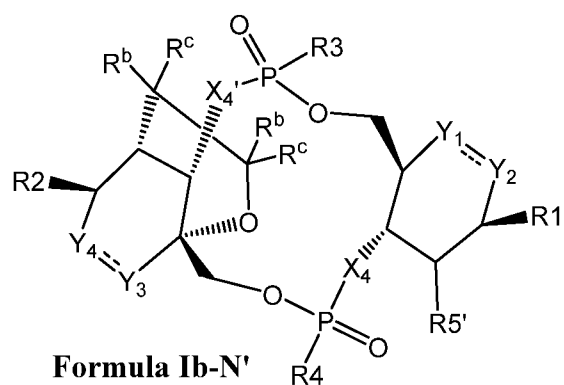
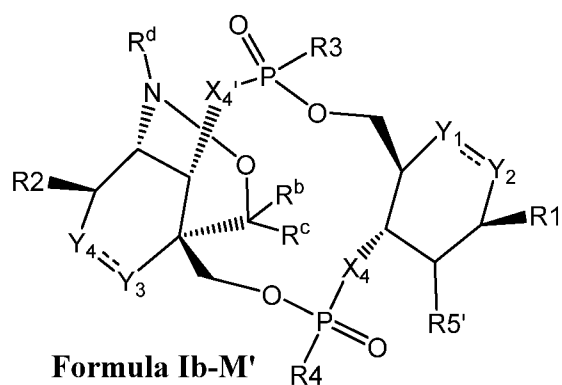
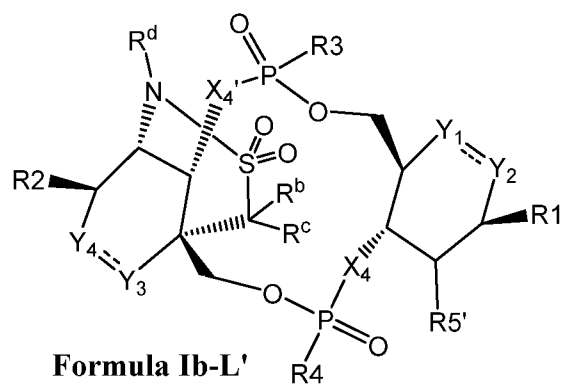
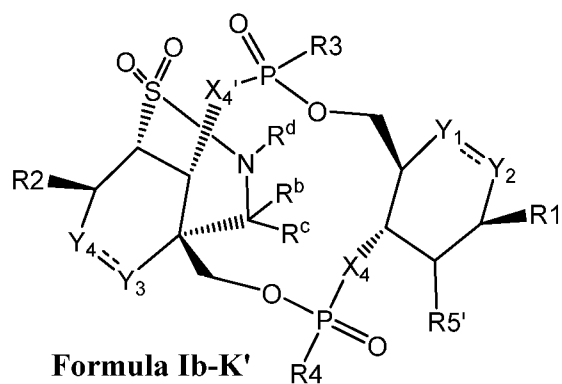
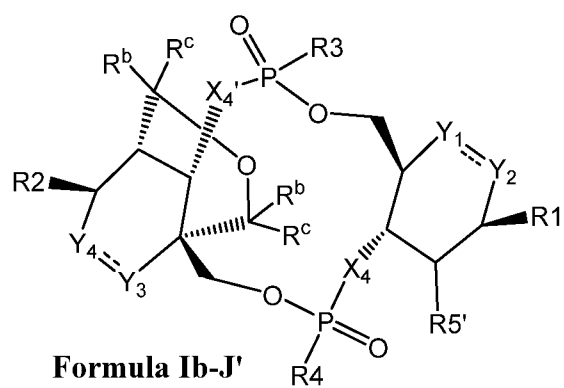
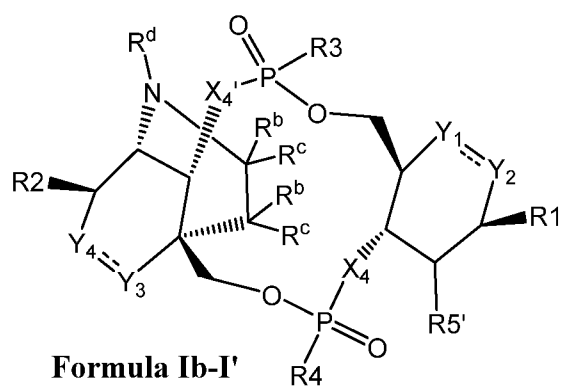
[0014] In some embodiments, the compound of Formula Ia-A is a compound of Formula Ia-A', the compound of Formula Ia-B is a compound of Formula Ia-B', the compound of Formula Ia-C is a compound of Formula Ia-C', the compound of Formula Ia-D is a compound of Formula Ia-D', the compound of Formula Ia-E is a compound of Formula Ia-E', the compound of Formula Ia-F is a compound of Formula Ia-F', the compound of Formula Ia-G is a compound of Formula Ia-G', the compound of Formula Ia-H is a compound of Formula Ia-H', the compound of Formula Ia-I is a compound of Formula Ia-I', the compound of Formula Ia-J is a compound of Formula Ia-J', the compound of Formula Ia-K is a compound of Formula Ia-K', the compound of Formula Ia-L is a compound of Formula Ia-L', the compound of Formula Ia-M is a compound of Formula Ia-M', the compound of Formula Ia-N is a compound of Formula Ia-N', the compound of Formula Ib-A is a compound of Formula Ib-A', the compound of Formula Ib-B is a compound of Formula Ib-B', the compound of Formula Ib-C is a compound of Formula Ib-C', the compound of Formula Ib-D is a compound of Formula Ib-D', the compound of Formula Ib-E is a compound of Formula Ib-E', the compound of Formula Ib-F is a compound of Formula Ib-F', the compound of Formula Ib-G is a compound of Formula Ib-G', the compound of Formula Ib-H is a compound of Formula Ib-H', the compound of Formula Ib-I is a compound of Formula Ib-I', the compound of Formula Ib-J is a compound of Formula Ib-J', the compound of Formula Ib-K is a compound of Formula Ib-K', the compound of Formula Ib-L is a compound of Formula Ib-L', the compound of Formula Ib-M is a compound of Formula Ib-M', the compound of Formula Ib-N is a compound of Formula Ib-N', the compound of Formula Ic-A is a compound of Formula Ic-A', the compound of Formula Ic-B is a compound of Formula Ic-B', the compound of Formula Ic-C is a compound of Formula Ic-C', the compound of Formula Ic-D is a compound of Formula Ic-D', the compound of Formula Ic-E is a compound of Formula Ic-E', the compound of Formula Ic-F is a compound of Formula Ic-F', the compound of Formula Ic-G is a compound of Formula Ic-G', the compound of Formula Ic-H is a compound of Formula Ic-H', the compound of Formula Ic-I is a compound of Formula Ic-I', the compound of Formula Ic-J is a compound of Formula Ic-J', the compound of Formula Ic-K is a compound of Formula Ic-K', the compound of Formula Ic-L is a compound of Formula Ic-L', the compound of Formula Ic-M is a compound of Formula Ic-M', the compound of Formula Ic-N is a compound of Formula Ic-N', the compound of Formula Id-A is a compound of Formula Id-A', the compound of Formula Id-B is a compound of Formula Id-B', the compound of Formula Id-C is a compound of

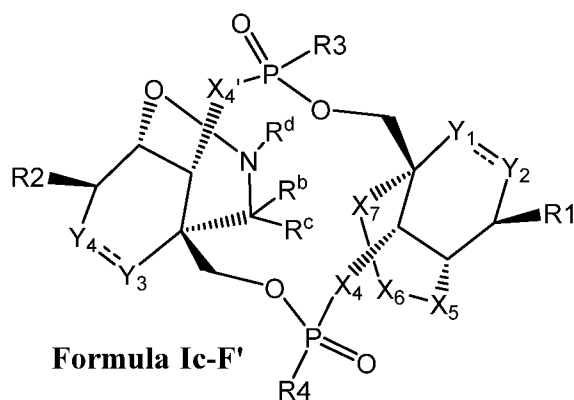
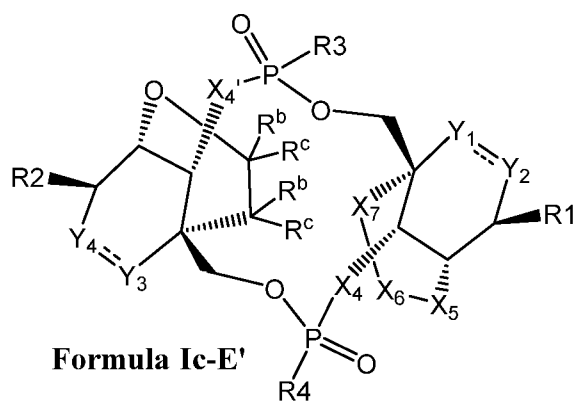
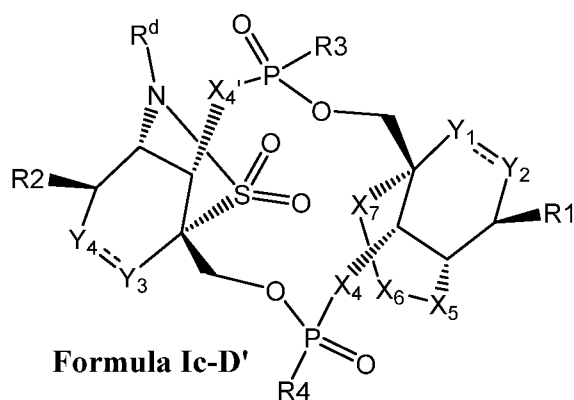
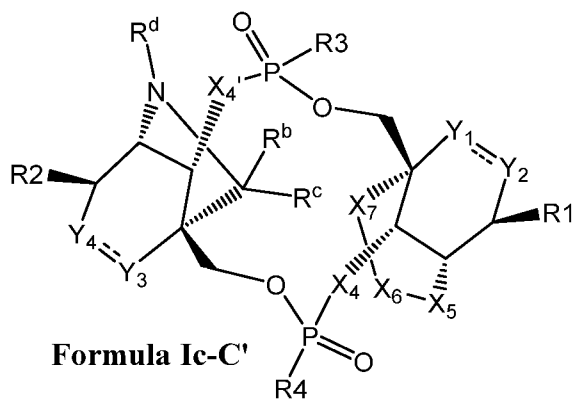
Formula Id-C', the compound of Formula Id-D is a compound of Formula Id-D', the compound of Formula Id-E is a compound of Formula Id-E', the compound of Formula Id-F is a compound of Formula Id-F', the compound of Formula Id-G is a compound of Formula Id-G', the compound of Formula Id-H is a compound of Formula Id-H', the compound of Formula Id-I is a compound of Formula Id-I', the compound of Formula Id-J is a compound of Formula Id-J', the compound of Formula Id-K is a compound of Formula Id-K', the compound of Formula Id-L is a compound of Formula Id-L', the compound of Formula Id-M is a compound of Formula Id-M' and the compound of Formula Id-N is a compound of Formula Id-N':

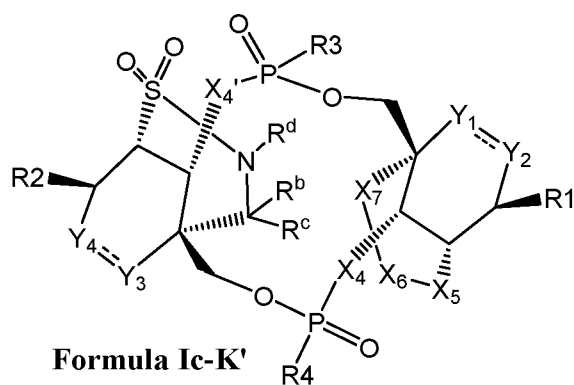
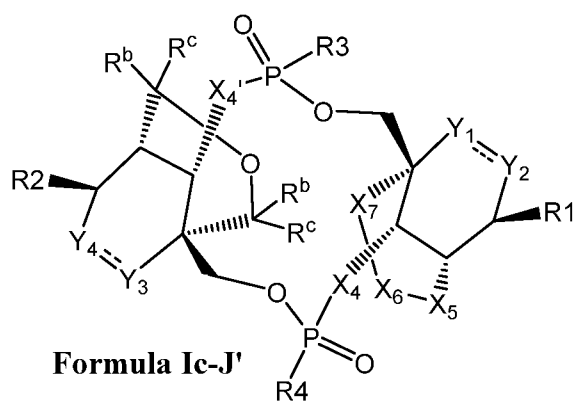
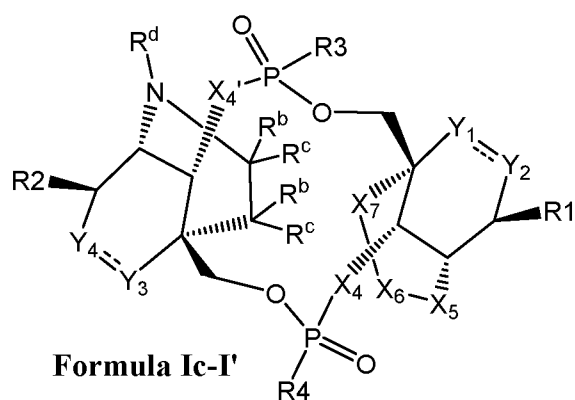
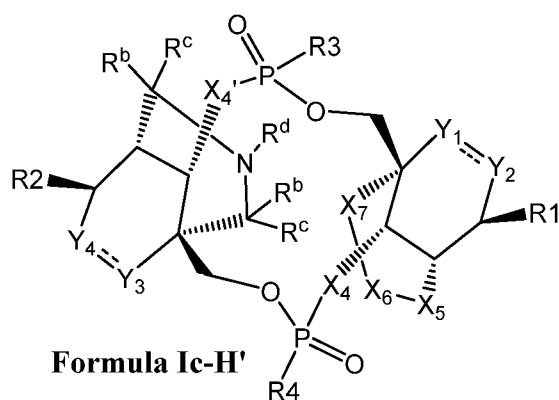
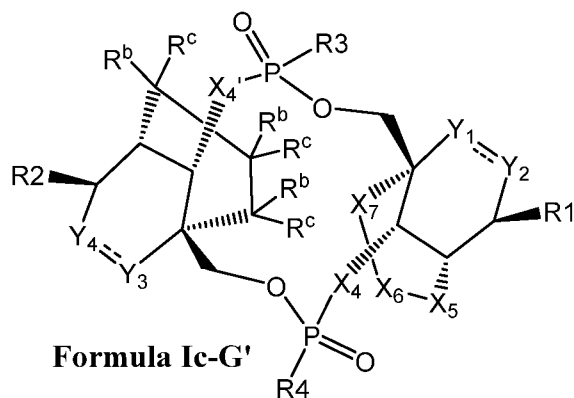


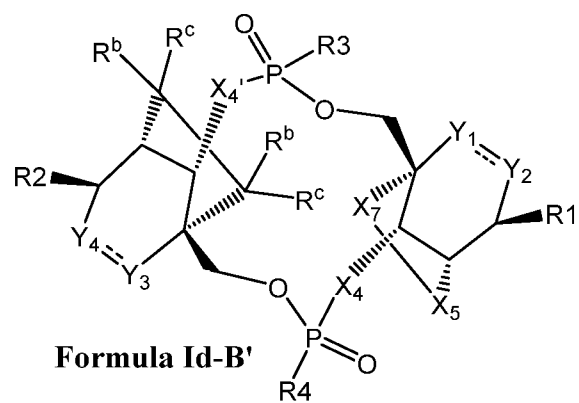
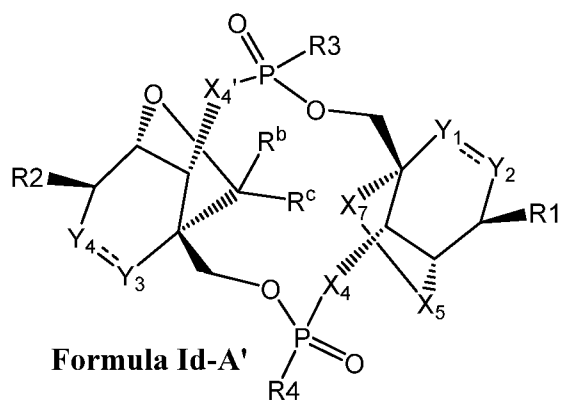
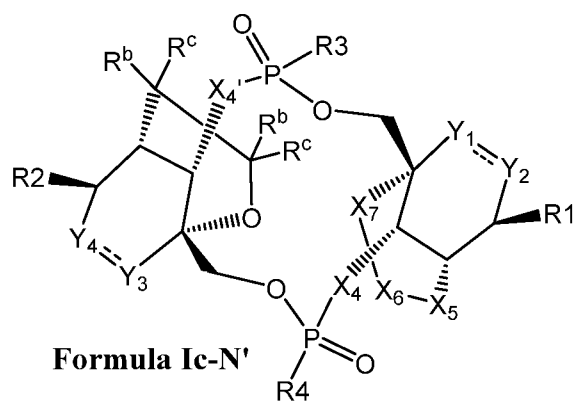
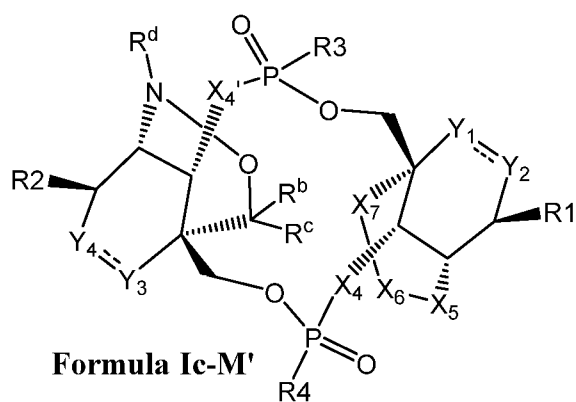
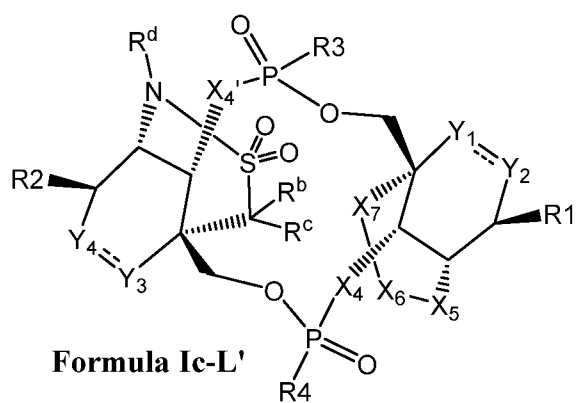


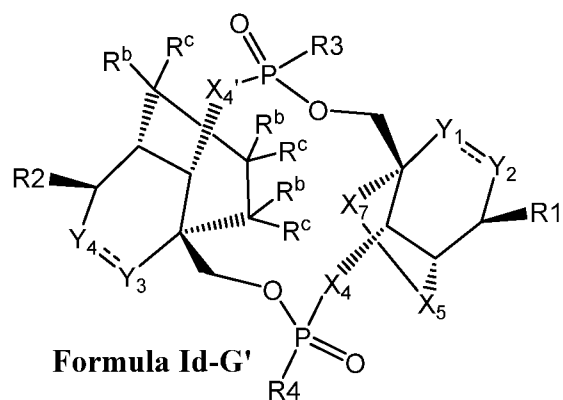
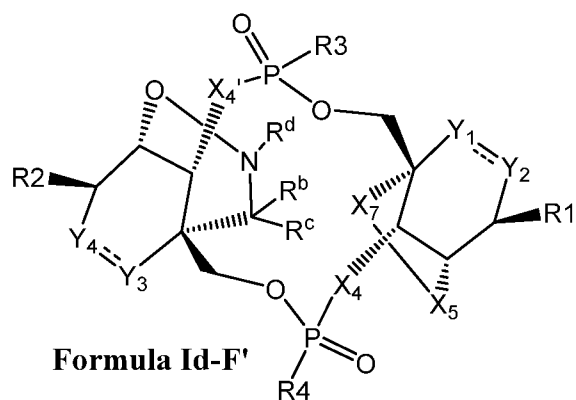
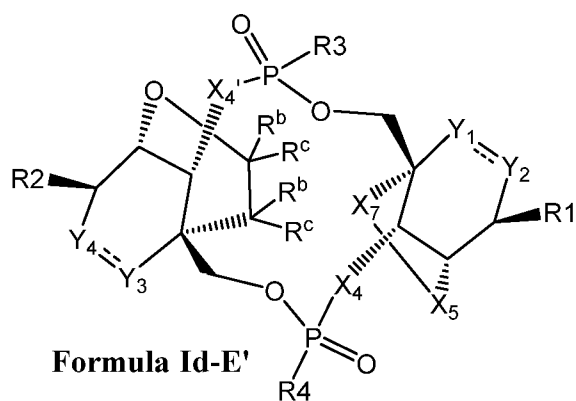
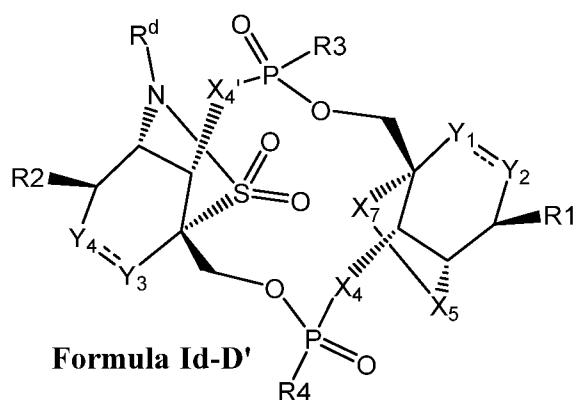
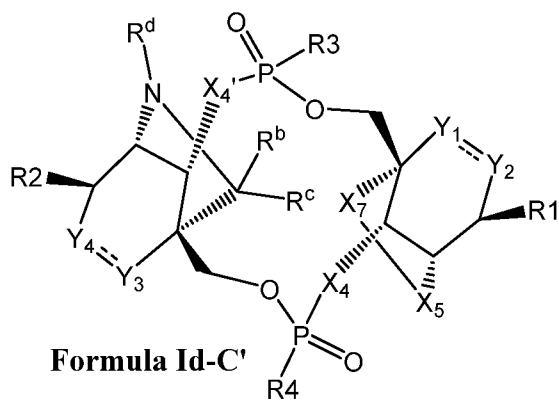


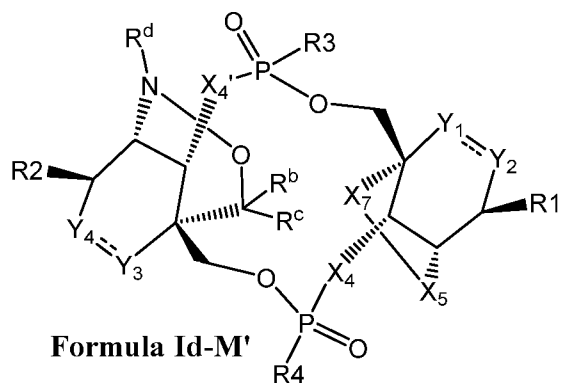
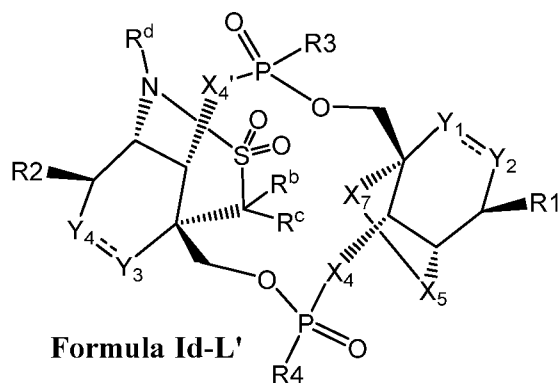
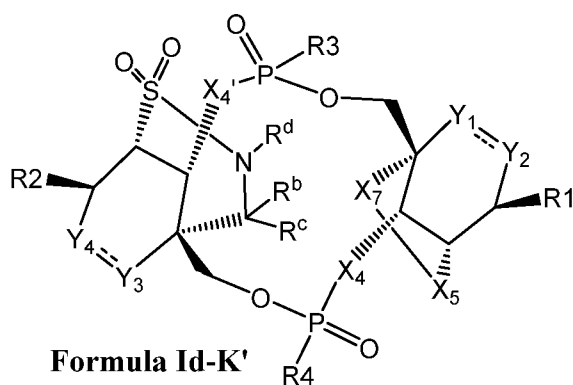
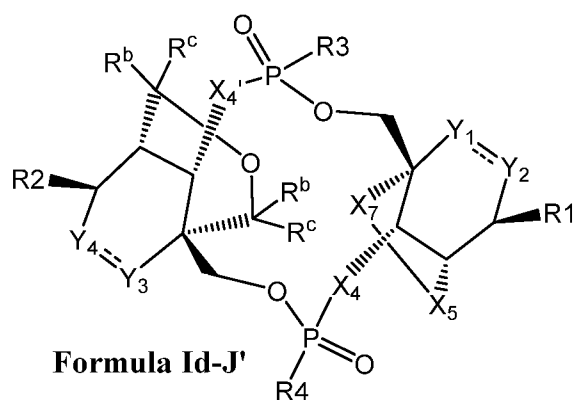
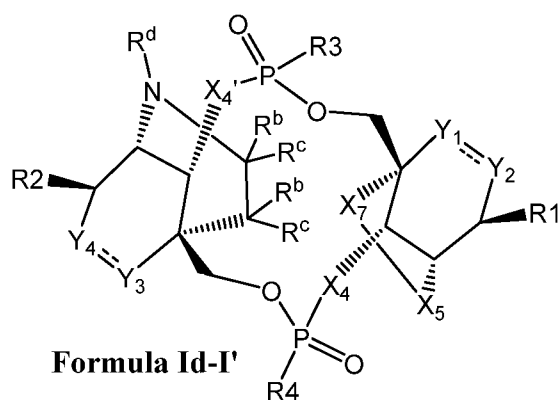
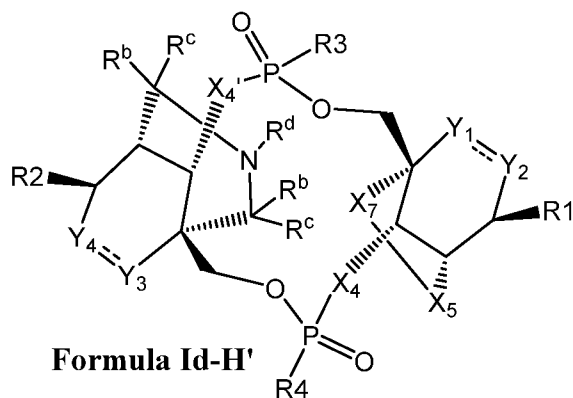


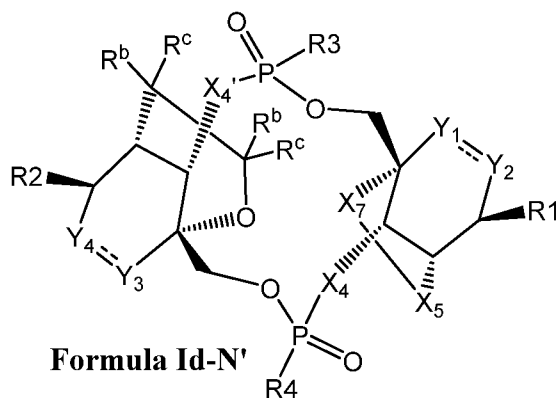












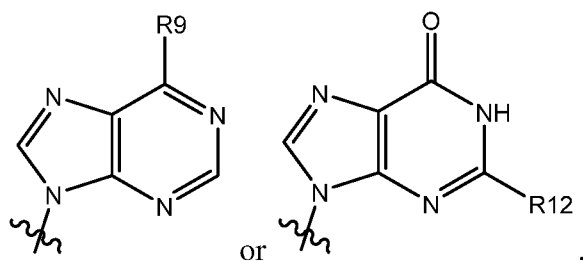
or

or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R^{5'} and R^{6'} are selected from the group consisting of -H, -OH, halogen and -OR^e, and R¹, R², R³, R⁴, R^b, R^c, R^d, R^e, Y₁, Y₂, Y₃, Y₄, X₁, X₂, X₃, X₄, X₄', X₅, X₆ and X₇ are as defined for Formula I. In some embodiments, R^{5'} and R^{6'} are selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl. In some embodiments, R^{5'} and R^{6'} are selected from the group consisting of -H, -OH, -F and -O-(C=O)-C₁₋₁₄alkyl. In some embodiments, R^{5'} and R^{6'} are -H, -OH or -F. In some embodiments, R^{5'} and R^{6'} are -OH or -F. In any of the above embodiments, the bond to R^{5'} or R^{6'} is preferably down into the paper, i.e. in the

above structures is represented as e.g.  R^{5'}, where the * represents the bond to the core ring.

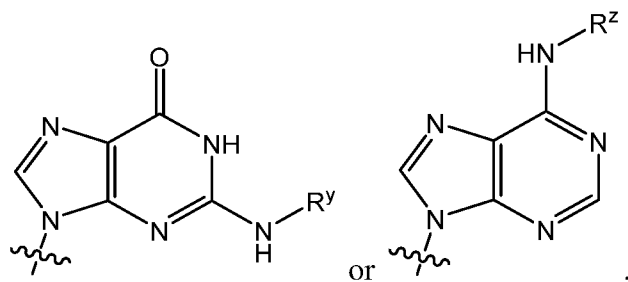
[0015] In a fifth embodiment of the first aspect, and any of the above embodiments thereof, R³ and R⁴ are independently -OH or -SH. In some embodiments, R³ and R⁴ are both -OH. In some embodiments, R³ and R⁴ are both -SH. In some embodiments, one of R³ and R⁴ is -OH and the other is -SH. In some embodiments, R³ and R⁴ are both -SH, wherein with reference to the stereochemistry at the phosphorus atoms, the compound is the substantially pure R_p,R_p isomer, the substantially pure the R_p,S_p isomer, the substantially pure S_p,R_p isomer, or the substantially pure S_p,S_p isomer.

[0016] In a sixth embodiment of the first aspect, and any of the above embodiments thereof, R¹ and R² are independently



wherein  indicates the bond of R1 or R2 to the ring as shown in Formula I and wherein R9 and R12 are independently -NH₂ or -NH-C₁₋₆alkyl, wherein -NH₂ is optionally substituted with a suitable nitrogen protecting group. In some embodiments, R9 and R12 are independently -NH₂, -NH-C₁₋₆alkyl, -N=CH-N(CH₃)₂, -NH-C(=O)-C₁₋₆alkyl or -NH-C(=O)-phenyl. In some embodiments, R9 is -NH₂ or -NH-C(=O)-phenyl and R12 is -NH₂ or -NH-C(=O)-isopropyl. In some embodiments R9 and R12 are both -NH₂.

[0017] In a seventh embodiment of the first aspect, and any of the above embodiments thereof, R1 and R2 are independently



wherein  indicates the bond of R1 or R2 to the ring as shown in Formula I and wherein R^y and R^z are independently -H, -C(=O)-C₁₋₆ alkyl, -C(=O)-phenyl or other suitable nitrogen protecting group. In some embodiments R^y and R^z are independently -H, -C(=O)-C₁₋₆alkyl or -C(=O)-phenyl. In some embodiments, R^y is -H or -C(=O)-isopropyl and R^z is -H or -C(=O)-phenyl. In some embodiments, R^y and R^z are both -H.

[0018] In an eighth embodiment of the first aspect, and any of the above embodiments thereof, Y₁ and Y₂, and Y₃ and Y₄, independently, are selected to provide -O-, -O-CH₂- or -CH=CH-. In some embodiments, Y₁ and Y₂, and Y₃ and Y₄, independently, are selected

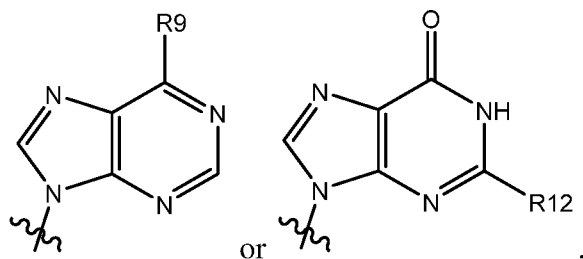
to provide -O- or -O-CH₂-. In some embodiments, Y₁ and Y₃ are O and Y₂ and Y₄ are absent.

[0019] In a ninth embodiment of the first aspect, and any of the above embodiments thereof, the bridging portion, i.e. -X₁-X₂-X₃- or -X₁-X₃-, and when present -X₅-X₆-X₇- or -X₅-X₇-, are independently selected from the group consisting of -CH₂-CH₂-CH₂-; -CH₂-N(R^{d'})-CH₂-, where R^{d'} at each occurrence is independently -H or a suitable nitrogen protecting group; -CH₂-O-CH₂-; -C(R^{b'}R^{c'})-CH₂-O-, where R^{b'} and R^{c'} at each occurrence are independently -H, -CH₃, or together form =CH₂; -O-CH₂-CH₂-; -O-N(R^{d'})-CH₂- or -O-N(R^{d'})-C(=O)-, where R^{d'} at each occurrence is independently -H, -CH₃ or a suitable nitrogen protecting group; -S(=O)₂-N(R^{d'})-CH₂-, where R^{d'} at each occurrence is independently -H or -C₁₋₃alkyl or a suitable nitrogen protecting group; -NH-CH₂-CH₂- or -N(R^{d'})-C(=O)-CH₂-, where R^{d'} at each occurrence is independently -H, -CH₃ or a suitable nitrogen protecting group; -N(R^{d'})-O-CH₂- or -N(R^{d'})-S(=O)₂-CH₂-, where R^{d'} at each occurrence is independently -H or -C₁₋₃alkyl or a suitable nitrogen protecting group; -C(R^{b'}R^{c'})-C(R^{b''}R^{c''})-, where R^{b'} and R^{c'} at each occurrence are independently -H, -F, -NH₂, -CH₃, or together form =CH₂, =CHF or =O, and where R^{b''} and R^{c''} at each occurrence are independently -H, -F, -OH, -OCH₃, -CH₃, -CH₂F, -CHF₂, -CF₃, or together form =CH₂, =CHF or =O; -O-C(R^{b'}R^{c'})-, where R^{b'}, R^{c'} at each occurrence are independently -H, -CH₃, -CH₂F, -CHF₂, -CF₃, or -CH₂OCH₃, or together form =CH₂, or together with the carbon to which they are attached form a 3-6 membered heterocycloalkyl or C₃₋₆cycloalkyl spirocyclic ring; -N(R^{d'})-CH₂-, where R^{d'} at each occurrence is independently -H or -C₁₋₆alkoxy or a suitable nitrogen protecting group, wherein the alkyl chain of C₁₋₆alkoxy is optionally substituted with 1, 2 or 3 -F or with -OCH₃; -N(R^{d'})-C(=O)-, where R^{d'} at each occurrence is independently -H, -CH₃ or a suitable nitrogen protecting group; or -N(R^{d'})-S(=O)₂-, where R^{d'} at each occurrence is independently -H or -C₁₋₃alkyl or a suitable nitrogen protecting group. In some embodiments, the bridging portion is -X₁-X₃- and when present -X₅-X₇-, wherein -X₁-X₃- and -X₅-X₇- are independently -O-C(R^{b'}R^{c'})-, where R^{b'}, R^{c'} at each occurrence are independently -H, -CH₃, -CH₂F, -CHF₂, -CF₃, or -CH₂OCH₃, or together form =CH₂, or together with the carbon to which they are attached form a cyclopropyl spirocyclic ring, preferably wherein -X₁-X₃- and when present -X₅-X₇- are -O-CH₂-.

[0020] In a tenth embodiment of the first aspect, and any of the above embodiments thereof, X_4' and X_4 are both -O-.

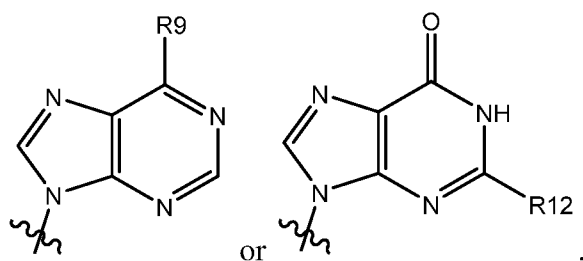
[0021] In an eleventh embodiment of the first aspect, and any of the above embodiments thereof, X_4 and X_4' are -O-, R_3 and R_4 are independently -OH or -SH, Y_1 and Y_3 are O and Y_2 and Y_4 are absent. In some embodiments, X_4 and X_4' are -O-, one of R_3 and R_4 is -OH and the other of R_3 and R_4 is -SH, Y_1 and Y_3 are O and Y_2 and Y_4 are absent. In some embodiments, X_4 and X_4' are -O-, R_3 and R_4 are both -OH, Y_1 and Y_3 are O and Y_2 and Y_4 are absent. In some embodiments, X_4 and X_4' are -O-, R_3 and R_4 are both -SH, Y_1 and Y_3 are O and Y_2 and Y_4 are absent. In some embodiments, X_4 and X_4' are -O-, R_3 and R_4 are both -SH, wherein with reference to the stereochemistry at the phosphorus atoms, the compound is the substantially pure Rp,Rp isomer, the substantially pure Rp,Sp isomer, the substantially pure Sp,Rp isomer, or the substantially pure Sp,Sp isomer, Y_1 and Y_3 are O and Y_2 and Y_4 are absent.

[0022] In a twelfth embodiment of the first aspect, and any of the above embodiments thereof, X_4 and X_4' are -O-, R_3 and R_4 are independently -OH or -SH, Y_1 and Y_3 are O, Y_2 and Y_4 are absent, and R_1 and R_2 are independently

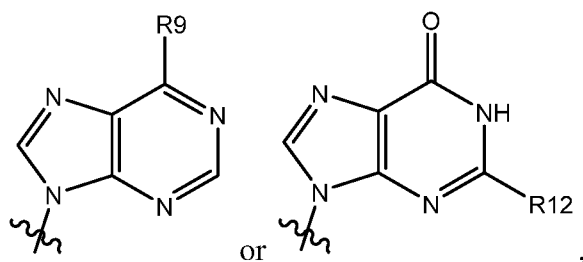


wherein  indicates the bond of R_1 or R_2 to the ring as shown in Formula I and

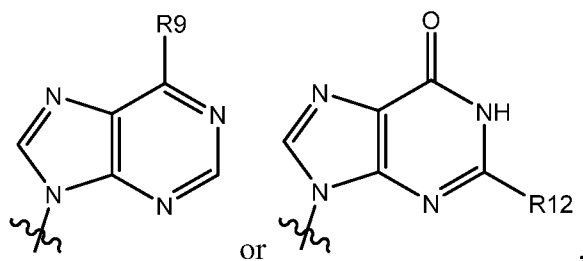
wherein R_9 and R_{12} are independently -NH₂, -NH-C₁₋₆alkyl, -N=CH-N(CH₃)₂, -NH-C(=O)-C₁₋₆alkyl or -NH-C(=O)-phenyl. In some embodiments, X_4 and X_4' are -O-, one of R_3 and R_4 is -OH and the other of R_3 and R_4 is -SH, Y_1 and Y_3 are O, Y_2 and Y_4 are absent, and R_1 and R_2 are independently



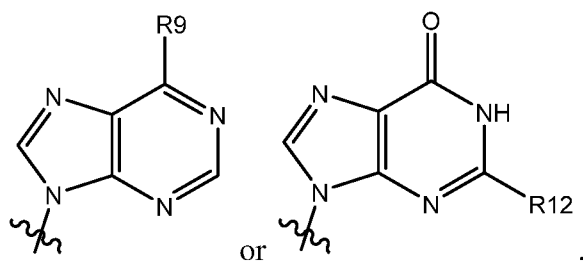
wherein  indicates the bond of R1 or R2 to the ring as shown in Formula I and wherein R9 and R12 are independently -NH₂, -NH-C₁₋₆alkyl, -N=CH-N(CH₃)₂, -NH-C(=O)-C₁₋₆alkyl or -NH-C(=O)-phenyl. In some embodiments, X₄ and X₄' are -O-, R3 and R4 are both -OH, Y₁ and Y₃ are O, Y₂ and Y₄ are absent, and R1 and R2 are independently



wherein  indicates the bond of R1 or R2 to the ring as shown in Formula I and wherein R9 and R12 are independently -NH₂, -NH-C₁₋₆alkyl, -N=CH-N(CH₃)₂, -NH-C(=O)-C₁₋₆alkyl or -NH-C(=O)-phenyl. In some embodiments, X₄ and X₄' are -O-, R3 and R4 are both -SH, Y₁ and Y₃ are O, Y₂ and Y₄ are absent, and R1 and R2 are independently



wherein  indicates the bond of R1 or R2 to the ring as shown in Formula I and wherein R9 and R12 are independently -NH₂, -NH-C₁₋₆alkyl, -N=CH-N(CH₃)₂, -NH-C(=O)-C₁₋₆alkyl or -NH-C(=O)-phenyl. In some embodiments, X₄ and X₄' are -O-, R3 and R4 are both -SH, wherein with reference to the stereochemistry at the phosphorus atoms, the compound is the substantially pure Rp,Rp isomer, the substantially pure Rp,Sp isomer, the substantially pure Sp,Rp isomer, or the substantially pure Sp,Sp isomer, Y₁ and Y₃ are O, Y₂ and Y₄ are absent, and R1 and R2 are independently



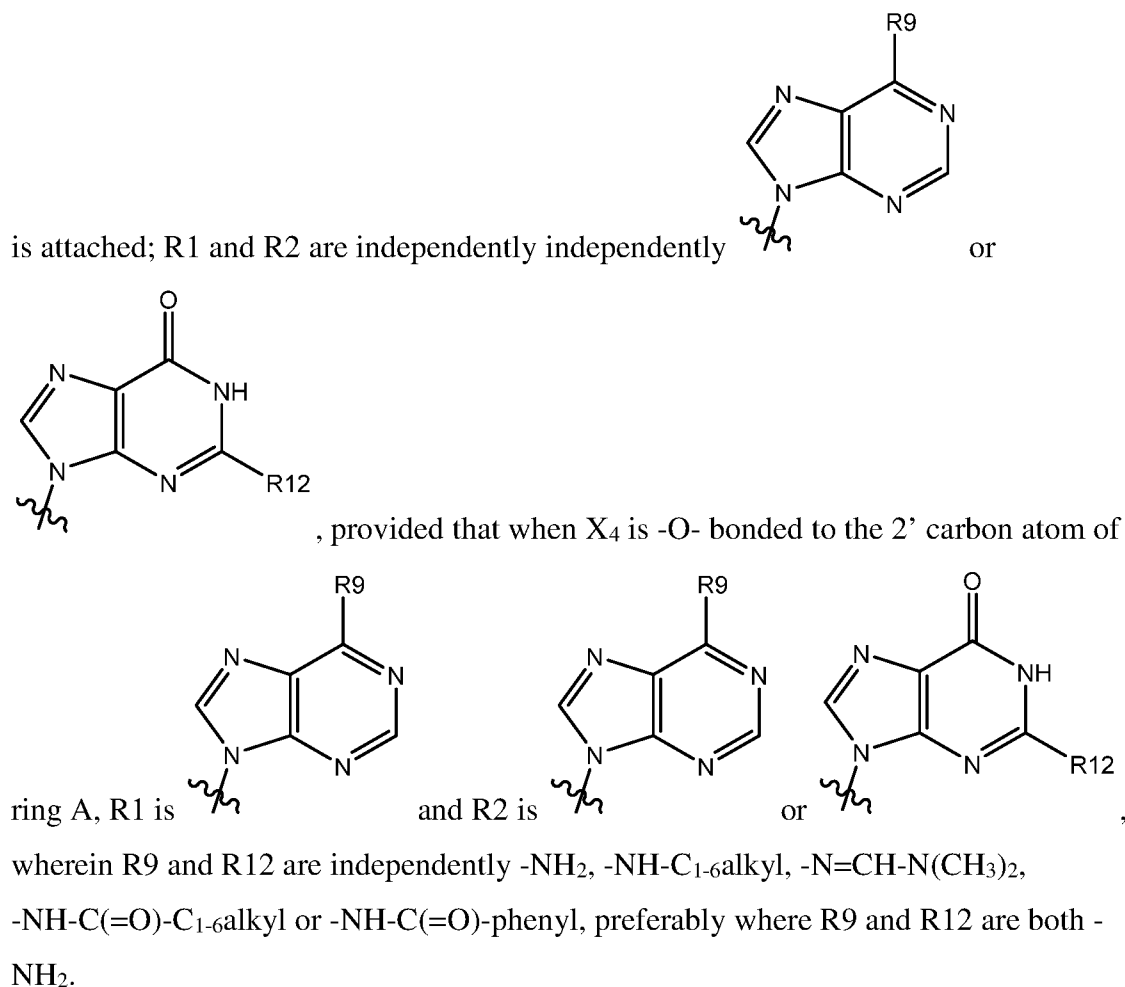
wherein  indicates the bond of R1 or R2 to the ring as shown in Formula I and wherein R9 and R12 are independently -NH₂, -NH-C₁₋₆alkyl, -N=CH-N(CH₃)₂, -NH-C(=O)-C₁₋₆alkyl or -NH-C(=O)-phenyl.

[0023] In any of the above embodiments of the first aspect, the bridging portions -X₁-X₂-X₃- or -X₁-X₃- are excluded wherein X₂ is absent and -X₁-X₃- provide -O-C(R^bR^c)-.

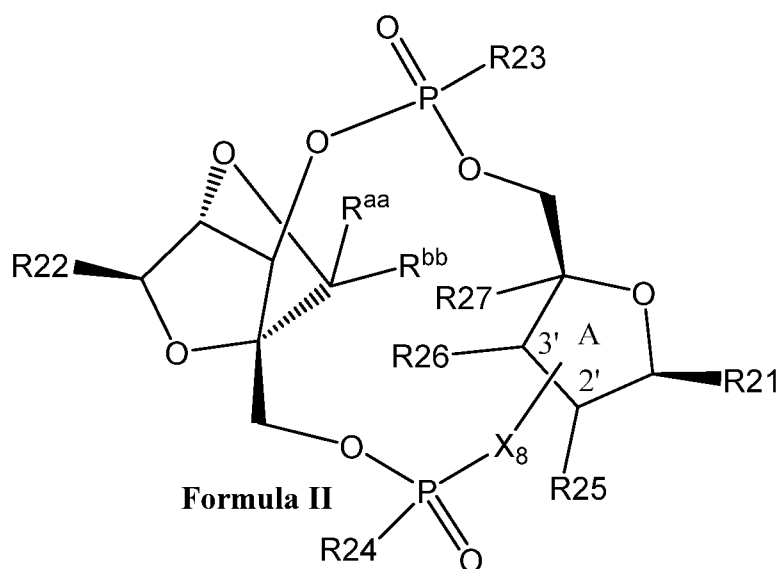
[0024] In any of the above embodiments of the first aspect, the bridging portions -X₁-X₂-X₃- or -X₁-X₃- are selected such that X₁, X₂, and X₃ are all present, X₁ and X₂ are -C(R^bR^c)-, -N(R^d), -O- or -S(=O)₂-, and X₃ is -C(R^bR^c)- or -O-, selected to provide -X₁-X₂-X₃- as -C(R^bR^c)-C(R^bR^c)-C(R^bR^c)-, -C(R^bR^c)-N(R^d)-C(R^bR^c)-, -C(R^bR^c)-O-C(R^bR^c)-, -C(R^bR^c)-C(R^bR^c)-O-, -O-C(R^bR^c)-C(R^bR^c)-, -O-N(R^d)-C(R^bR^c)-, -S(=O)₂-N(R^d)-C(R^bR^c)-, -N(R^d)-C(R^bR^c)-C(R^bR^c)-, -N(R^d)-O-C(R^bR^c)- or -N(R^d)-S(=O)₂-C(R^bR^c)-; or X₂ is absent, X₁ and X₃ are both present, X₁ is -C(R^bR^c)- or -N(R^d) and X₃ is -C(R^bR^c)- or -S(=O)₂-, selected to provide -X₁-X₃- as -C(R^bR^c)-C(R^bR^c)-, -N(R^d)-C(R^bR^c)- or -N(R^d)-S(=O)₂-.

[0025] In any of the above embodiments of the first aspect, when X₂ is absent and X₁ and X₃ are both present, then X₁ is -C(R^bR^c)- or -N(R^d) and X₃ is -C(R^bR^c)- or -S(=O)₂-, selected to provide -X₁-X₃- as -C(R^bR^c)-C(R^bR^c)-, -N(R^d)-C(R^bR^c)- or -N(R^d)-S(=O)₂-; and when X₄ is -O- or -NH- bonded to the 3' carbon atom of ring A, R₆ is absent, and R₅ and R₇ join to form -X₅-X₆-X₇-, resulting in a fused ring system with X₅ bonded to the 2' carbon atom of ring A and X₇ bonded to the carbon atom of ring A to which R₇ is attached, and X₆ is absent and X₅ and X₇ are both present, then X₅ is -C(R^bR^c)- or -N(R^d) and X₇ is -C(R^bR^c)- or -S(=O)₂-, selected to provide -X₅-X₇- as -C(R^bR^c)-C(R^bR^c)-, -N(R^d)-C(R^bR^c)- or -N(R^d)-S(=O)₂-.

[0026] In any of the eleventh or twelfth embodiments of the first aspect, X_2 is absent and X_1 and X_3 provide $-O-C(R^bR^c)-$; X_4 is $-O-$ bonded to the 2' carbon atom of ring A, R_5 is absent, R_6 is selected from the group consisting of $-H$, $-OH$, halogen and $-OR^e$, and R_7 is $-H$; or X_4 is $-O-$ bonded to the 3' carbon atom of ring A, R_6 is absent, and R_5 is selected from the group consisting of $-H$, $-OH$, halogen and $-OR^e$, and R_7 is $-H$; or X_4 is $-O-$ bonded to the 3' carbon atom of ring A, R_6 is absent, and R_5 and R_7 join to form $-X_5-X_7-$ to provide $-O-C(R^aR^b)-$, resulting in a fused ring system with $-O-$ bonded to the 2' carbon atom of ring A and $-C(R^aR^b)-$ bonded to the carbon atom of ring A to which R_7



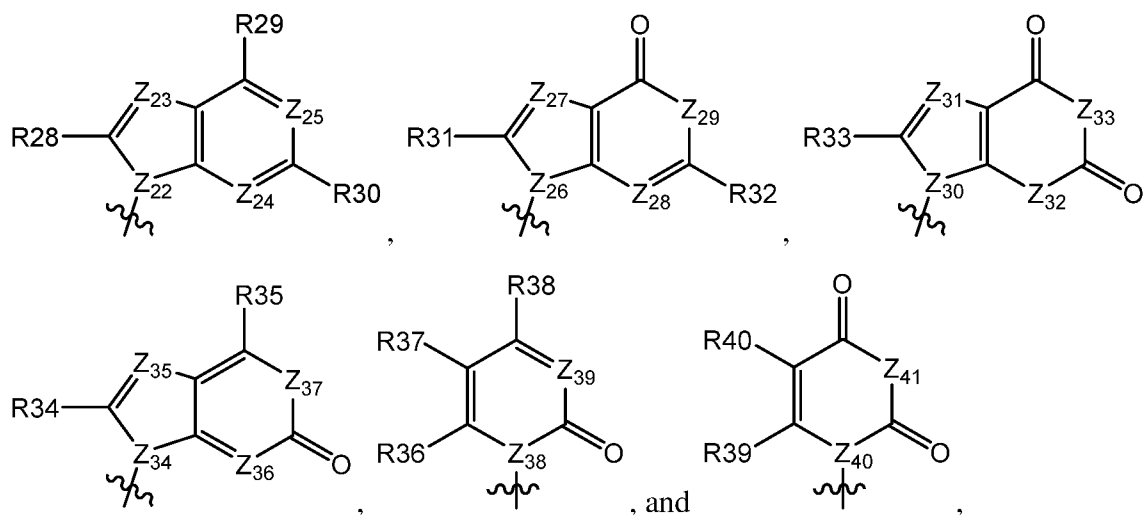
[0027] In a second aspect, the present invention provides a locked nucleic acid cyclic dinucleotide ("LNA-CDN") compound of Formula II:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof,

wherein:

R21 and R22 are independently selected from the group consisting of:



wherein  indicates the bond of R21 or R22 to the ring as shown in Formula II;

R23 and R24 are independently -OH, -OR^{cc}, -SH or -SR^{cc};

X₈ is -O- bonded to either the 2' or the 3' carbon atom of the indicated ring A;

wherein:

when X₈ is -O- bonded to the 2' carbon atom of ring A:

R25 is absent, R26 is selected from the group consisting of -H, -OH, halogen and -OR^{dd}, and R27 is -H;

when X₈ is -O- bonded to the 3' carbon atom of ring A:

R₂₆ is absent, R₂₅ is selected from the group consisting of -H, -OH, halogen and -OR^{dd}, and R₂₇ is -H; or

R₂₆ is absent, and R₂₅ and R₂₇ join to form -O-C(R^{aa}R^{bb})-, resulting in a fused ring system with -O- bonded to the 2' carbon atom of ring A and -C(R^{aa}R^{bb})- bonded to the carbon atom of ring A to which R₂₇ is attached;

R^{aa} and R^{bb}, at each occurrence, are independently selected from the group consisting of -H, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R' and -L₅-R^{ee}; or R^{aa} and R^{bb} together on a carbon are =O, =CR^{ff}R^{ff}, =N-OR^{gg}, =N-R^{gg}, or =N-NR^{gg}R^{gg}; or R^{aa} and R^{bb} together with the carbon to which they are attached form a C₃₋₆cycloalkyl or 3 to 6 membered heterocycloalkyl spirocyclic ring; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

R^{cc} at each occurrence are independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl and -C(=O)R^{hh}; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

R^{dd} is selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl and -C(=O)R^{hh}; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk}; or R^{dd} is a suitable oxygen protecting group;

R^{ee} at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

R^{ff} at each occurrence is independently selected from the group consisting of -H, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

R^{gg} at each occurrence is independently selected from the group consisting of -H, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

R^{hh} at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

R' at each occurrence is selected from the group consisting of -CN, -NO₂, -OH, -SH, -NH₂,
 -C(=W)-WH, -C(=W)-NH₂, -C(=W)-NH-WH, -C=N-OH, -C(=NH)-NH₂,
 -W-C(=W)-WH, -W-C(=W)-NH₂, -S(=O)₂-NH₂, -S(=O)₂-NH-W-, -NH-NH₂,
 -NH-WH, -NH-C(=W)-WH, -NH-C(=W)-NH₂, -NH-S(=O)₂-NH₂, and
 -NH-C(=NH)-NH₂;

L₅ is selected from the group consisting of -O-, -S-, -S(=O)-, -S(=O)₂-, -C(=W)-, -C(=W)-W-, -C(=W)-NRⁱⁱ-, -C(=W)-NRⁱⁱ-W-, -C=N-O-, -C(=NRⁱⁱ)NRⁱⁱ-, -C(NRⁱⁱRⁱⁱ)=N-, -W-C(=W)-, -W-C(=W)-W-, -W-C(=W)-NRⁱⁱ-, -S(=O)₂-NRⁱⁱ-, -S(=O)₂-NRⁱⁱ-W-, -NRⁱⁱ-, -N=CR^{ff}-, -NRⁱⁱ-W-, -NRⁱⁱ-NRⁱⁱ-, -NRⁱⁱ-C(=NRⁱⁱ)NRⁱⁱ-, -NRⁱⁱ-C(NRⁱⁱRⁱⁱ)=N-, -NRⁱⁱ-C(=W)-, -NRⁱⁱ-C(=W)-W-, -NRⁱⁱ-C(=W)-NRⁱⁱ-, -NRⁱⁱ-S(=O)₂-NRⁱⁱ- and -NRⁱⁱ-S(=O)₂-;

Rⁱⁱ at each occurrence is independently selected from the group consisting of -H, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

R^{jj} at each occurrence is independently selected from the group consisting of =O, halogen, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R' and -L₆-R^{mmm}, when substituted on an available carbon atom, and carbocyclyl, heterocyclyl, aryl, heteroaryl, -R'' and -L₇-R^{mmm}, when substituted on an available nitrogen atom; wherein carbocyclyl, heterocyclyl, aryl and heteroaryl are optionally independently substituted with one or more -R^{kk}.

- R^{kk} at each occurrence is independently selected from the group consisting of =O, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-R'$ and $-L_6-R^{mm}$, when substituted on an available carbon atom, and alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-R''$ and $-L_7-R^{mm}$, when substituted on an available nitrogen atom;
- R'' at each occurrence is selected from the group consisting of -OH, -SH, -NH₂, -C(=W)-WH, -C(=W)-NH₂, -C(=NH)-NH₂, and -S(=O)₂-NH₂;
- L_6 is selected from the group consisting of -O-, -S-, -S(=O)-, -S(=O)₂-, -C(=W)-, -C(=W)-W-, -C(=W)-NR^{oo}-, -C(=W)-NR^{oo}-W-, -C=N-O-, -C(=NR^{oo})NR^{oo}-, -C(NR^{oo}R^{oo})=N-, -W-C(=W)-, -W-C(=W)-W-, -W-C(=W)-NR^{oo}-, -S(=O)₂-NR^{oo}-, -S(=O)₂-NR^{oo}-W-, -NR^{oo}-, -N=CR^{pp}-, -NR^{oo}-W-, -NR^{oo}-NR^{oo}-, -NR^{oo}-C(=NR^{oo})NR^{oo}-, -NR^{oo}-C(NR^{oo}R^{oo})=N-, -NR^{oo}-C(=W)-, -NR^{oo}-C(=W)-W-, -NR^{oo}-C(=W)-NR^{oo}-, -NR^{oo}-S(=O)₂-NR^{oo}-, and -NR^{oo}-S(=O)₂-;
- L_7 is selected from the group consisting of -O-, -S-, -NR^{oo}-, -C(=W)-, -C(=W)-W-, -C(=W)-NR^{oo}-, -C(=NR^{oo})NR^{oo}-, -C(NR^{oo}R^{oo})=N-, -S(=O)₂-NR^{oo}-, and -S(=O)₂-;
- each W is independently O or S;
- R^{mm} and R^{nn} , at each occurrence, are independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently substituted with one or more $-R^{qq}$; carbocyclyl or heterocyclyl are optionally independently substituted with one or more $-R^{rr}$; and aryl or heteroaryl are optionally independently substituted with one or more $-R^{ss}$;
- R^{oo} at each occurrence is independently selected from the group consisting of -H, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently substituted with one or more $-R^{qq}$; carbocyclyl or heterocyclyl are optionally independently substituted with one or more $-R^{rr}$; and aryl or heteroaryl are optionally independently substituted with one or more $-R^{ss}$;
- R^{pp} at each occurrence is independently selected from the group consisting of -H, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently substituted with one or more $-R^{qq}$; carbocyclyl or heterocyclyl are

optionally independently substituted with one or more -R^{rr}; and aryl or heteroaryl are optionally independently substituted with one or more -R^{ss};

R^{qq} at each occurrence is independently selected from the group consisting of halogen, =O, -OH, -SH, -NH₂, -CN, C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino, and N-linked-heterocycloalkyl; wherein C₁₋₆alkyl, alone or as part of C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, or C₁₋₆dialkylamino, is optionally substituted with one or more substituents independently selected from the group consisting of -OH, -SH, -NH₂, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylthio, C₁₋₆haloalkylthio, C₁₋₆alkylsulfinyl, C₁₋₆haloalkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆haloalkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl;

R^{rr} at each occurrence is independently selected from the group consisting of halogen, =O, -OH, -SH, -NH₂, -CN, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl; wherein C₂₋₆alkenyl, C₂₋₆alkynyl, and C₁₋₆alkyl, alone or as part of C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, or C₁₋₆dialkylamino, is optionally substituted with one or more substituents independently selected from the group consisting of -OH, -SH, -NH₂, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylthio, C₁₋₆haloalkylthio, C₁₋₆alkylsulfinyl, C₁₋₆haloalkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆haloalkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl; and

R^{ss} at each occurrence is independently selected from the group consisting of halogen, -OH, -SH, -NH₂, -CN, -NO₂, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino, and N-linked-heterocycloalkyl; wherein C₂₋₆alkenyl, C₂₋₆alkynyl, and C₁₋₆alkyl, alone or as part of C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, or C₁₋₆dialkylamino, is optionally substituted with one or more substituents independently selected from the group consisting of -OH, -SH, -NH₂, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylthio, C₁₋₆haloalkylthio, C₁₋₆alkylsulfinyl, C₁₋₆haloalkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆haloalkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl;

Z₂₂, Z₂₆, Z₃₀, Z₃₄, Z₃₈ and Z₄₀ are independently $\text{---}\overset{\text{---}}{\underset{|}{\text{C}}}\text{H---}$ or $\text{---}\overset{\text{---}}{\underset{|}{\text{N}}}\text{---}$;

Z₂₉, Z₃₂, Z₃₃, Z₃₇ and Z₄₁ are independently -CR^{tt}R^{uu}-, -O- or -NR^{vv}-;

Z₂₃, Z₂₄, Z₂₅, Z₂₇, Z₂₈, Z₃₁, Z₃₅, Z₃₆ and Z₃₉ are independently =CR^{tt}- or =N-;

R^{tt} and R^{uu}, at each occurrence, are independently selected from the group consisting of -H, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R' and -L₅-R^{ee}; or R^{tt} and R^{uu} together are =O, =CR^{ff}R^{ff}, =N-OR^{gg}, =N-R^{gg}, or =N-NR^{gg}R^{gg}; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

each R^{vv} is independently selected from the group consisting of -H, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R'' and -L₈-R^{ww}; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

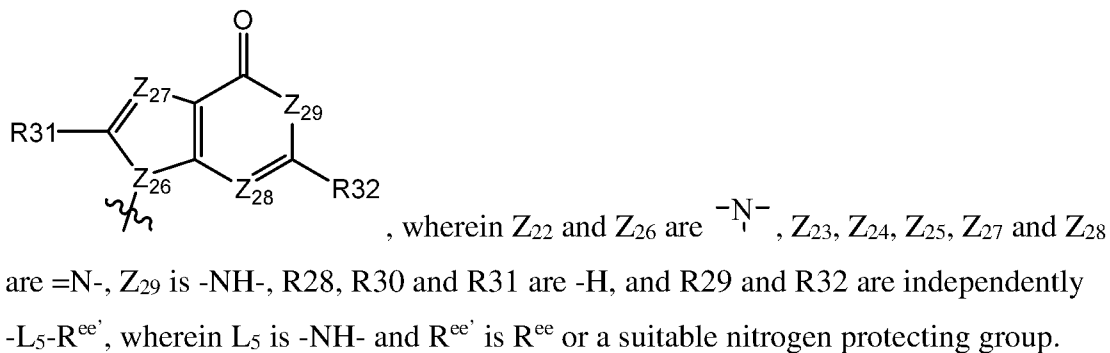
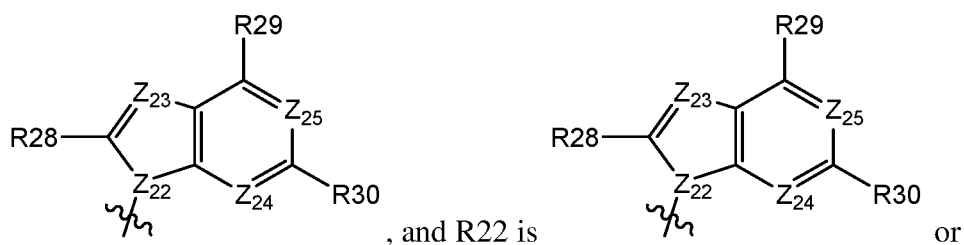
L₈ is selected from the group consisting of -O-, -S-, -NR^{xx}-, -C(=W)-, -C(=W)-W-, -C(=W)-NR^{xx}-, -C(=NR^{xx})NR^{xx}-, -C(NR^{xx}R^{xx})=N-, -S(=O)₂-NR^{xx}-, and -S(=O)₂-;

R^{ww} at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

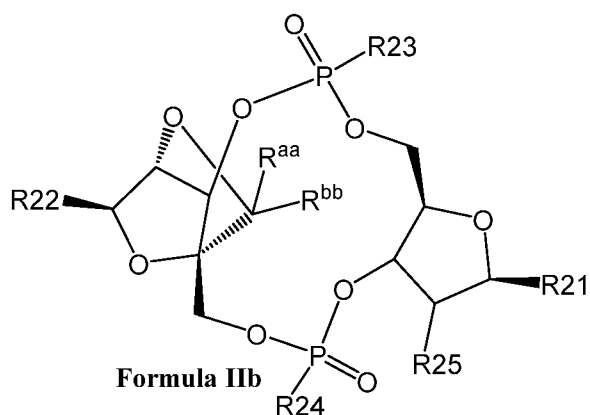
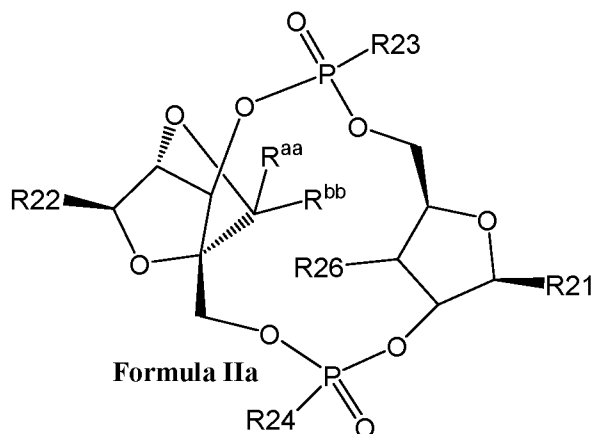
R^{xx} at each occurrence is independently selected from the group consisting of -H, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, R₃₃, R₃₄, R₃₅, R₃₆, R₃₇, R₃₈, R₃₉ and R₄₀ are independently selected from the group consisting of -H, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R' and -L₅-R^{ee'}; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk}, wherein R^{ee'} is R^{ee} or a suitable nitrogen protecting group when L₅ is -NH-;

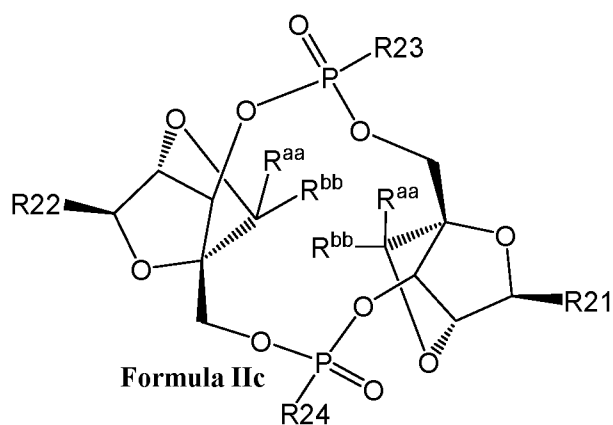
provided that when X_8 is -O- bonded to the 2' carbon atom of ring A, R21 is



[0028] In a first embodiment of the second aspect, the compound of Formula II is a compound selected from the group consisting of a compound of Formula IIa, a compound of Formula IIb, and a compound of Formula IIc:



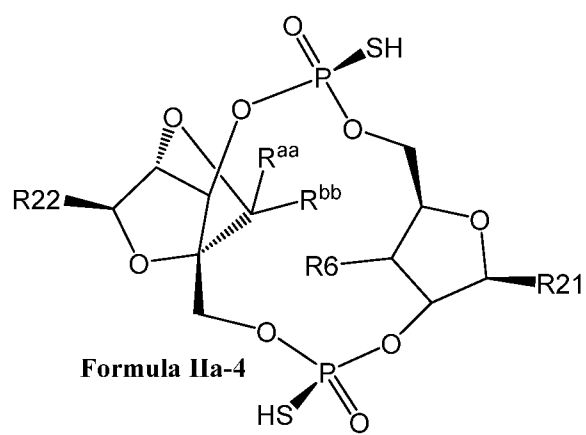
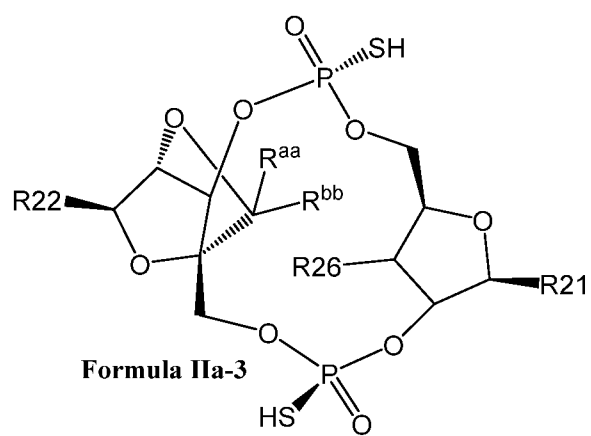
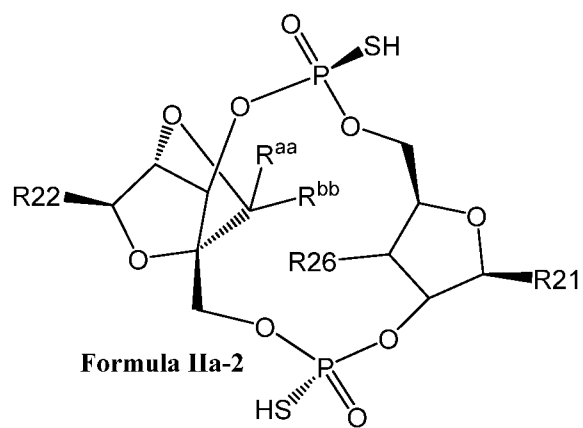
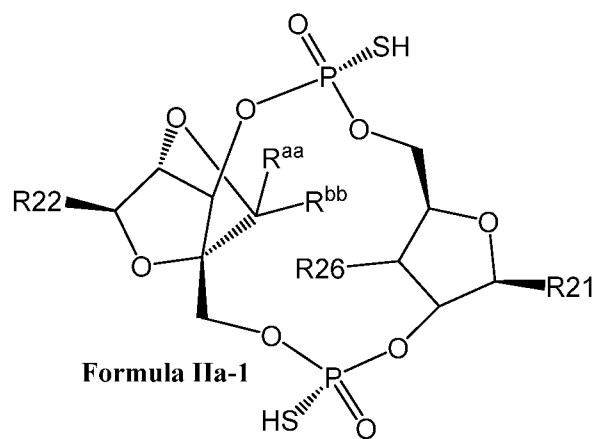
and

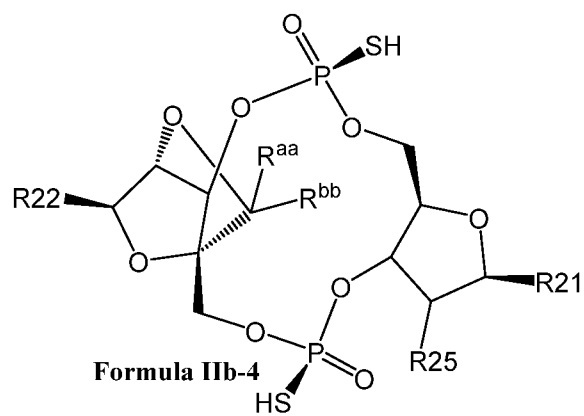
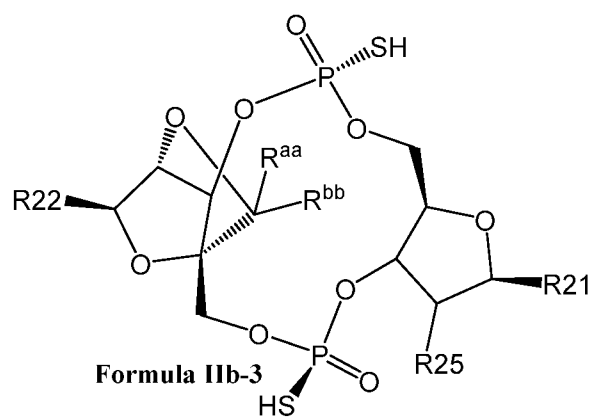
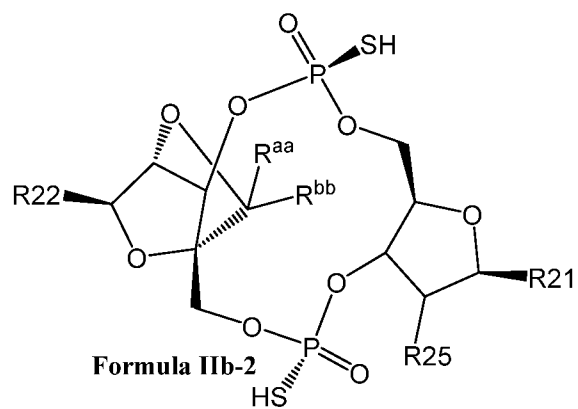
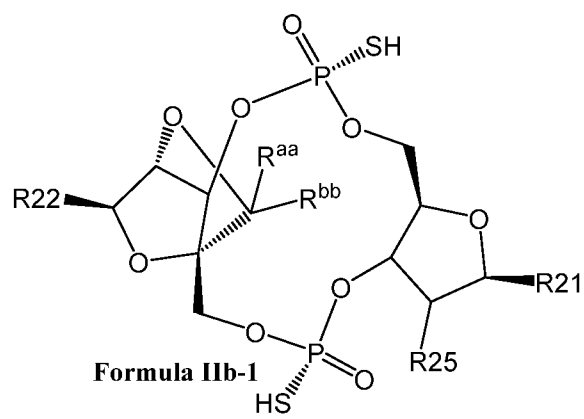


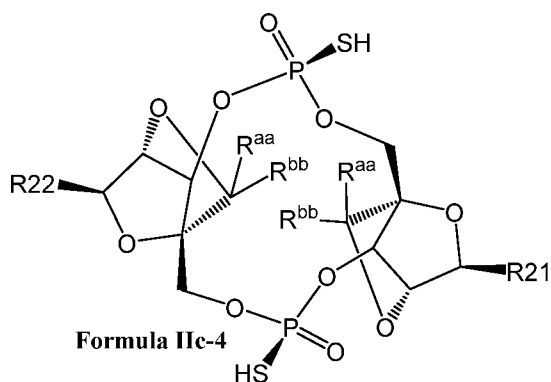
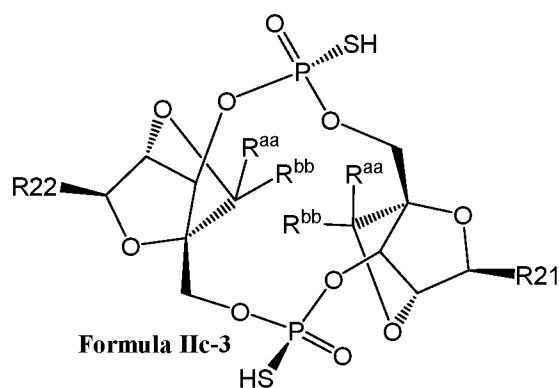
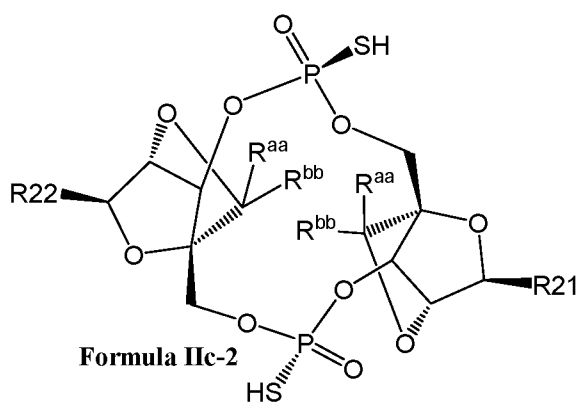
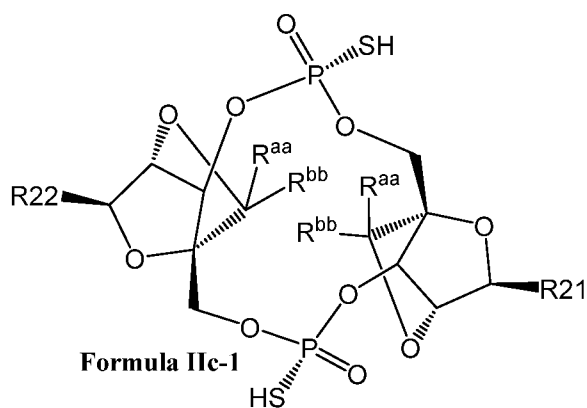
;

or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R21, R22, R23, R24, R25, R26, R^{aa} and R^{bb} are as defined for Formula II.

[0029] In a second embodiment of the second aspect, the compound of Formula II is a compound selected from the group consisting of a compound of Formula IIa-1, a compound of Formula IIa-2, a compound of Formula IIa-3, a compound of Formula IIa-4, a compound of Formula IIb-1, a compound of Formula IIb-2, a compound of Formula IIb-3, a compound of Formula IIb-4, a compound of Formula IIc-1, a compound of Formula IIc-2, a compound of Formula IIc-3, and a compound of Formula IIc-4:





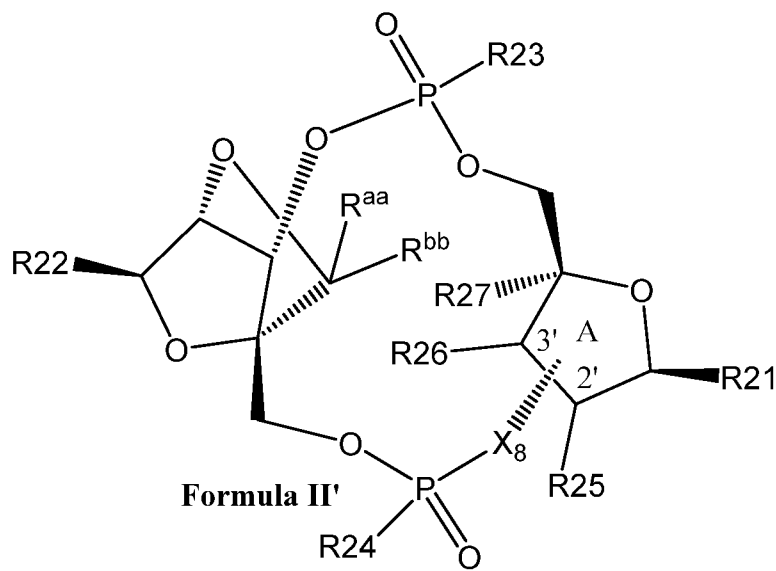


, and

;

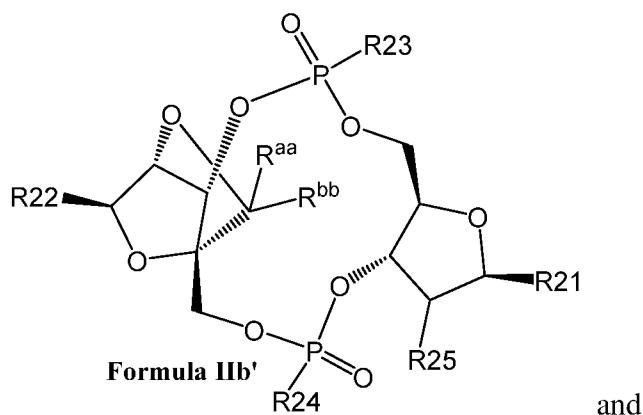
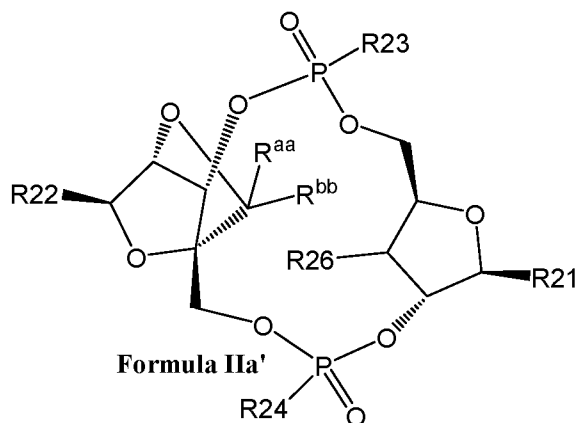
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R₂₁, R₂₂, R₂₅, R₂₆, R^{aa} and R^{bb} are as defined for Formula II.

[0030] In a third embodiment of the second aspect, the compound of Formula II is a compound of Formula II':

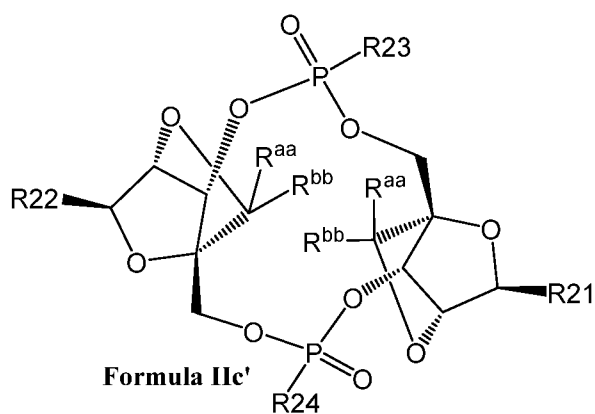


or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein X₈, R₂₁, R₂₂, R₂₃, R₂₄, R₂₅, R₂₆, R₂₇, R^{aa} and R^{bb} are as defined for Formula II.

[0031] In a fourth embodiment of the second aspect, the compound of Formula II is a compound selected from the group consisting of a compound of Formula IIa', a compound of Formula IIb', and a compound of Formula IIc':



and

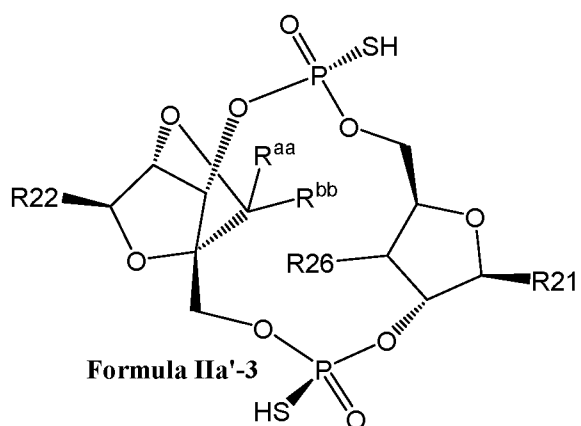
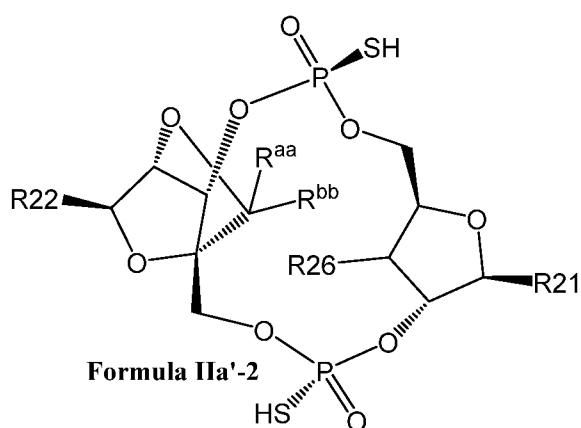
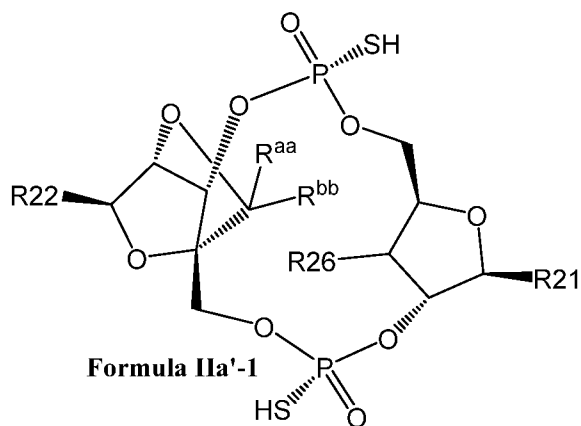


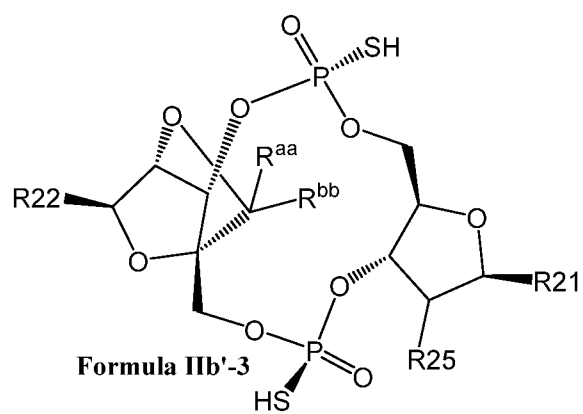
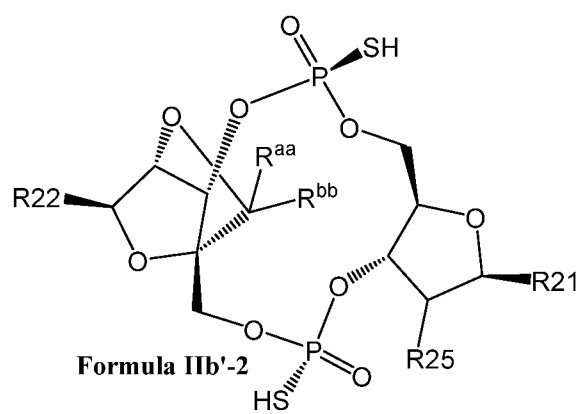
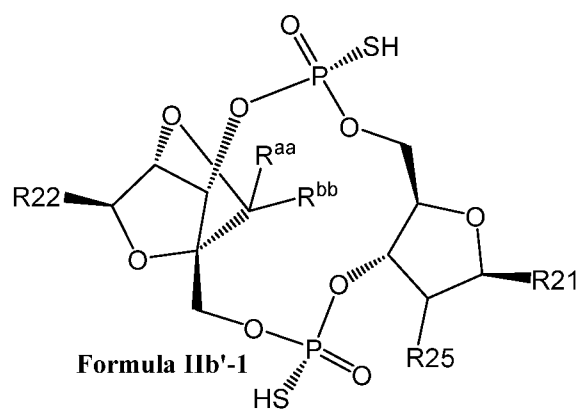
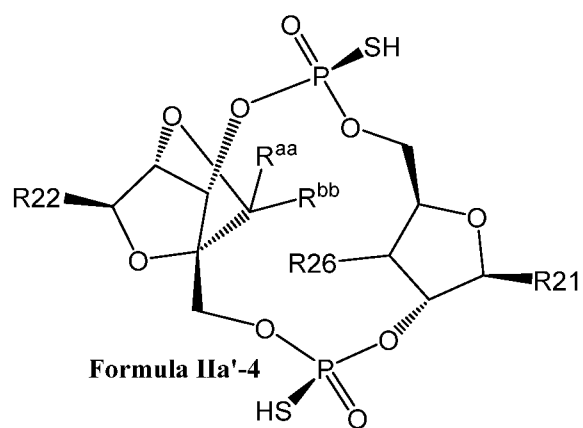
;

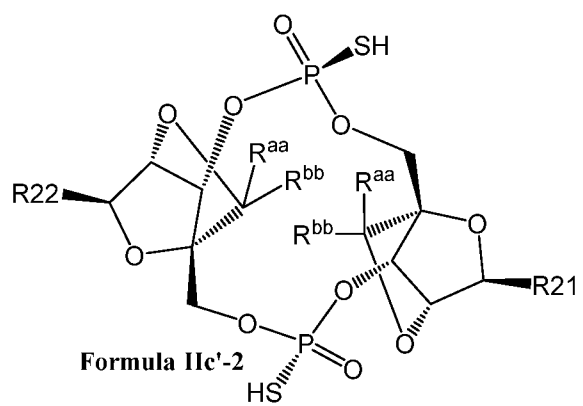
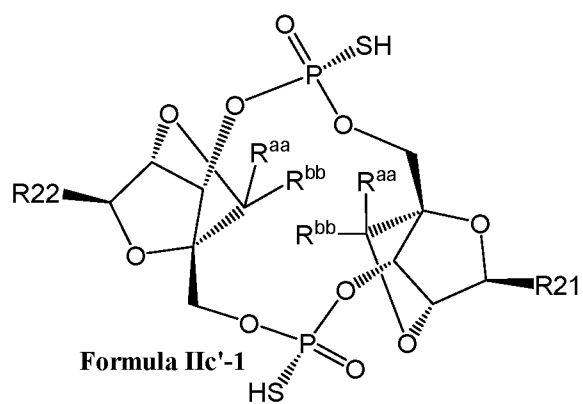
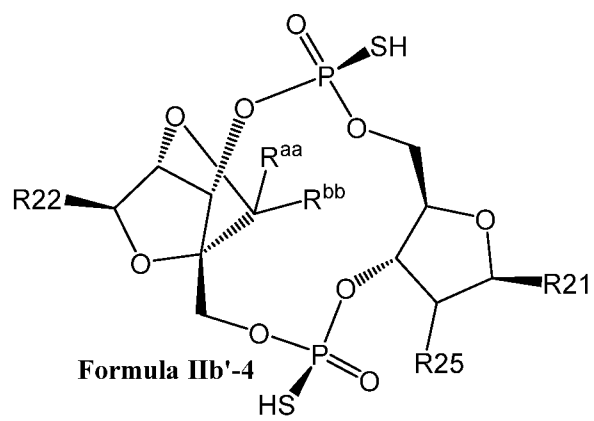
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R21, R22, R23, R24, R25, R26, R^{aa} and R^{bb} are as defined for Formula II.

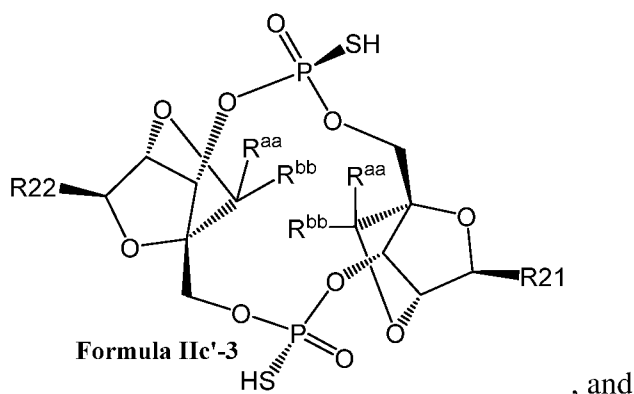
[0032] In a fifth embodiment of the second aspect, the compound of Formula II is a compound selected from the group consisting of a compound of Formula IIa'-1, a compound of Formula IIa'-2, a compound of Formula IIa'-3, a compound of Formula IIa'-4, a compound of Formula IIb'-1, a compound of Formula IIb'-2, a compound of Formula IIb'-3, a compound of Formula IIb'-4, a compound of Formula IIc'-1, a

compound of Formula IIc'-2, a compound of Formula IIc'-3, and a compound of Formula IIc'-4:

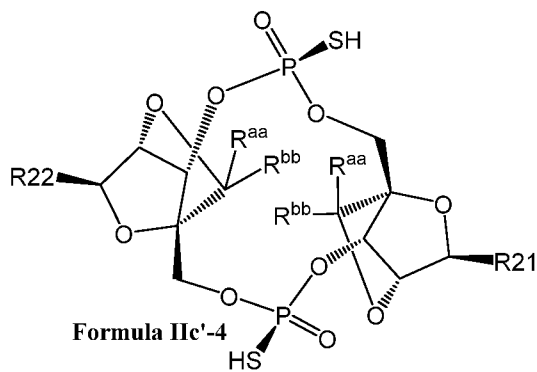








, and



;

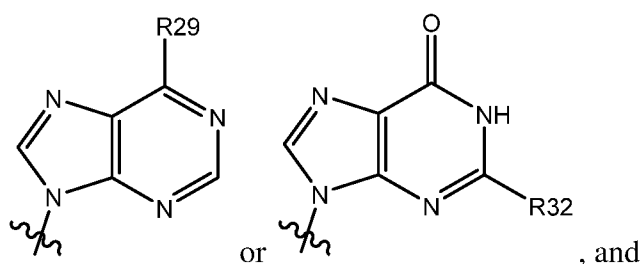
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R²¹, R²², R²⁵, R²⁶, R^{aa} and R^{bb} are as defined for Formula II. In some embodiment of the compounds of Formula IIa'-1, Formula IIa'-2, Formula IIa'-3, Formula IIa'-4, Formula IIb'-1, Formula IIb'-2, Formula IIb'-3, or Formula IIb'-4, the bond to R²⁵ or R²⁶ is preferably down into the paper, i.e. in

the above structures is represented as e.g.  R²⁵, where the * represents the bond to the core ring.

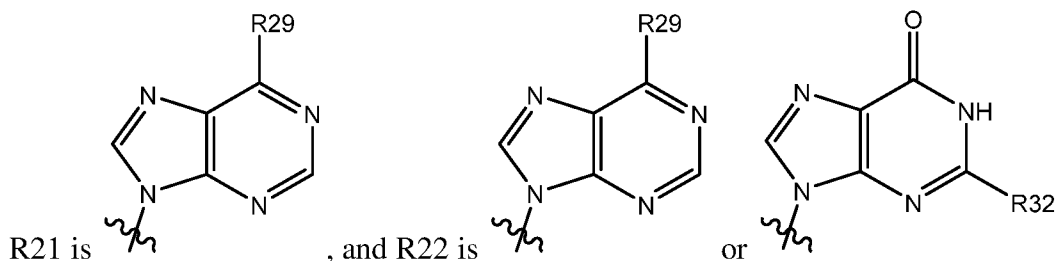
[0033] In a sixth embodiment of the second aspect, and any of the above embodiments thereof, R²³ and R²⁴ are independently -OH or -SH. In some embodiments, R²³ and R²⁴ are both -OH. In some embodiments, one of R²³ and R²⁴ is -OH and the other is -SH. In some embodiments, R²³ and R²⁴ are both -SH. In some embodiments, R²³ and R²⁴ are both -SH, wherein with reference to the stereochemistry at the phosphorous atoms, the compound is the substantially pure Rp,Rp isomer, the substantially pure Rp,Sp isomer, the substantially pure Sp,Rp isomer, or the substantially pure Sp,Sp isomer.

[0034] In a seventh embodiment of the second aspect, and any of the above embodiments thereof, when X₈ is -O- bonded to the 3' carbon atom of ring A, R²¹ and

R22 are independently



when X₈ is -O- bonded to the 2' carbon atom of ring A,



wherein R29 and R32 are independently -NH₂ or -NH-C₁₋₆alkyl, wherein -NH₂ is optionally substituted with a suitable nitrogen protecting group. In some embodiments, R29 and R32 are independently -NH₂, -NH-C₁₋₆alkyl, -N=CH-N(CH₃)₂, -NH-C(=O)-C₁₋₆alkyl or -NH-C(=O)-phenyl. In some embodiments, R29 is -NH₂ or -NH-C(=O)-phenyl and R32 is -NH₂ or -NH-C(=O)-isopropyl. In some embodiments R29 and R32 are both -NH₂.

[0035] In an eighth embodiment of the second aspect, and any of the above embodiments thereof, when R25 or R26 are selected from the group consisting of -H, -OH, halogen and -OR^{dd}, R25 or R26 are selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl. In some embodiments, when R25 or R26 are selected from the group consisting of -H, -OH, halogen and -OR^{dd}, R25 and R26 are selected from the group consisting of -H, -OH, -OTBS, -F and -O-(C=O)-C₁₋₁₄alkyl. In some embodiments, when R25 or R26 are selected from the group consisting of -H, -OH, halogen and -OR^{dd}, R25 and R26 are -H, -OH, -OTBS or -F, in some embodiments -H, -OH or -F, in some embodiments -OH or -F.

[0036] In a ninth embodiment of the second aspect, and any of the above embodiments thereof:

R^{aa} and R^{bb}, at each occurrence, are independently selected from the group consisting of -H, -CN, -OH, -NH₂, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, -O-R^{a'},

-OC(=O)-R^{a'} and -NH-C(=O)-R^{b'}, wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally substituted with 1, 2 or 3 substituents independently selected from the group consisting of -OH, -NH₂, -CN, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl; or R^{aa} and R^{bb} together are =O, =CH₂ or with the carbon to which they are attached form a C₃₋₆cycloalkyl or 3 to 6 membered heterocycloalkyl spirocyclic ring;

R^{cc} at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, and -C(=O)-R^{c'}; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally substituted with 1, 2 or 3 substituents independently selected from the group consisting of -OH, -NH₂, -CN, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl;

R^{dd} is selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, and -C(=O)-R^{c'}; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally substituted with 1, 2 or 3 substituents independently selected from the group consisting of -OH, -NH₂, -CN, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl; or R^{dd} is a suitable oxygen protecting group;

R^{a'} at each occurrence is independently selected from the group consisting of phenyl, toluenyl, alkyl, alkenyl, alkynyl and heteroalkyl, wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally substituted with 1, 2 or 3 substituents independently selected from the group consisting of -OH, -NH₂, -CN, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl;

R^{b'} at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl and heteroalkyl, wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally substituted with 1, 2 or 3 substituents independently selected from the group consisting of -OH, -NH₂, -CN, -O-phenyl, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl; and

R^{c'} at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl and heteroalkyl, wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally substituted with 1, 2 or 3 substituents independently selected from the group consisting of -OH, -NH₂, -CN, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl.

[0037] In tenth embodiment of the second aspect, and any of the of the above embodiments thereof, R^{aa} and R^{bb} at each occurrence are independent -H or -C₁₋₆alkyl,

wherein alkyl is optionally substituted with 1, 2 or 3 -F or with -C₁₋₆alkoxy; or together form =CH₂, or together with the carbon to which they are attached form a 3-6 membered heterocycloalkyl or C₃₋₆cycloalkyl spirocyclic ring. In some embodiments R^{aa} and R^{bb} at each occurrence are independently -H, -CH₃, -CH₂F, -CHF₂, -CF₃, or -CH₂OCH₃; or together form =CH₂; or together with the carbon to which they are attached form a cyclopropyl spirocyclic ring. In some embodiments, R^{aa} and R^{bb} at each occurrence are independently -H or -C₁₋₆alkyl. In some embodiment, R^{aa} and R^{bb} are both -H.

[0038] In an eleventh embodiment of the second aspect, and any of the above embodiments thereof:

R23 and R24 are independently selected from the group consisting of -OH, -SH,

-O-C₁₋₁₄alkyl, -S-C₁₋₁₄alkyl, -O-(C=O)-C₁₋₁₄alkyl and -S-C(=O)-C₁₋₁₄alkyl;

X₈ is -O- bonded to either the 2' or the 3' carbon atom of the indicated ring A;

wherein:

when X₁ is -O- bonded to the 2' carbon atom of ring A:

R25 is absent, R26 is selected from the group consisting of -H, -OH, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl, and R27 is -H;

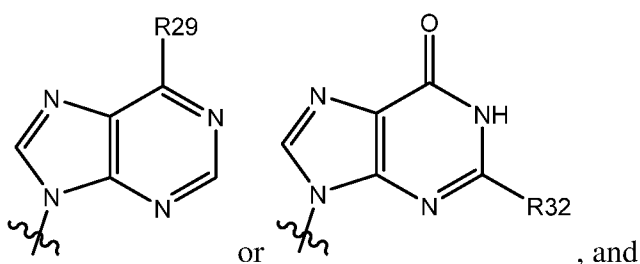
when X₈ is -O- bonded to the 3' carbon atom of ring A:

R26 is absent, R25 is selected from the group consisting of -H, -OH, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl, and R27 is -H; or

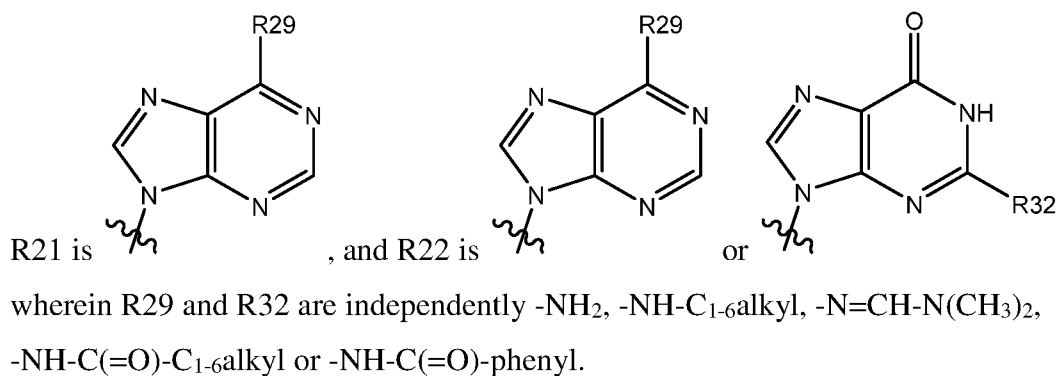
R26 is absent, and R25 and R27 join to form -O-C(R^{aa}R^{bb})-, resulting in a fused ring system with -O- bonded to the 2' carbon atom of ring A and -C(R^{aa}R^{bb})- bonded to the carbon atom of ring A to which R27 is attached;

R^{aa} and R^{bb}, at each occurrence, are -H; and

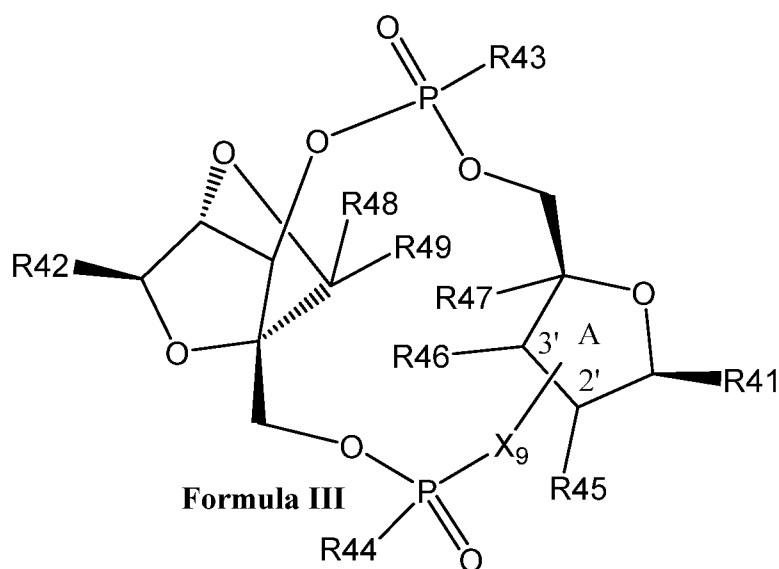
when X₈ is -O- bonded to the 3' carbon atom of ring A, R21 and R22 are independently



when X₈ is -O- bonded to the 2' carbon atom of ring A,



[0039] In a third aspect, the present invention provides a locked nucleic acid cyclic dinucleotide (“LNA-CDN”) compound of Formula III:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof,

wherein:

R43 and R44 are independently selected from the group consisting of -OH, -SH,

-O-C₁₋₁₄alkyl, -S-C₁₋₁₄alkyl, -O-(C=O)-C₁₋₁₄alkyl and -S-C(=O)-C₁₋₁₄alkyl;

X₉ is -O- bonded to either the 2' or the 3' carbon atom of the indicated ring A;

wherein:

when X₉ is -O- bonded to the 2' carbon atom of ring A:

R45 is absent, R46 is selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen,

-O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl and R47 is -H;

when X₉ is -O- bonded to the 3' carbon atom of ring A:

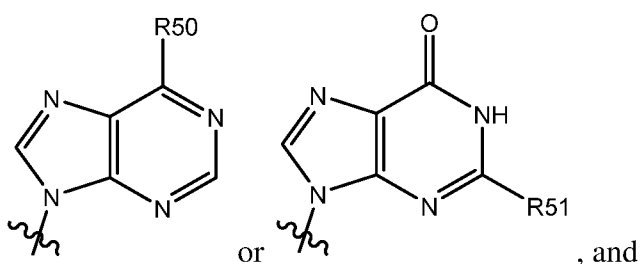
R46 is absent, R45 is selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl, and R47 is -H; or

R46 is absent, and R45 and R47 join to form -O-C(R48R49)-, resulting in a fused ring system with -O- bonded to the 2' carbon atom of ring A and -C(R48R49)- is bonded to the carbon atom of ring A to which R47 is attached;

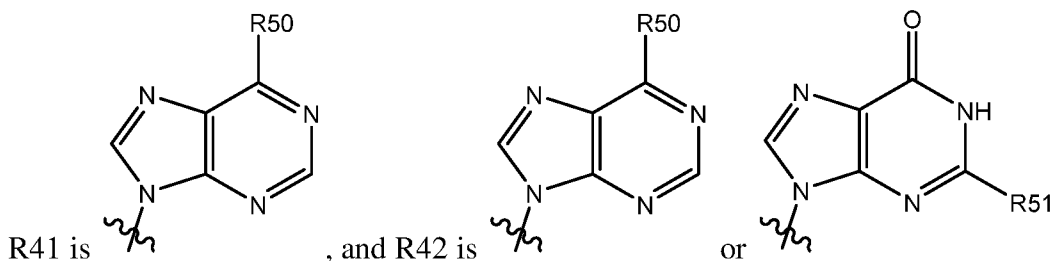
R48 and R49 at each occurrence are independently -H or -C₁₋₆alkyl, wherein alkyl is optionally substituted with 1, 2 or 3 -F or with -C₁₋₆alkoxy; or R48 and R49 on the same carbon together form a C₃₋₆cycloalkyl spirocyclic ring;

and

when X₉ is -O- bonded to the 3' carbon atom of ring A, R41 and R42 are independently

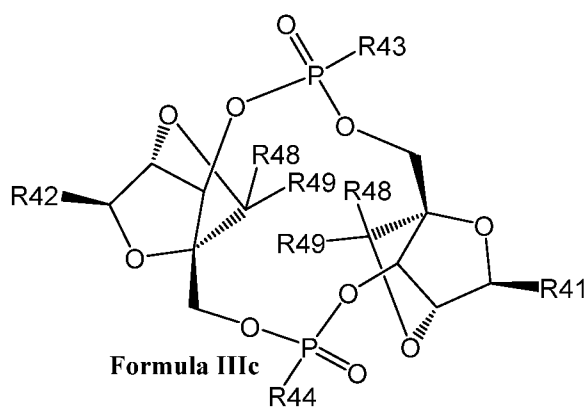
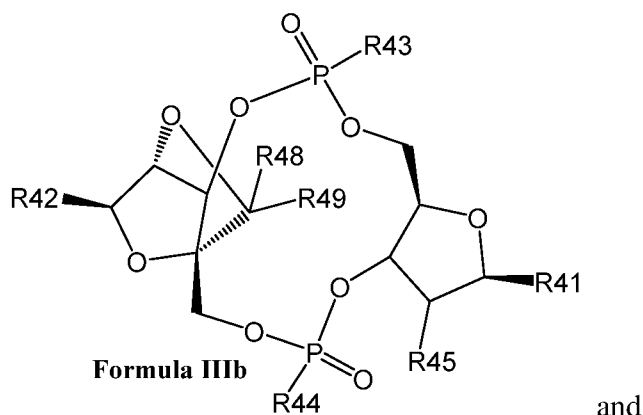
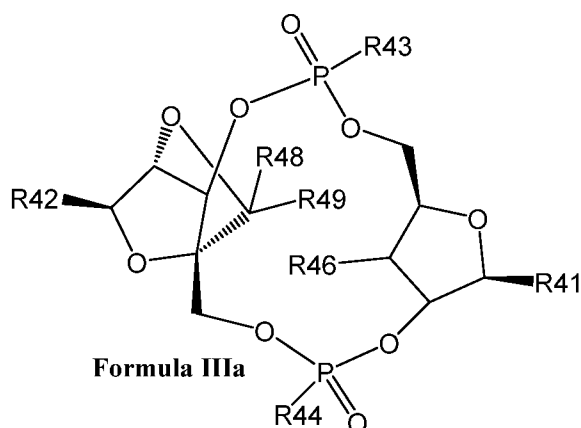


when X₉ is -O- bonded to the 2' carbon atom of ring A,



wherein  indicates the bond of R41 or R42 to the ring as shown in Formula III, and wherein R50 and R51 are independently -NH₂ or -NH-C₁₋₆alkyl, wherein -NH₂ is optionally substituted with a suitable nitrogen protecting group.

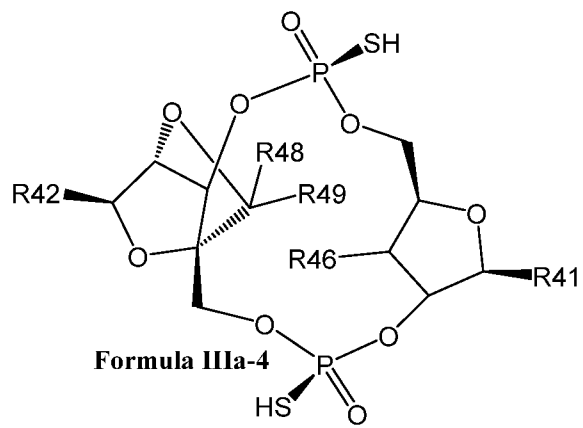
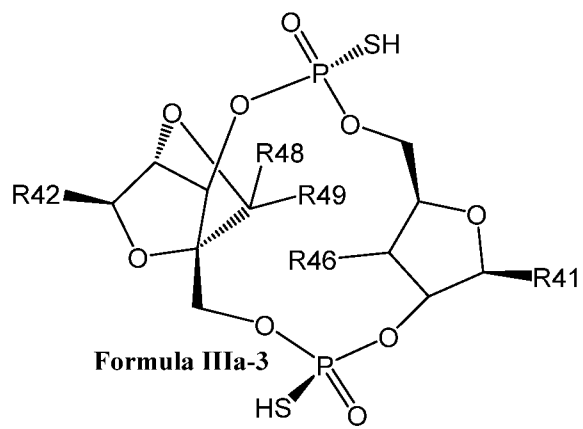
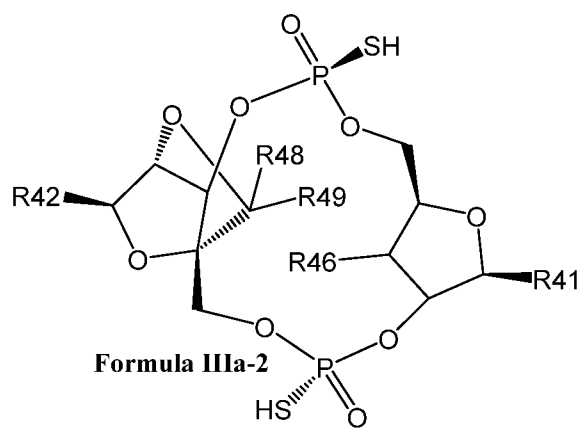
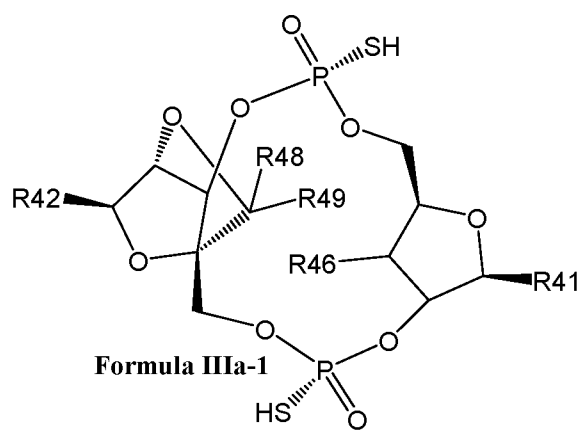
[0040] In a first embodiment of the third aspect, the compound of Formula III is a compound selected from the group consisting of a compound of Formula IIIa, a compound of Formula IIIb, and a compound of Formula IIIc:

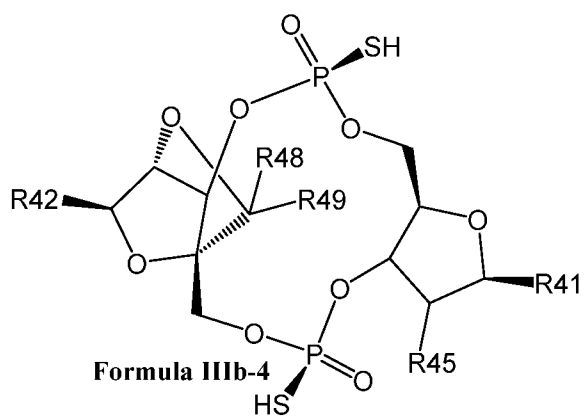
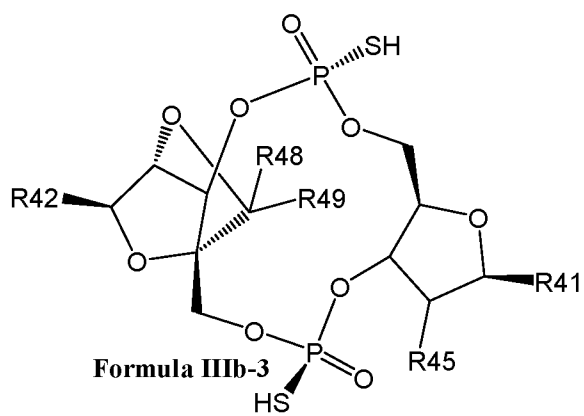
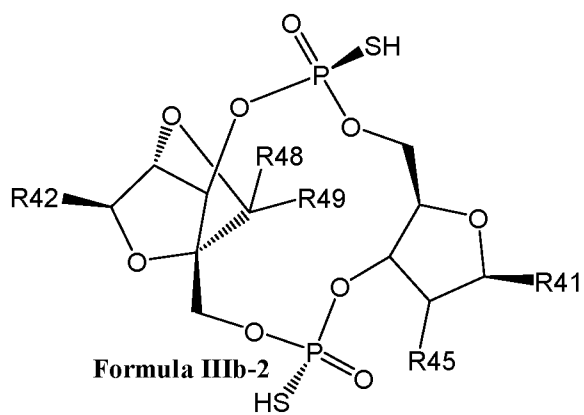
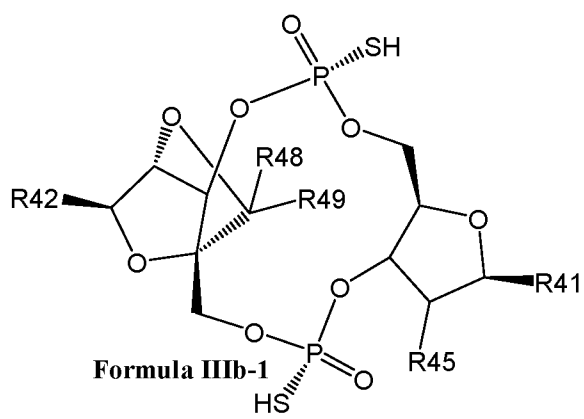


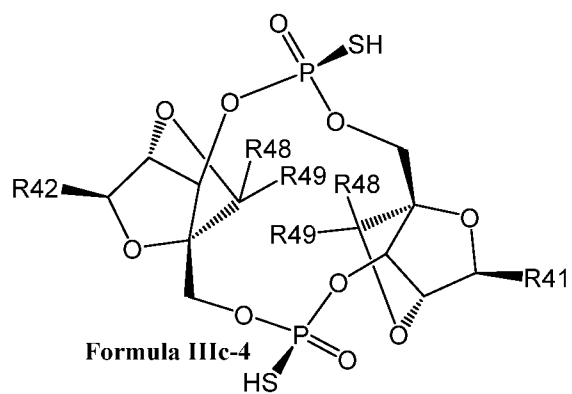
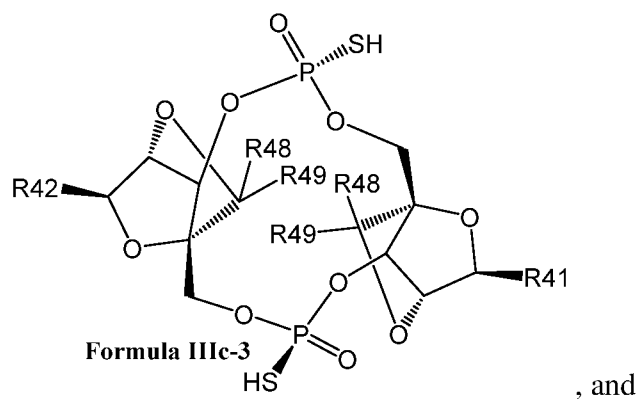
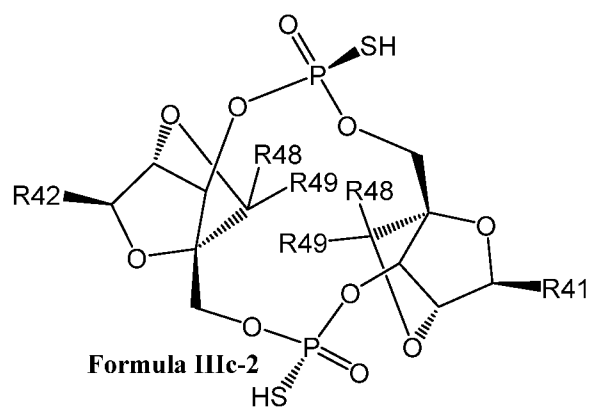
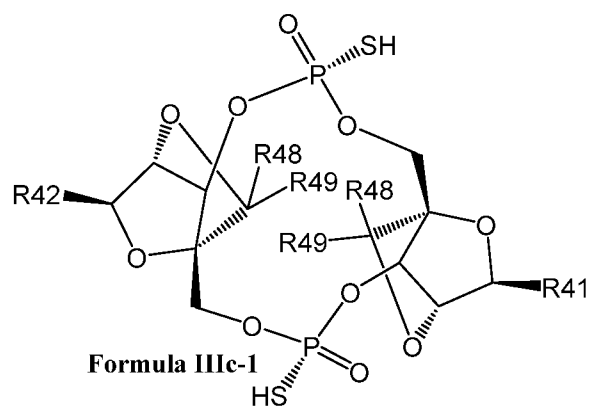
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R41, R42, R43, R44, R45, R46, R48 and R49 are as defined for Formula III.

[0041] In a second embodiment of the third aspect, the compound of Formula III is a compound selected from the group consisting of a compound of Formula IIIa-1, a compound of Formula IIIa-2, a compound of Formula IIIa-3, a compound of Formula IIIa-4, a compound of Formula IIIb-1, a compound of Formula IIIb-2, a compound of Formula IIIb-3, a compound of Formula IIIb-4, a compound of Formula IIIc-1, a compound of Formula IIIc-2, a compound of Formula IIIc-3, and a compound of Formula

IIIc-4:

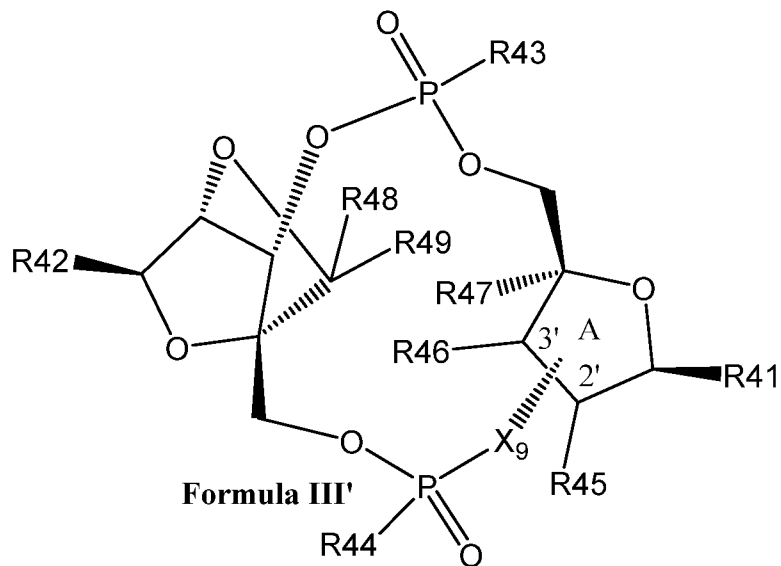






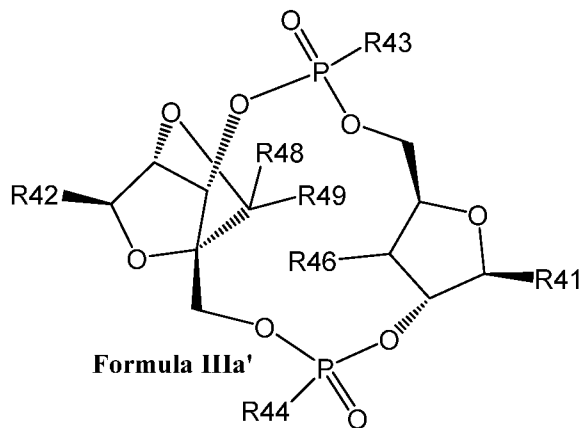
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R41, R42, R45, R46, R48 and R49 are as defined for Formula III.

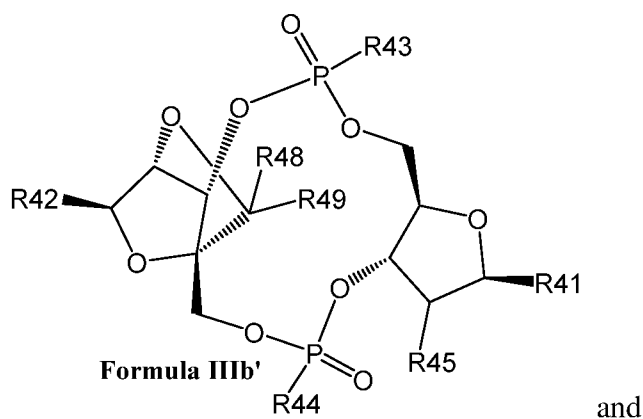
[0042] In a third embodiment of the third aspect, the compound of Formula III is a compound of Formula III':



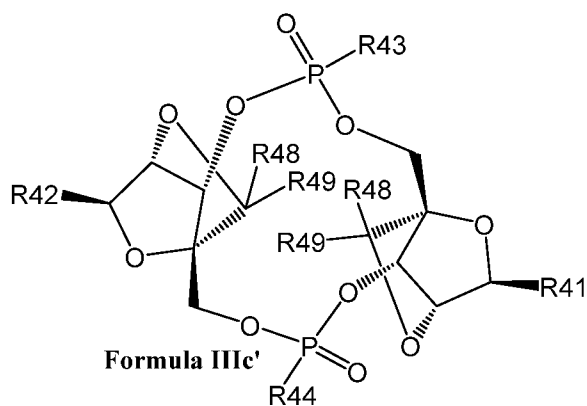
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein X₉, R41, R42, R43, R44, R45, R46, R47, R48 and R49 are as defined for Formula III.

[0043] In a fourth embodiment of the third aspect, the compound of Formula III is a compound selected from the group consisting of a compound of Formula IIIa', a compound of Formula IIIb' and a compound of Formula IIIc':





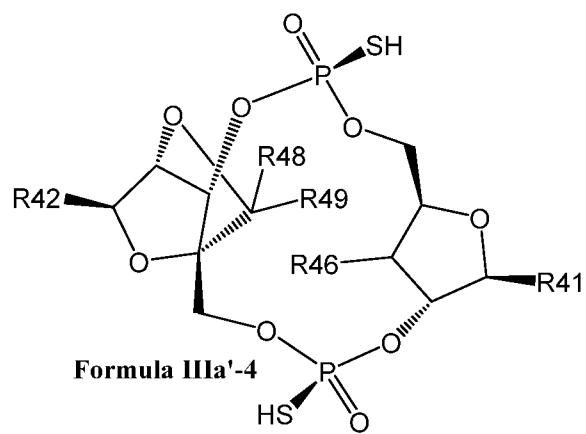
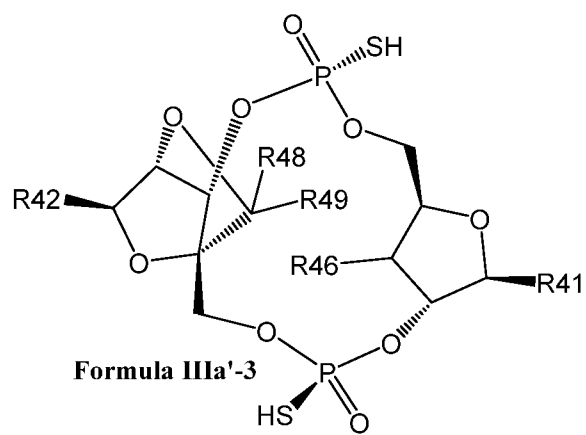
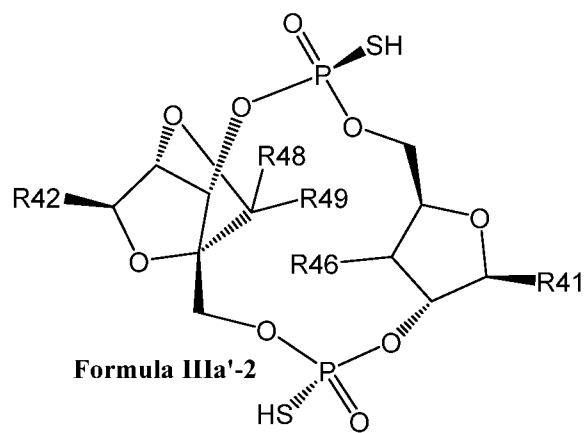
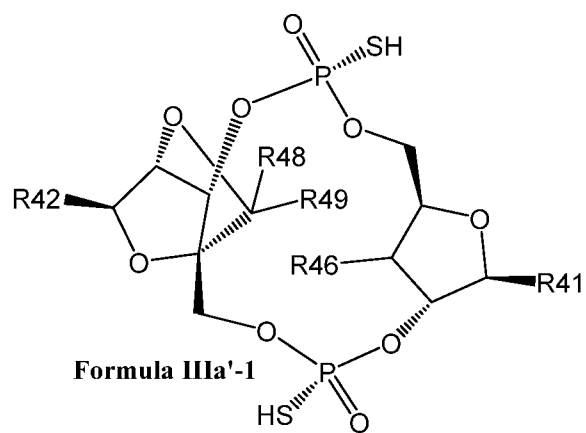
and

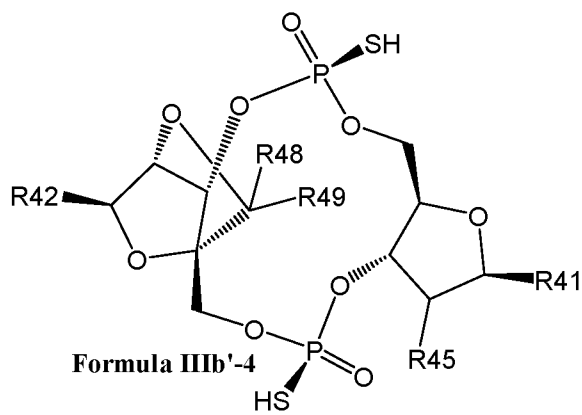
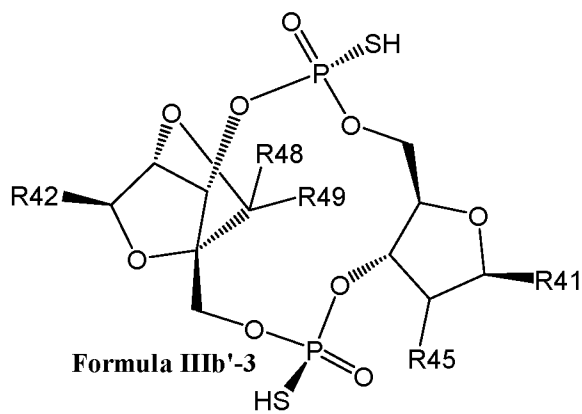
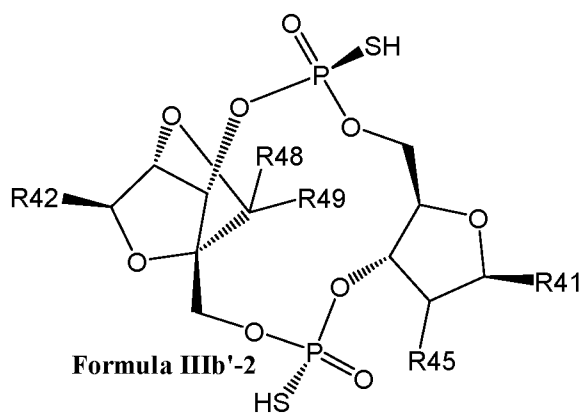
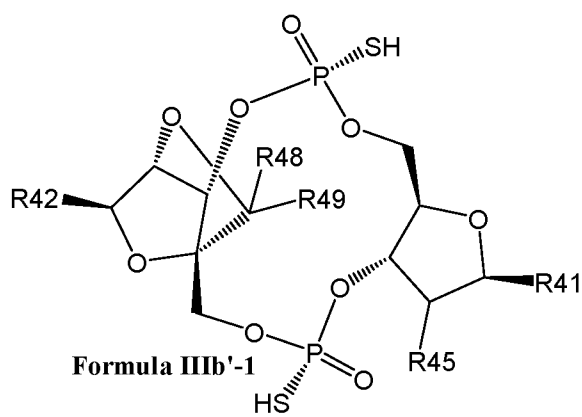


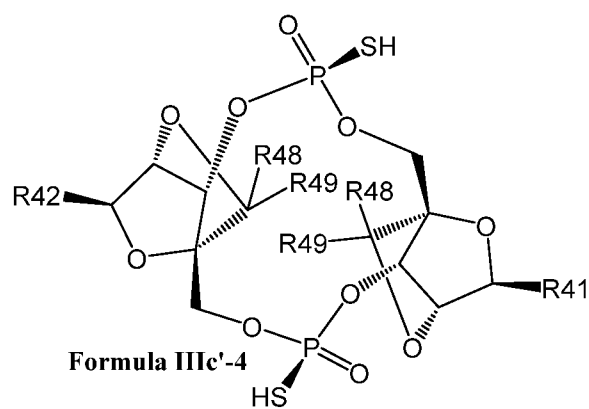
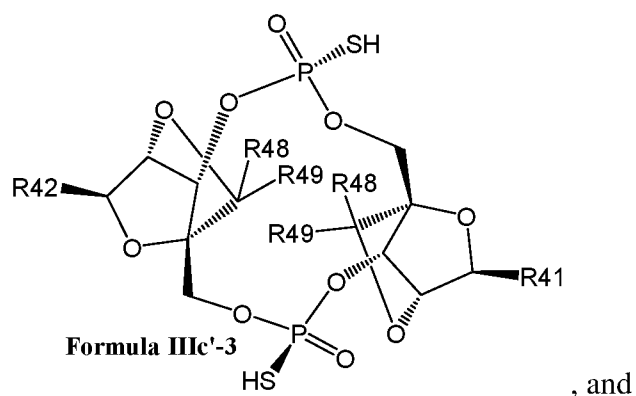
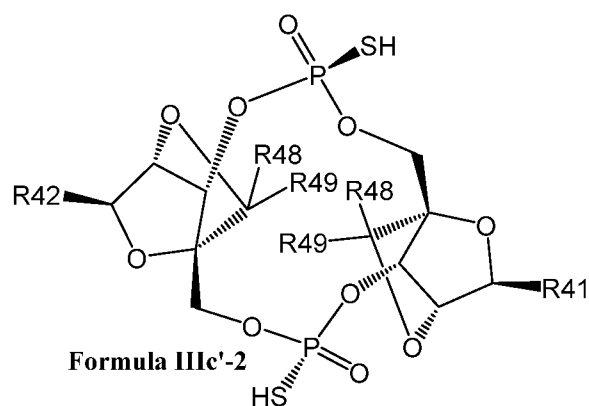
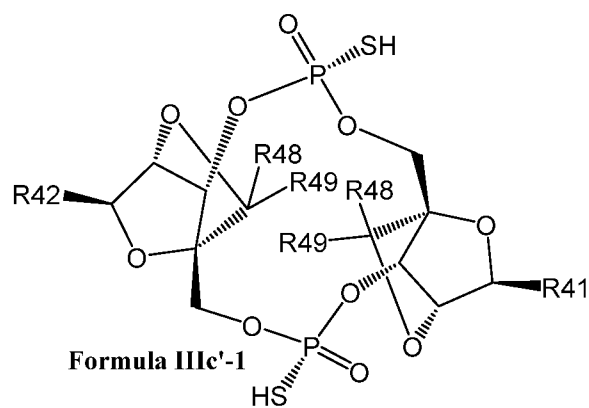
;

or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R41, R42, R43, R44, R45, R46, R48 and R49 are as defined for Formula III.

[0044] In a fifth embodiment of the third aspect, the compound of Formula III' is a compound selected from the group consisting of a compound of Formula IIIa'-1, a compound of Formula IIIa'-2, a compound of Formula IIIa'-3, a compound of Formula IIIa'-4, a compound of Formula IIIb'-1, a compound of Formula IIIb'-2, a compound of Formula IIIb'-3, a compound of Formula IIIb'-4, a compound of Formula IIIc'-1, a compound of Formula IIIc'-2, a compound of Formula IIIc'-3, and a compound of Formula IIIc'-4:







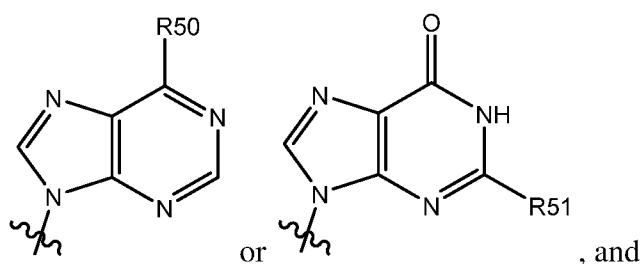
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R41, R42, R45, R46, R48 and R49 are as defined for Formula III. In some embodiment of the compounds of Formula IIIa'-1,

Formula IIIa'-2, Formula IIIa'-3, Formula IIIa'-4, Formula IIIb'-1, Formula IIIb'-2, Formula IIIb'-3, or Formula IIIb'-4, the bond to R45 or R46 is preferably down into the

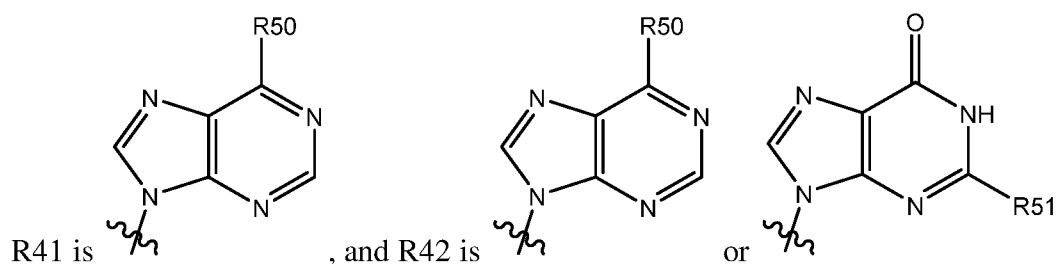
paper, i.e. in the above structures is represented as e.g.  R45, where the * represents the bond to the core ring.

[0045] In a sixth embodiment of the third aspect, and any of the above embodiments thereof, R43 and R44 are independently -OH or -SH. In some embodiments, R43 and R44 are both -OH. In some embodiments, R43 and R44 are both -SH. In some embodiments, one of R43 and R44 is -OH and the other is -SH. In some embodiments, R43 and R44 are both -SH. In some embodiments, R43 and R44 are both -SH, and the compound is substantially pure with respect to the Rp,Rp isomer, the Rp,Sp isomer, the Sp,Rp isomer, or the Sp,Sp isomer.

[0046] In a seventh embodiment of the third aspect, and any of the above embodiments thereof, when X₉ is -O- bonded to the 3' carbon atom of ring A, R41 and R42 are independently



when X₉ is -O- bonded to the 2' carbon atom of ring A,

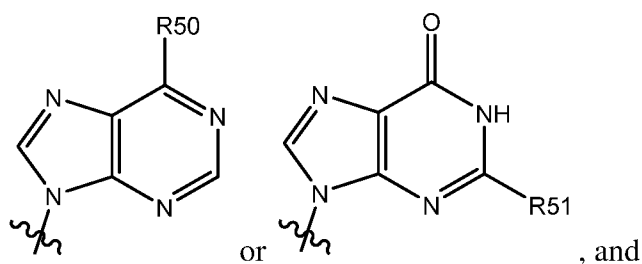


wherein R50 and R51 are independently -NH₂, -NH-C₁₋₆alkyl, -N=CH-N(CH₃)₂, -NH-C(=O)-C₁₋₆alkyl or -NH-C(=O)-phenyl. In some embodiments, R50 is -NH₂ or -NH-C(=O)-phenyl and R51 is -NH₂ or -NH-C(=O)-isopropyl. In some embodiments R50 and R51 are both -NH₂.

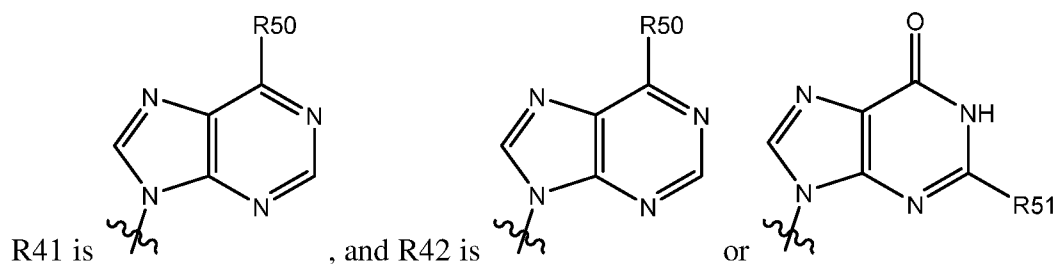
[0047] In an eighth embodiment of the third aspect, and any of the above embodiments thereof, when R45 or R46 are selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl, R45 or R46 are selected from the group consisting of -H, -OH, -OTBS, -F and -O-(C=O)-C₁₋₁₄alkyl, or R45 and R46 are -H, -OH, -OTBS or -F, or R45 and R46 are -H, -OH or -F or R45 and R46 are -OH or -F.

[0048] In a ninth embodiment of the third aspect, and any of the above embodiments thereof, R48 and R49 at each occurrence are independently -H or -C₁₋₆alkyl. In some embodiments, R48 and R49 at each occurrence are -H.

[0049] In a tenth embodiment of the third aspect, R43 and R44 are both -SH; when X₉ is -O- bonded to the 3' carbon atom of ring A, R41 and R42 are independently

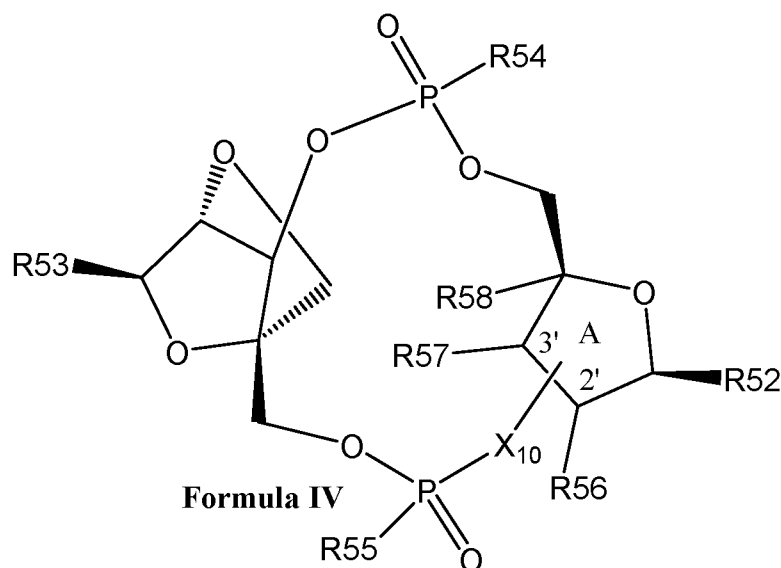


when X₉ is -O- bonded to the 2' carbon atom of ring A,



wherein each R50 is independently -NH₂ or -NH-C(=O)-phenyl and each R51 is independently -NH₂ or -NH-C(=O)-isopropyl, preferably where R50 and R52 are both -NH₂; when R45 or R46 are selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl, R45 or R46 are selected from the group consisting of -H, -OH, -OTBS, -F and -O-(C=O)-C₁₋₁₄alkyl, or R45 and R46 are -H, -OH or -F, preferably where R45 or R46 are -OH or -F; and R48 and R49 at each occurrence are independently -H or -C₁₋₆alkyl, preferably where R48 and R49 are both -H.

[0050] In a fourth aspect, the present invention provides a locked nucleic acid cyclic dinucleotide ("LNA-CDN") compound of Formula IV:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof,

wherein:

R54 and R55 are independently -OH or -SH;

X₁₀ is -O- bonded to either the 2' or the 3' carbon atom of the indicated ring A;

wherein:

when X₁₀ is -O- bonded to the 2' carbon atom of ring A:

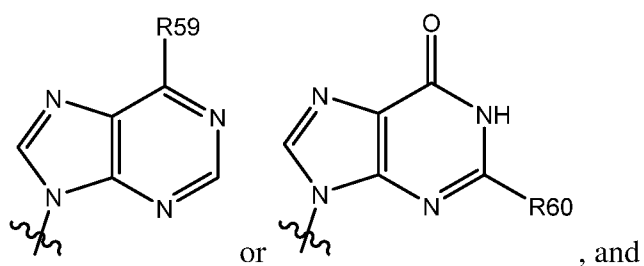
R56 is absent, R57 is selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, and halogen, and R58 is -H;

when X₁₀ is -O- bonded to the 3' carbon atom of ring A:

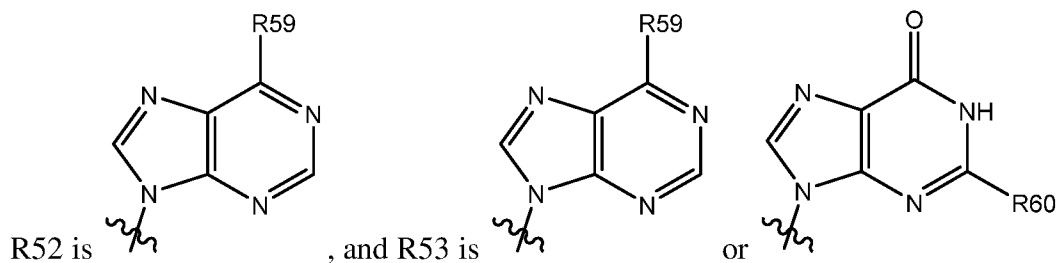
R57 is absent, R56 is selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, and halogen, and R58 is -H; or

R57 is absent, and R56 and R58 join to form -O-CH₂-, resulting in a fused ring system with -O- bonded to the 2' carbon atom of ring A and -CH₂- bonded to the carbon atom of ring A to which R58 is attached; and

when X₁₀ is -O- bonded to the 3' carbon atom of ring A, R52 and R53 are independently

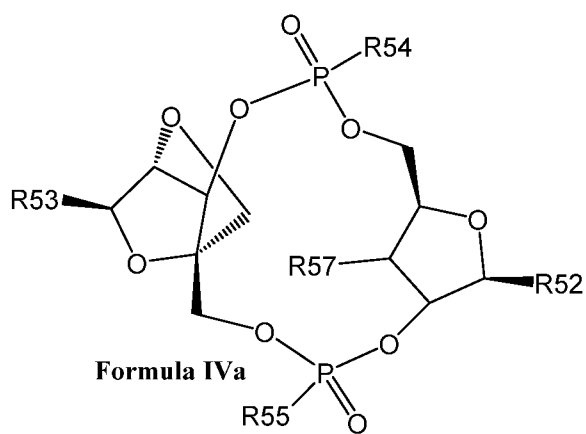


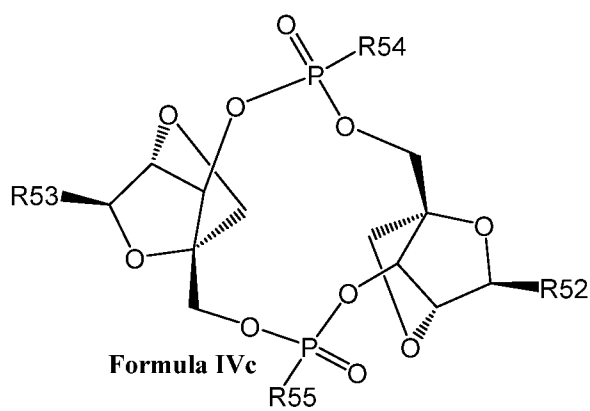
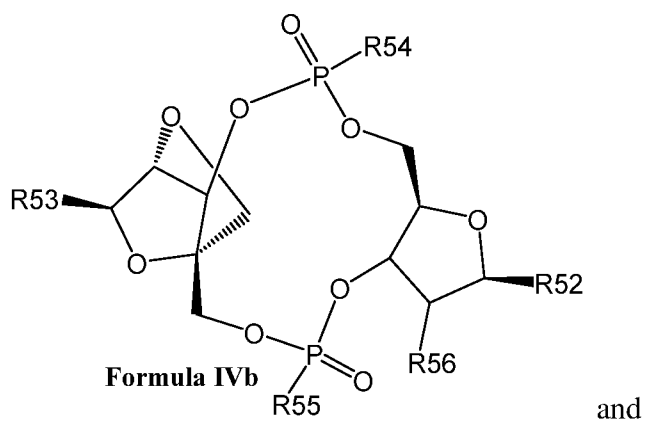
when X₁₀ is -O- bonded to the 2' carbon atom of ring A,



wherein  indicates the bond of R52 or R53 to the ring as shown in Formula IV, and wherein R59 and R60 are independently -NH₂ optionally substituted with a suitable nitrogen protecting group.

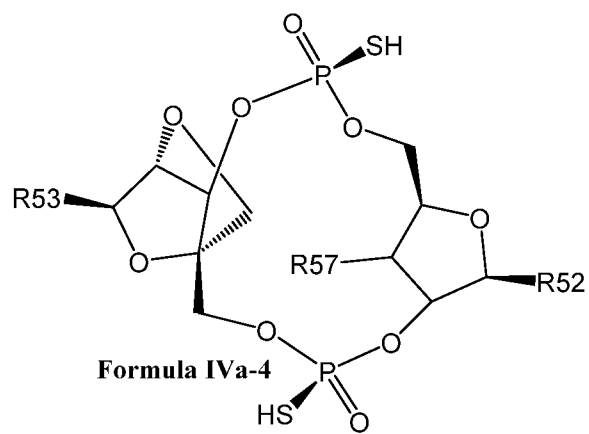
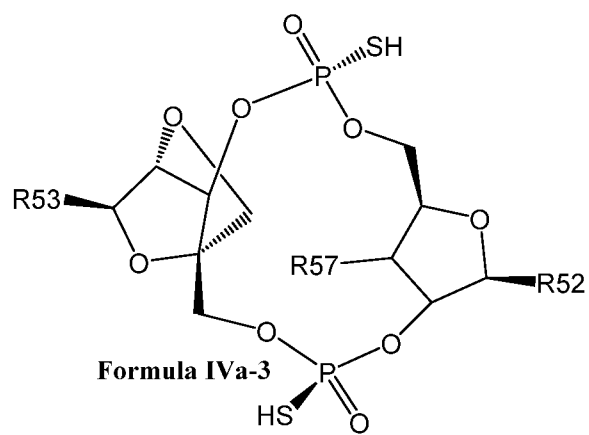
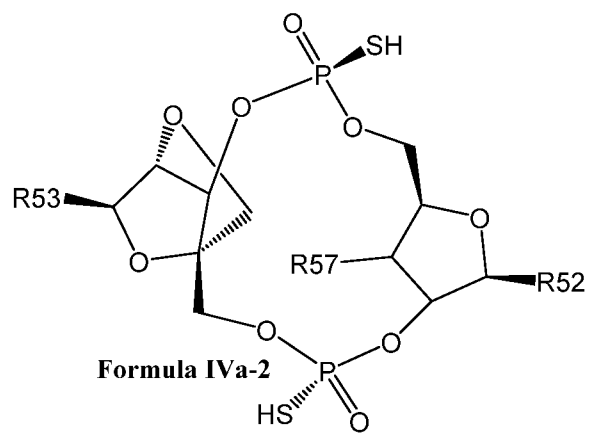
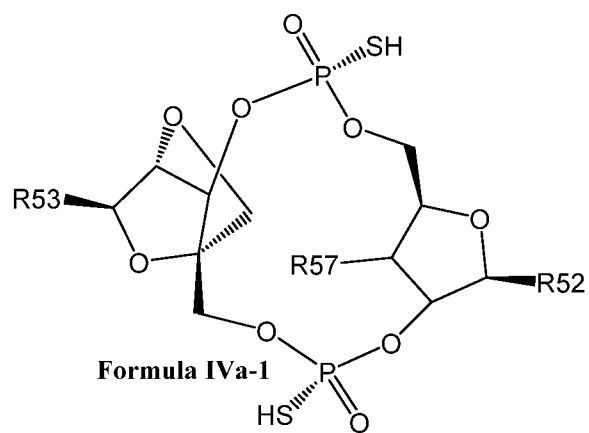
[0051] In a first embodiment of the fourth aspect, the compound of Formula IV is a compound selected from the group consisting of a compound of Formula IVa, a compound of Formula IVb and a compound of Formula IVc:

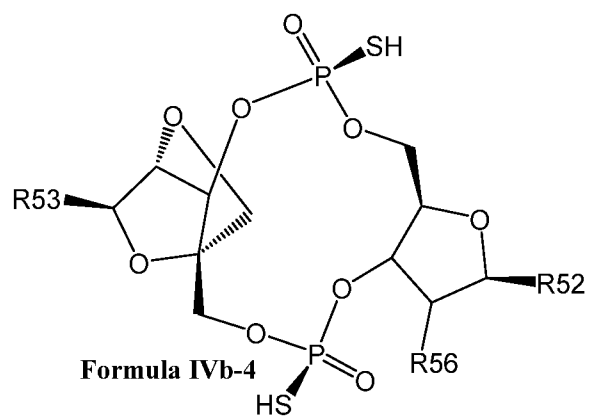
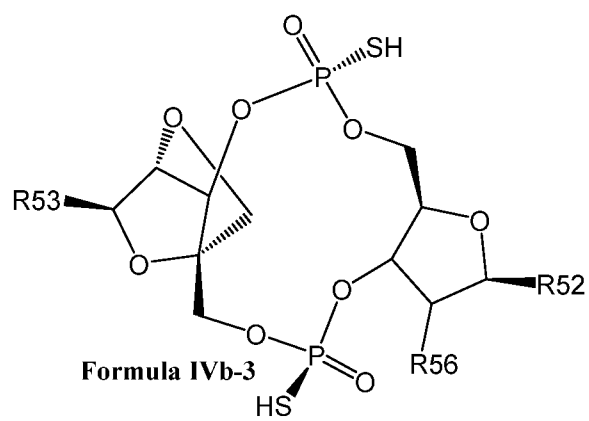
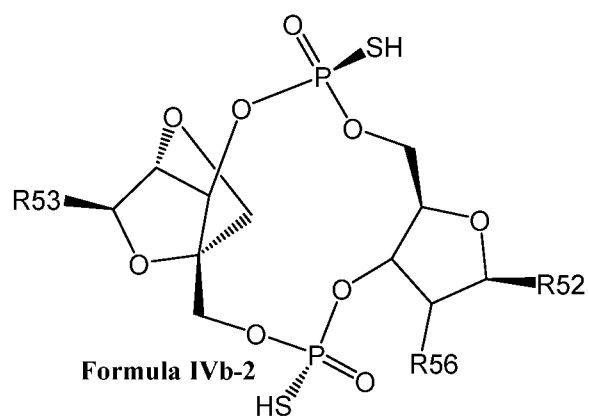
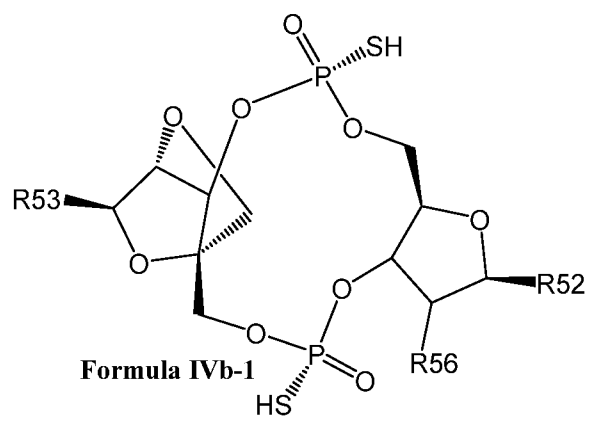


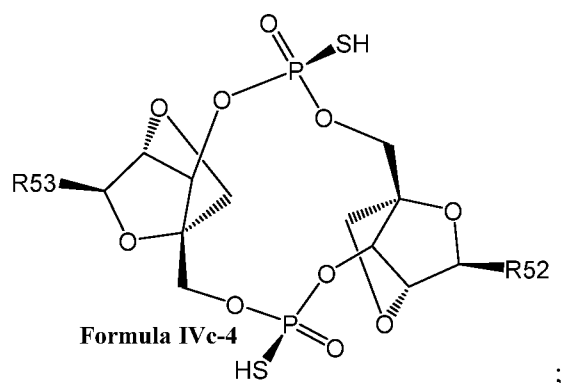
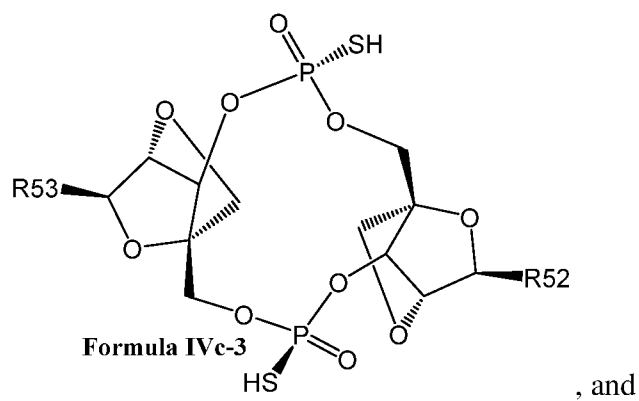
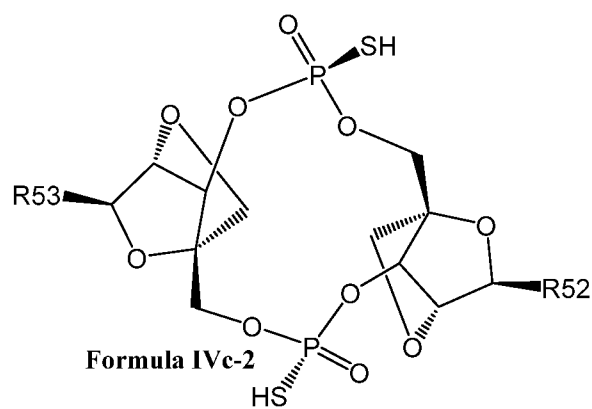
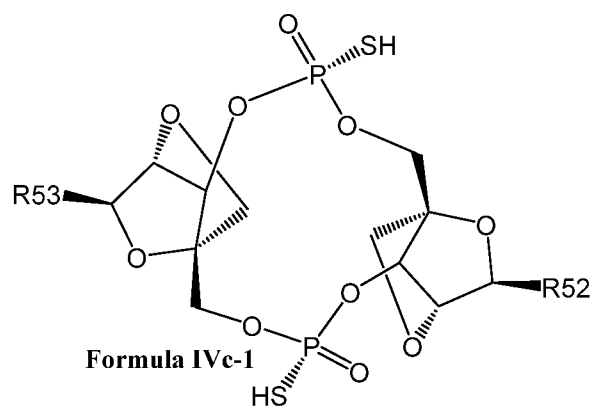


or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R52, R53, R54, R55, R56 and R57 are as defined for Formula IV.

[0052] In a second embodiment of the fourth aspect, the compound of Formula IV is a compound selected from the group consisting of a compound of Formula IVa-1, a compound of Formula IVa-2, a compound of Formula IVa-3, a compound of Formula IVa-4, a compound of Formula IVb-1, a compound of Formula IVb-2, a compound of Formula IVb-3, a compound of Formula IVb-4, a compound of Formula IVc-1, a compound of Formula IVc-2, a compound of Formula IVc-3, and a compound of Formula IVc-4:

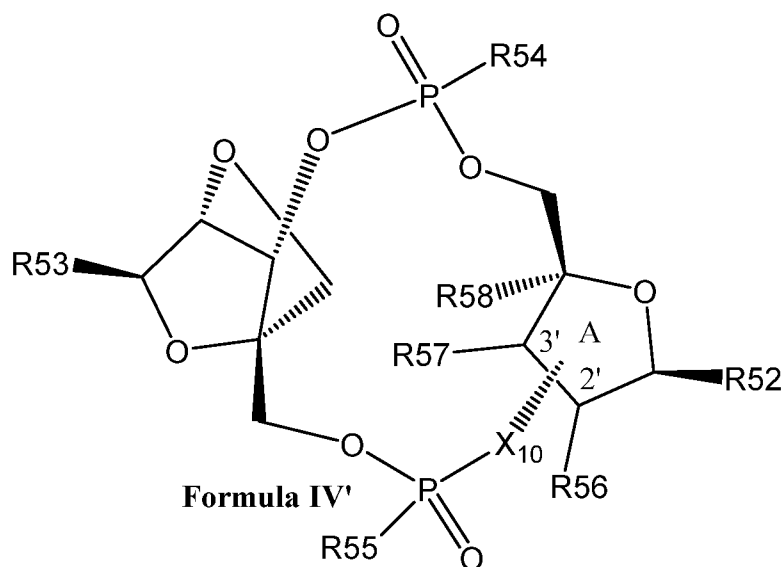






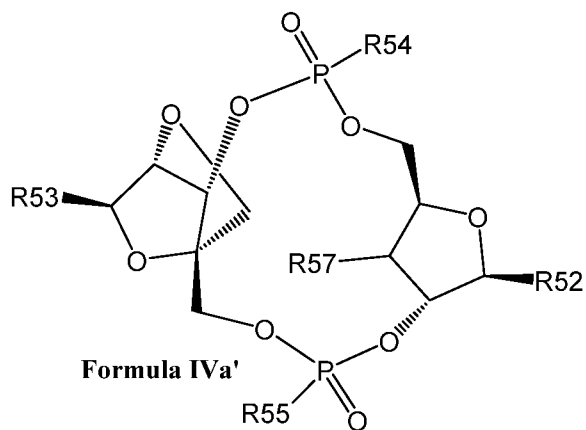
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R52, R53, R56 and R57 are as defined for Formula IV.

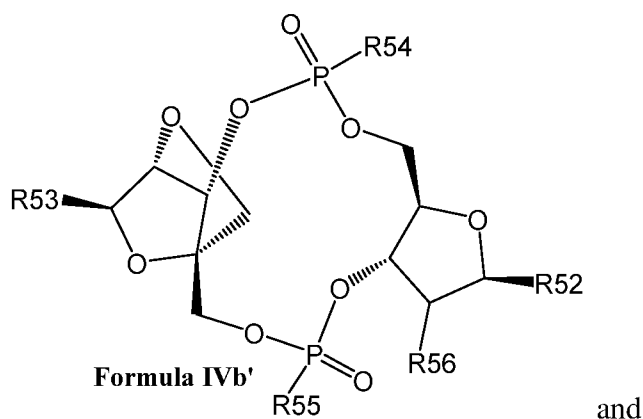
[0053] In a third embodiment of the fourth aspect, the compound of Formula IV is a compound of Formula IV':



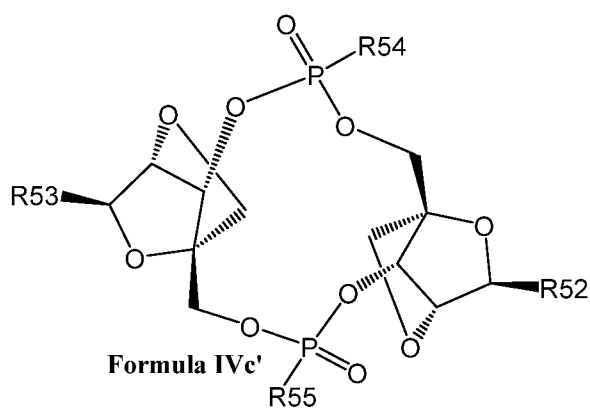
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein X₁₀, R52, R53, R54, R55, R56, R57 and R58 are as defined for Formula IV.

[0054] In a fourth embodiment of the fourth aspect, the compound of Formula IV is a compound selected from the group consisting of a compound of Formula IVa', a compound of Formula IVb' and a compound of Formula IVc':





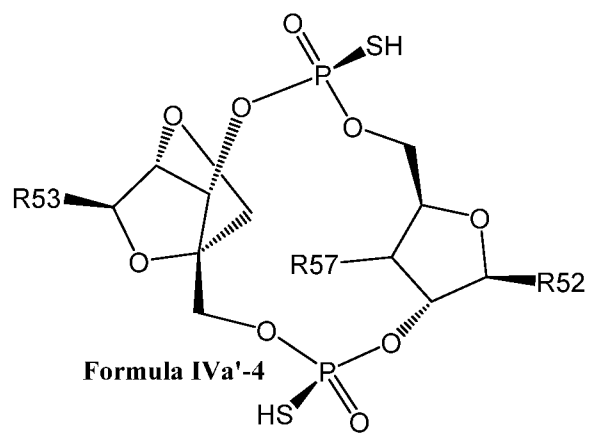
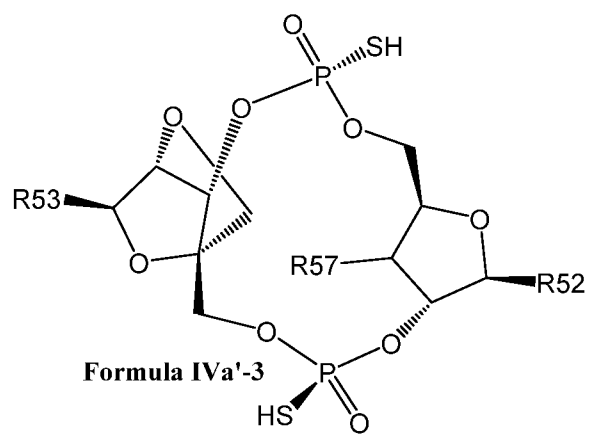
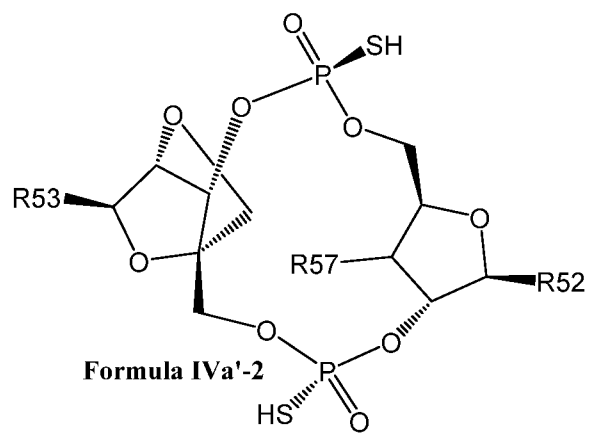
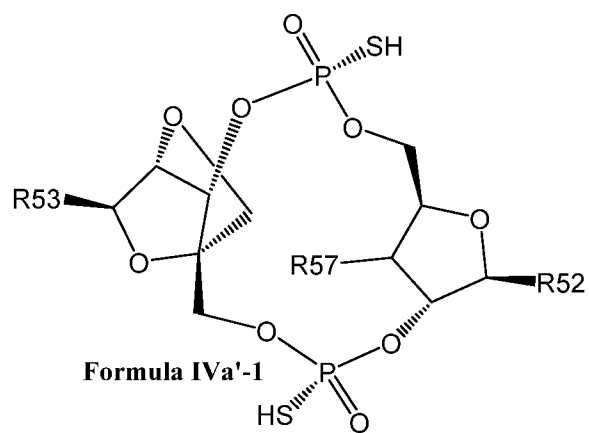
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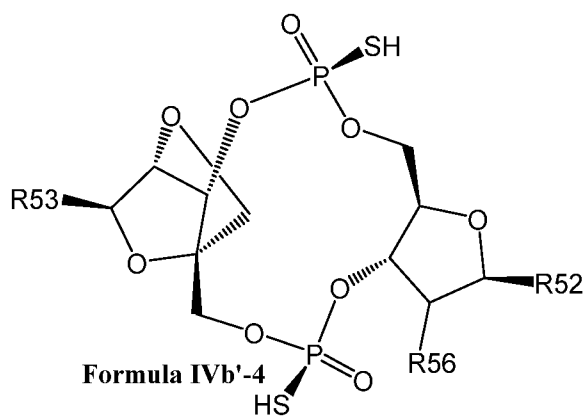
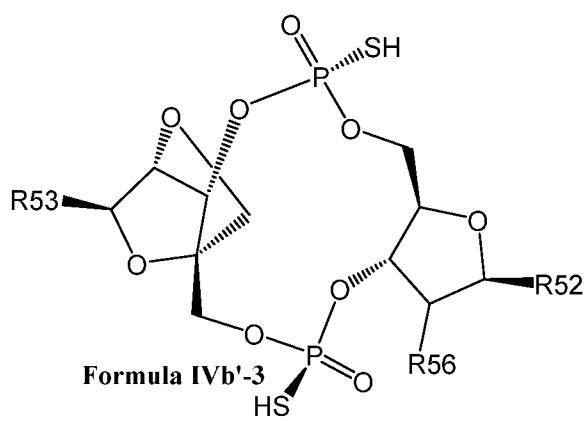
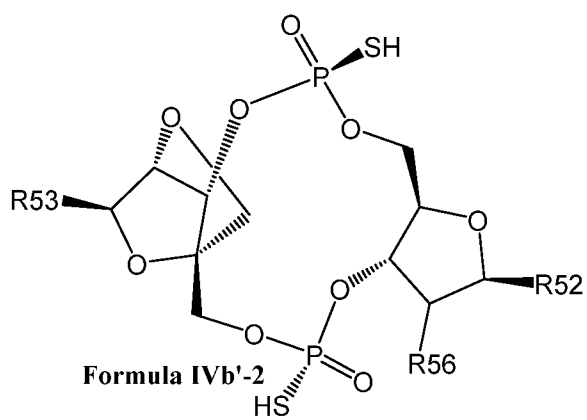
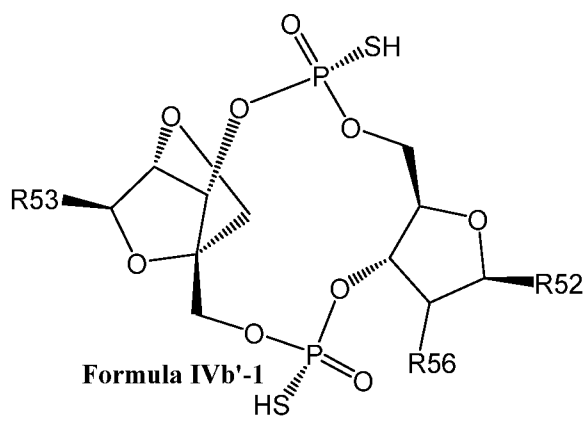


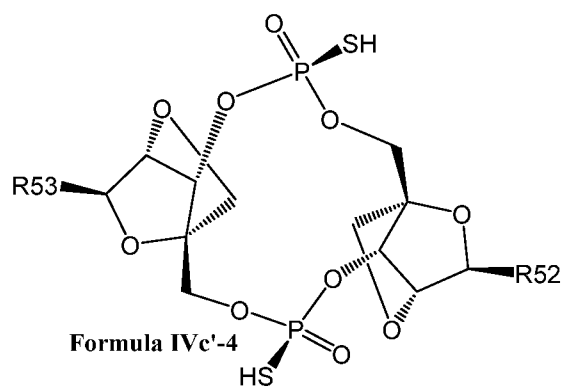
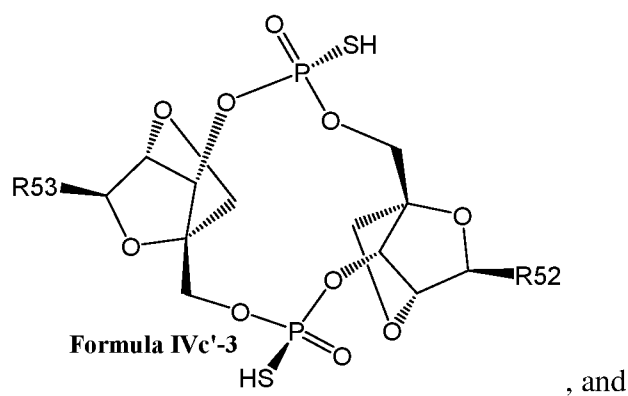
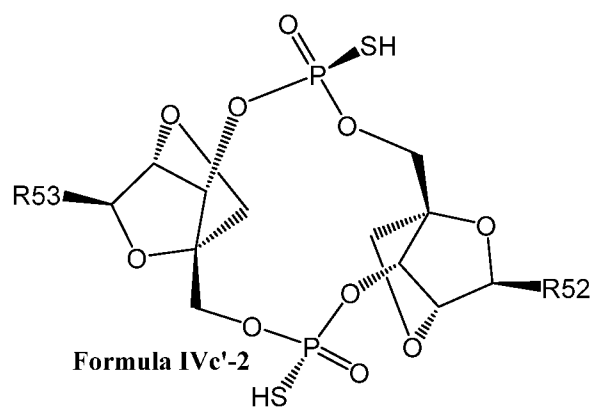
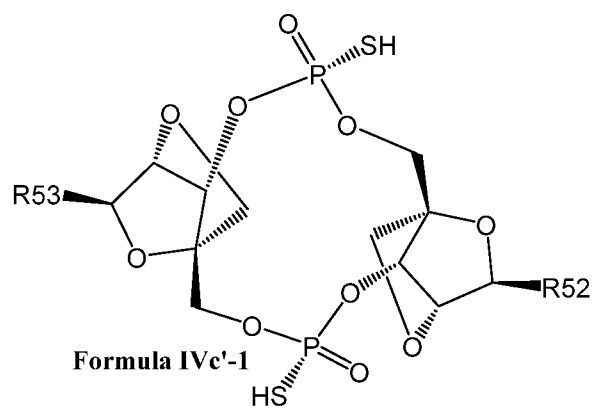
;

or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R52, R53, R54, R55, R56 and R57 are as defined for Formula IV.

[0055] In a fifth embodiment of the fourth aspect, the compound of Formula IV is a compound selected from the group consisting of a compound of Formula IVa'-1, a compound of Formula IVa'-2, a compound of Formula IVa'-3, a compound of Formula IVa'-4, a compound of Formula IVb'-1, a compound of Formula IVb'-2, a compound of Formula IVb'-3, a compound of Formula IVb'-4, a compound of Formula IVc'-1, a compound of Formula IVc'-2, a compound of Formula IVc'-3, and a compound of Formula IVc'-4:







or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R52, R53, R56 and R57 are as defined for Formula IV. In some embodiment of the compounds of Formula IVa'-1,

Formula IVa'-2, Formula IVa'-3, Formula IVa'-4, Formula IVb'-1, Formula IVb'-2, Formula IVb'-3, or Formula IVb'-4, the bond to R56 or R57 is preferably down into the

paper, i.e. in the above structures is represented as e.g.  R56, where the * represents the bond to the core ring.

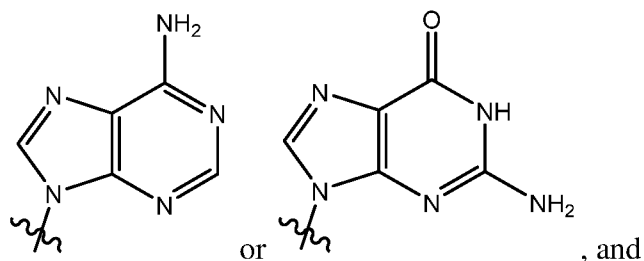
[0056] In a sixth embodiment of the fourth aspect, and any of the above embodiments thereof, R56 or R57 are -H, -OH, -OTBS or -F. In some embodiments R56 or R57 are -H, -OH or -F. In some embodiments R56 or R57 are -OH or -F.

[0057] In a seventh embodiment of the fourth aspect, and any of the above embodiments thereof, R54 and R55 are -SH, preferably wherein relative to the stereochemistry at each phosphorous atom the compound is the substantially pure Rp,Rp isomer, the substantially pure Rp,Sp isomer, the substantially pure Sp,Rp isomer, or the substantially pure Sp,Sp isomer.

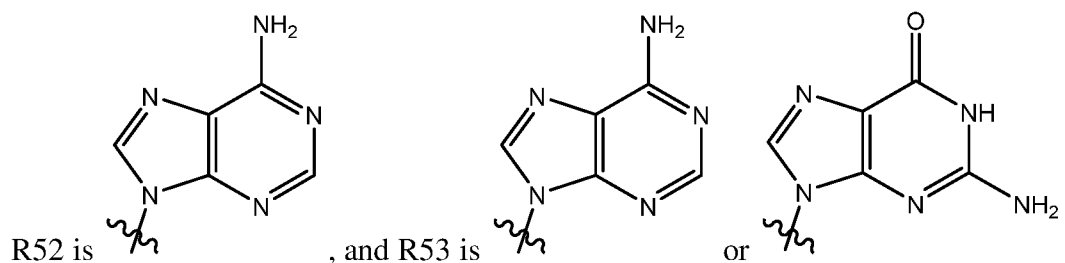
[0058] In an eighth embodiment of the fourth aspect, and any of the above embodiments thereof, each R59 is independently -NH₂ or -NH-C(=O)-phenyl and each R60 is independently -NH₂ or -NH-C(=O)-isopropyl. In some embodiments, R59 and R60 are -NH₂.

[0059] In a sixth embodiment of the fourth aspect, and any of the above embodiments thereof, R56 or R57 are -H, -OH or -F. In some embodiments, R54 and R55 are -SH and R56 or R57 are -H, -OH or -F. In some embodiments, R54 and R55 are -SH, R56 or R57 are -H, -OH or -F, and each occurrence of R59 and R60 is -NH₂.

[0060] In a seventh embodiment of the fourth aspect, and any of the above embodiments thereof, when X₁₀ is -O- bonded to the 3' carbon atom of ring A, R52 and R53 are independently



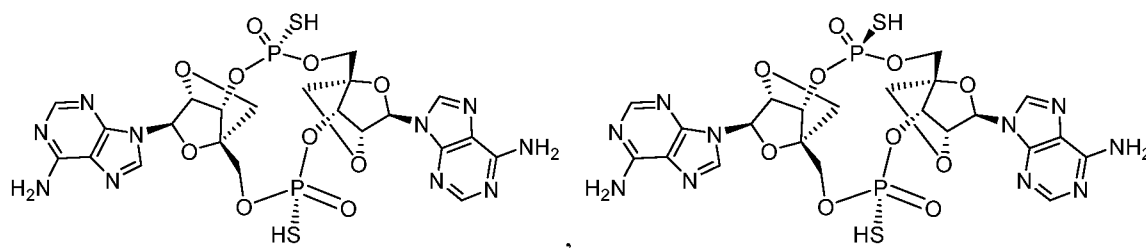
when X₁₀ is -O- bonded to the 2' carbon atom of ring A,

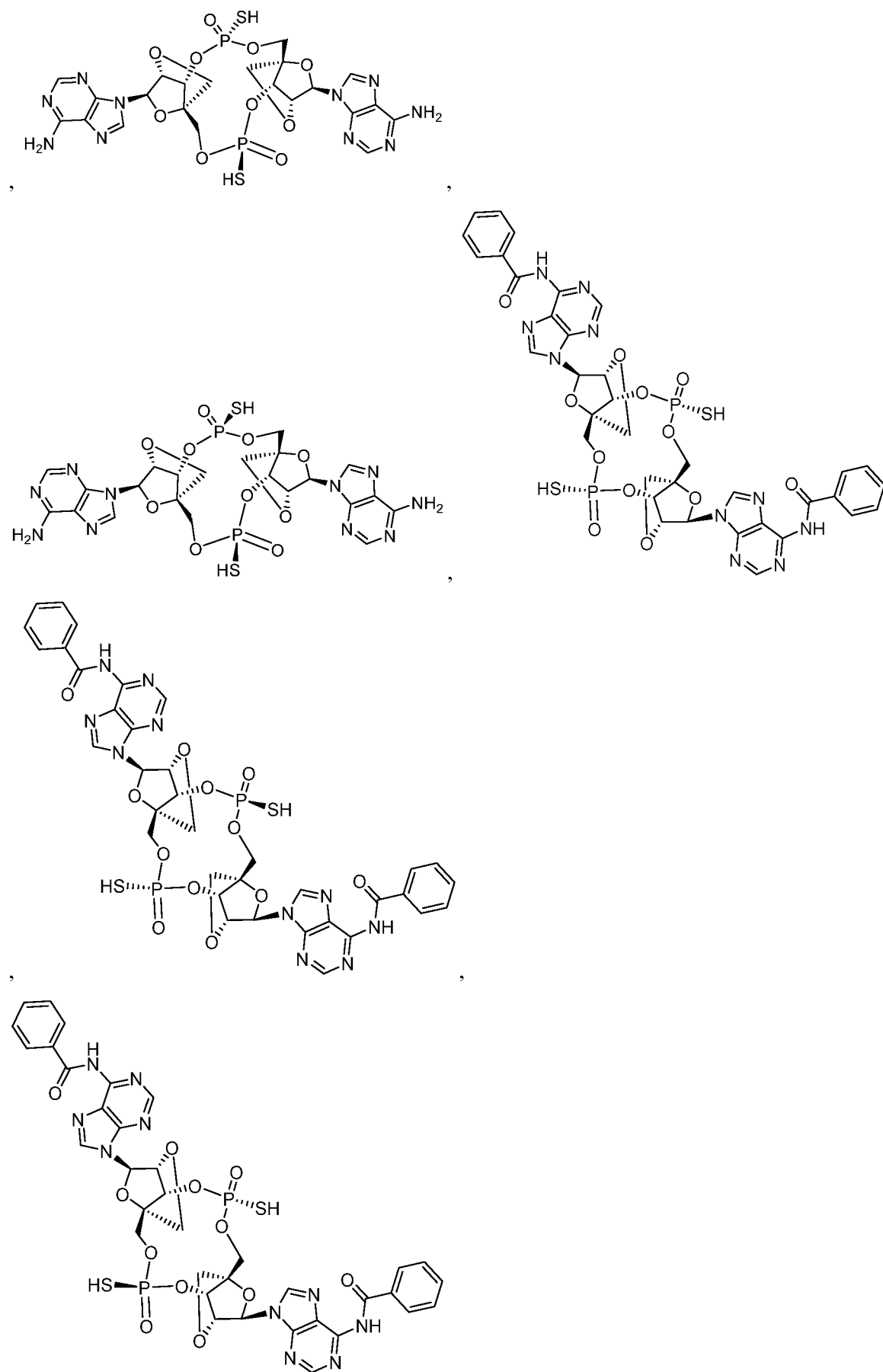


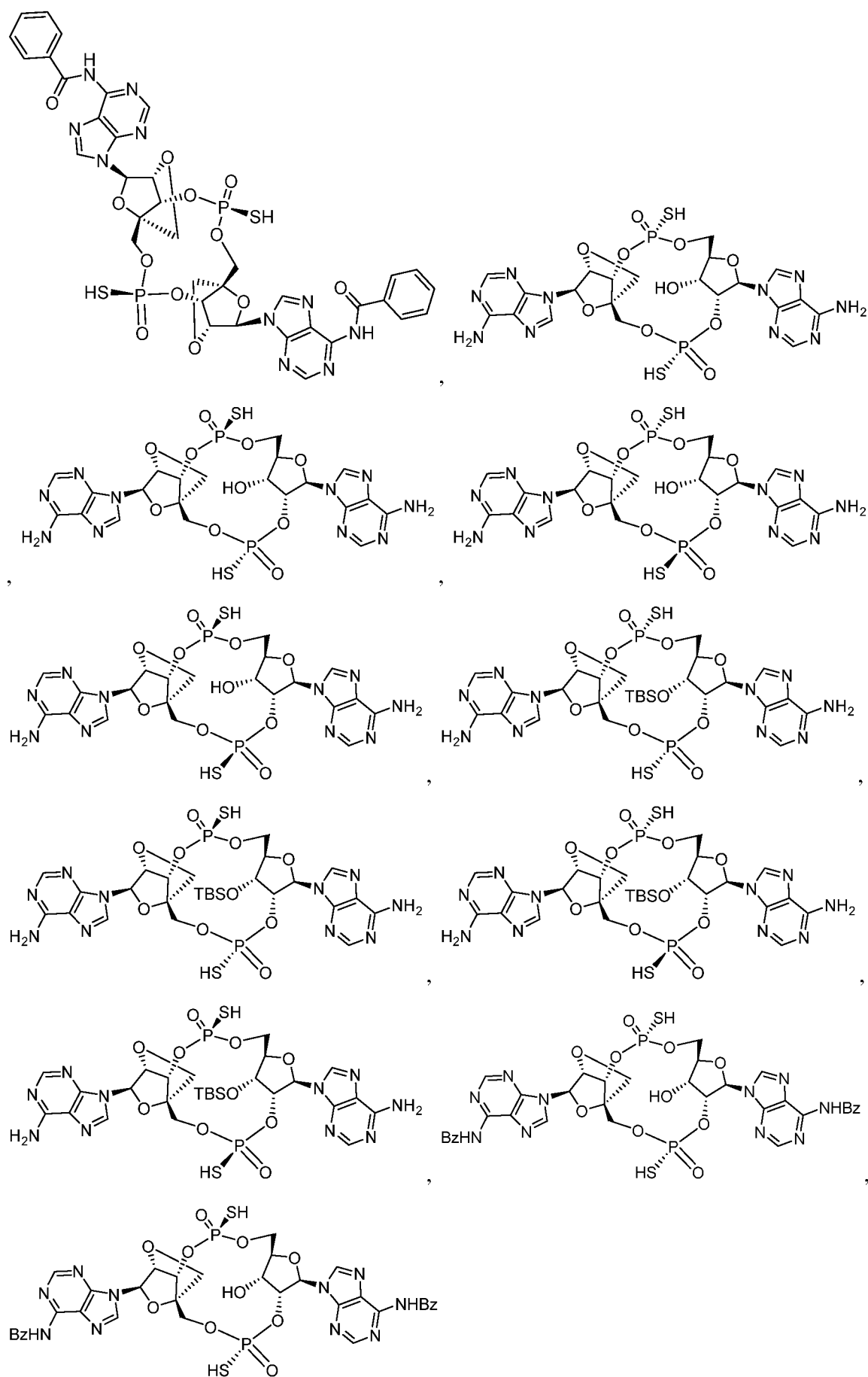
[0061] In any of the above aspects and embodiments, an oxygen protecting group is selected from the group consisting of a silyl protecting group, an ester forming protecting group and an ether forming protecting group. In a preferred embodiment, the oxygen protecting group is selected from the group consisting of TBS ($-\text{Si}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$), TMS ($-\text{Si}(\text{CH}_3)_3$), TES ($-\text{Si}(\text{CH}_2\text{CH}_3)_3$), TIPS ($-\text{Si}(\text{CH}(\text{CH}_3)_2)_3$), TBDPS ($-\text{Si}(\text{C}(\text{CH}_3)_3)(\text{phenyl})_2$), pivalyl ($-\text{C}(=\text{O})\text{C}(\text{CH}_3)_3$), acetyl ($-\text{C}(=\text{O})\text{CH}_3$), methyl ($-\text{CH}_3$), methoxymethyl ($-\text{CH}_2-\text{O}-\text{CH}_3$), t-butyl ($-\text{C}(\text{CH}_3)_3$), allyl ($-\text{CH}_2-\text{CH}=\text{CH}_2$), benzyl ($-\text{CH}_2$ -phenyl) and p-methoxybenzyl ($-\text{CH}_2$ -(4-methoxy-phenyl)). In some embodiments, the oxygen protecting group is TBS.

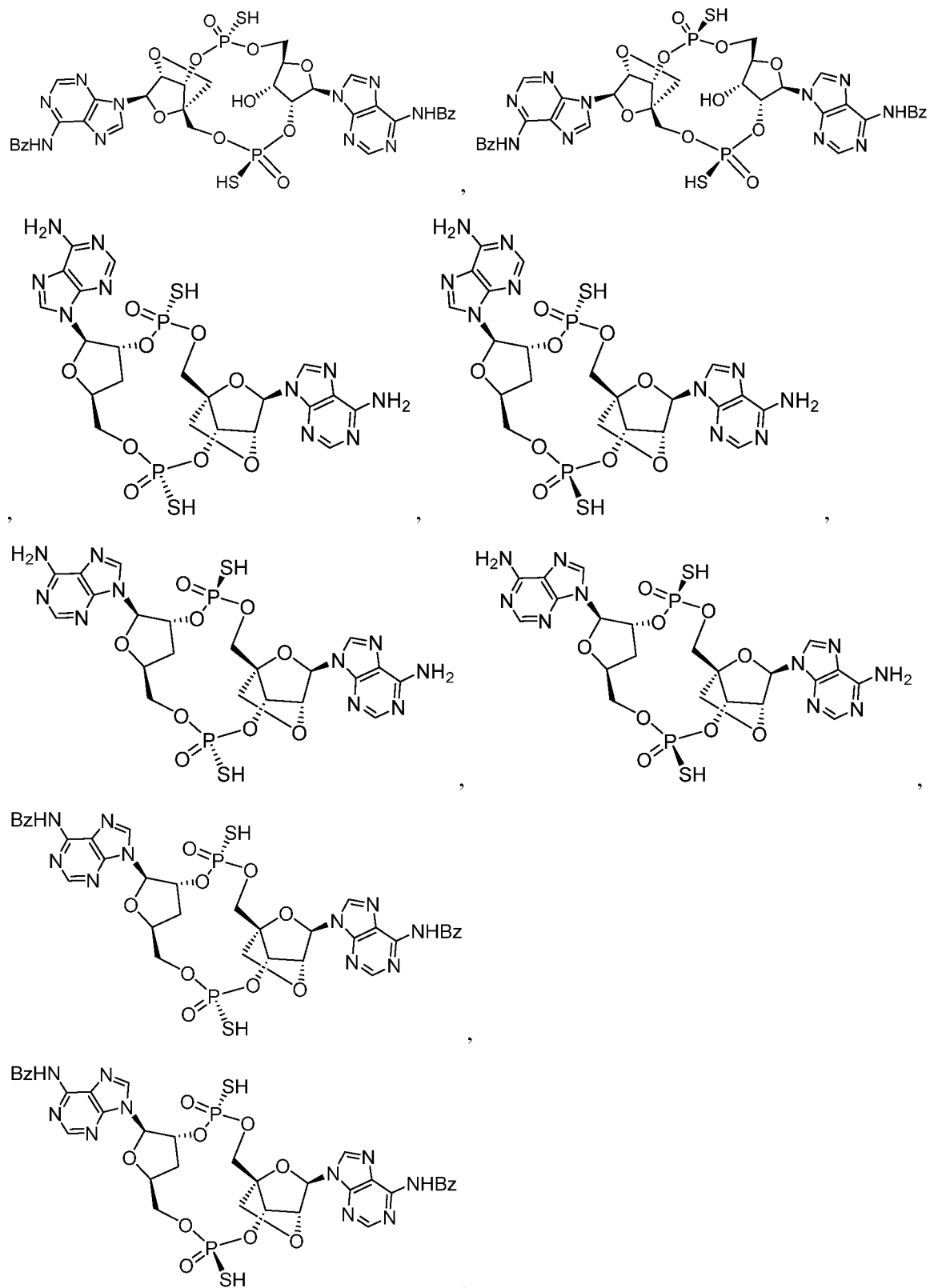
[0062] In any of the above aspects and embodiments, a nitrogen protecting group is selected from the group consisting of benzyl ($-\text{CH}_2$ -phenyl), benzoyl ($-\text{C}(=\text{O})$ -phenyl), isobutyryl ($-\text{C}(=\text{O})-\text{CH}(\text{CH}_3)_2$), acetyl ($-\text{C}(=\text{O})-\text{CH}_3$), t-butyl formate ($-\text{C}(=\text{O})\text{OC}(\text{CH}_3)_2$), and N'N'-dimethylformamidine ($=\text{CH}-\text{N}(\text{CH}_3)_2$, i.e. the protected nitrogen is $-\text{N}=\text{CH}-\text{N}(\text{CH}_3)_2$). In some embodiments the nitrogen protecting group is benzoyl or isobutyryl.

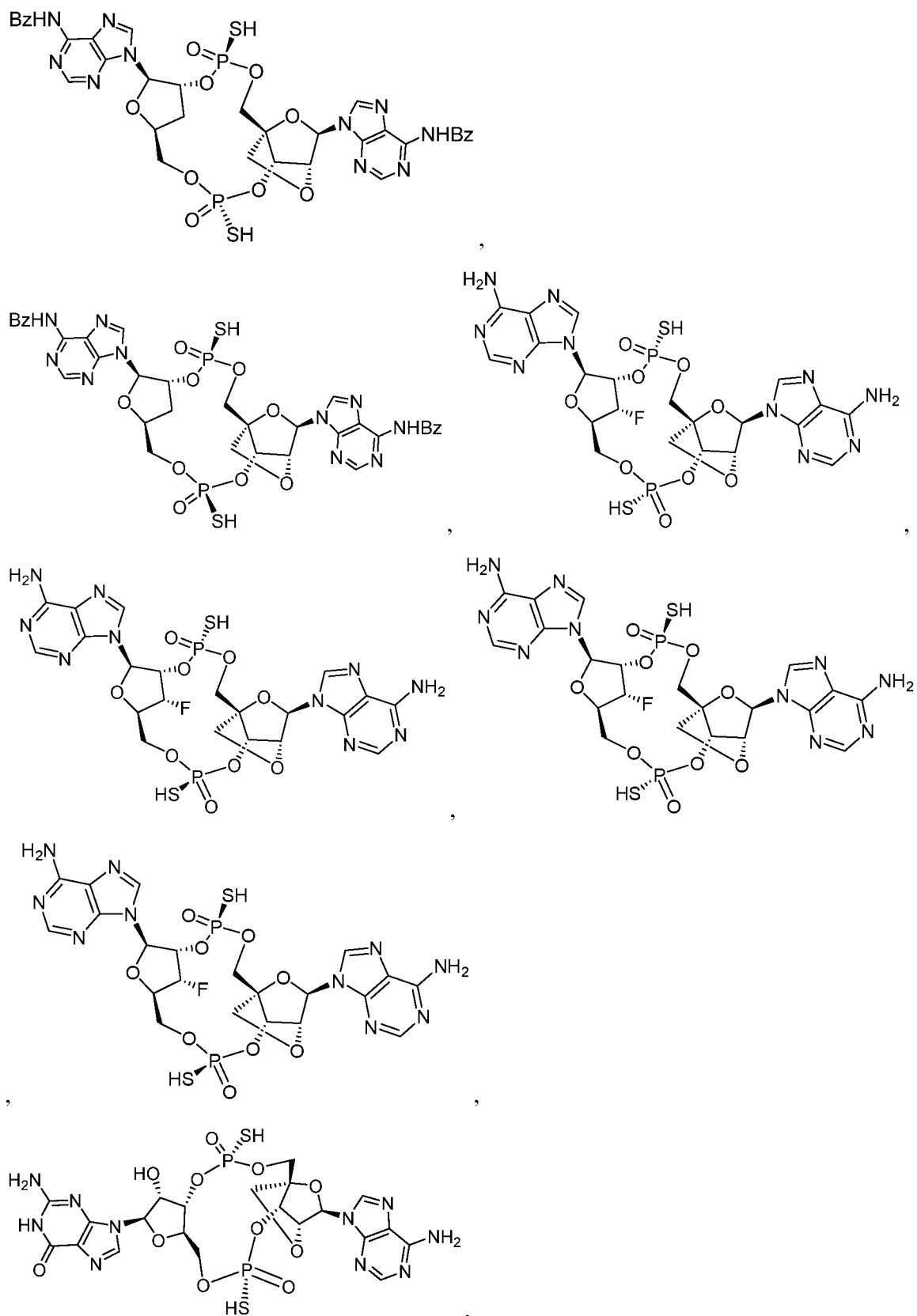
[0063] In some embodiments of any of the above aspects and embodiments, the LNA-CDN compound is selected from the group consisting of:

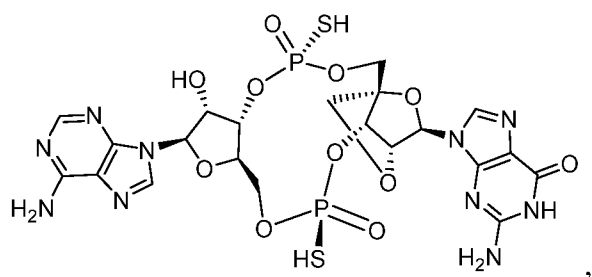
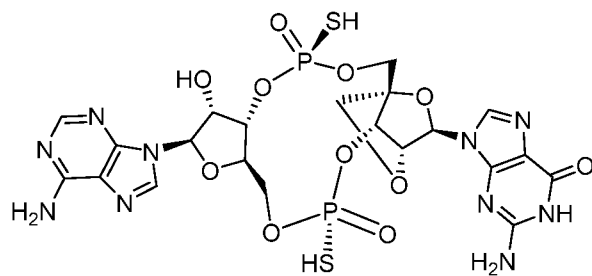
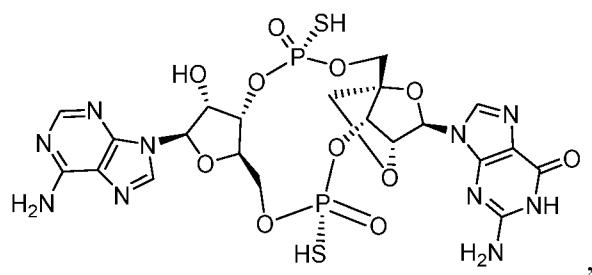
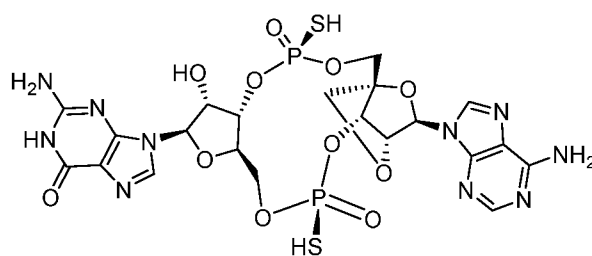
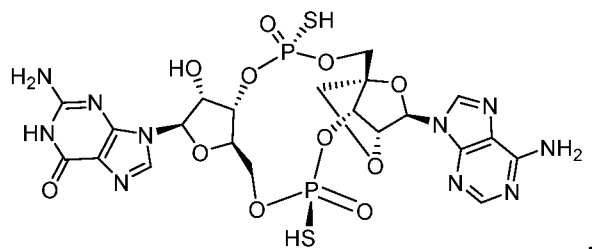
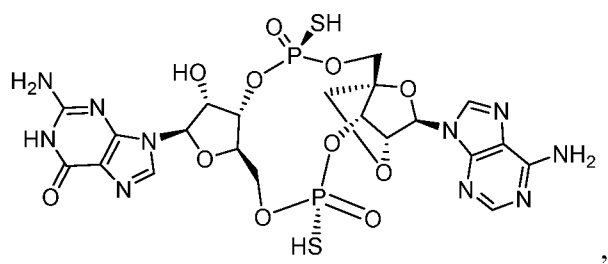


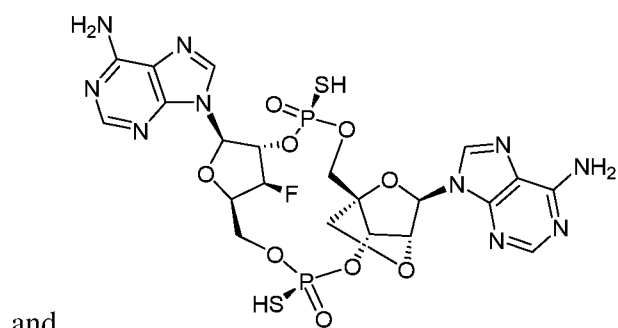
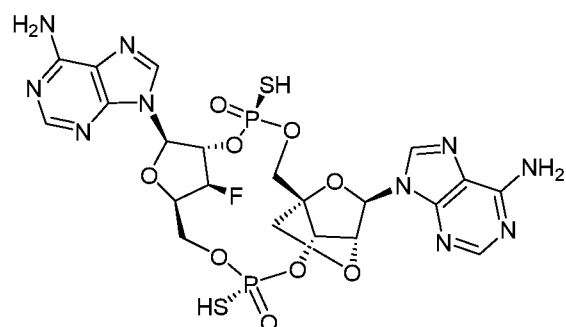
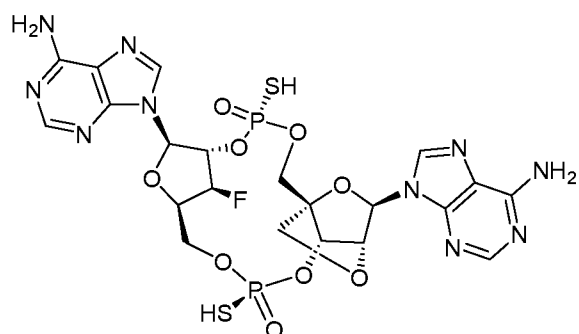
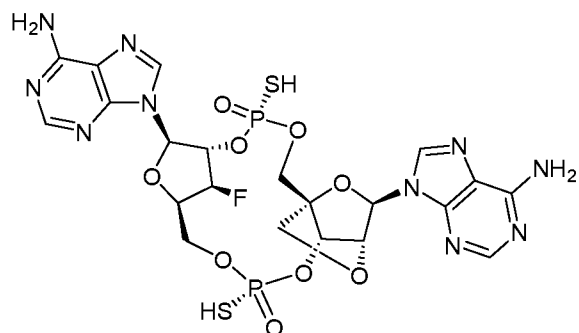
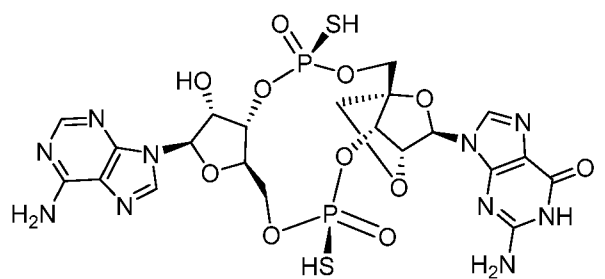








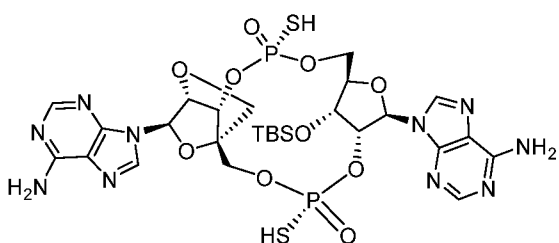
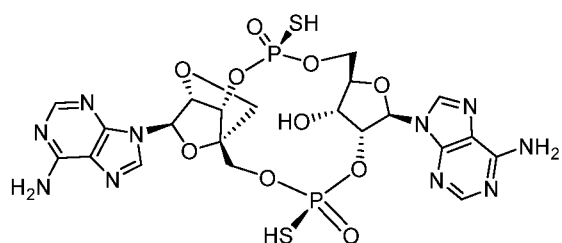
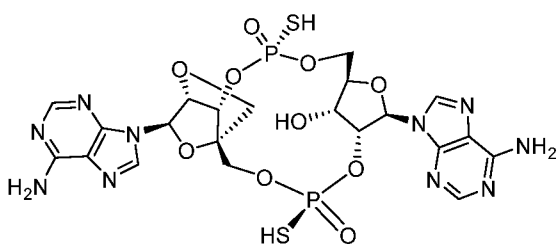
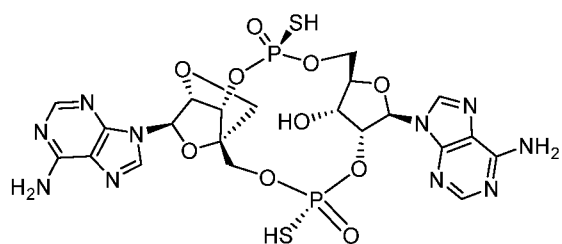
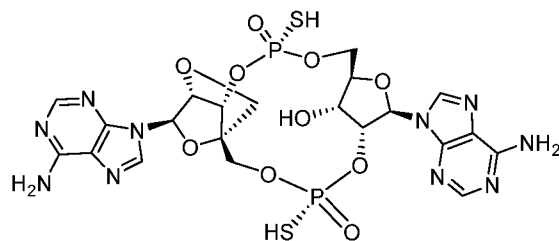
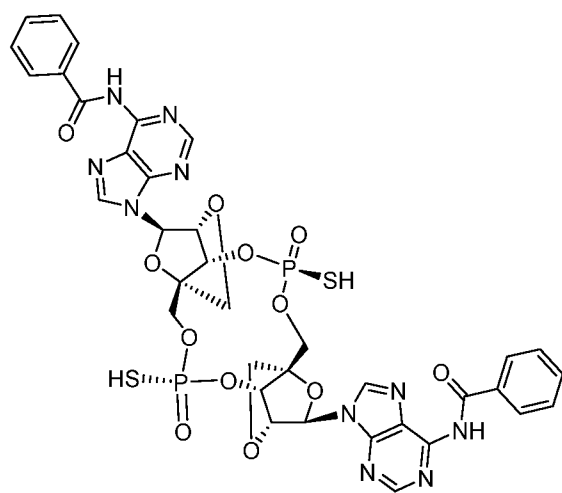
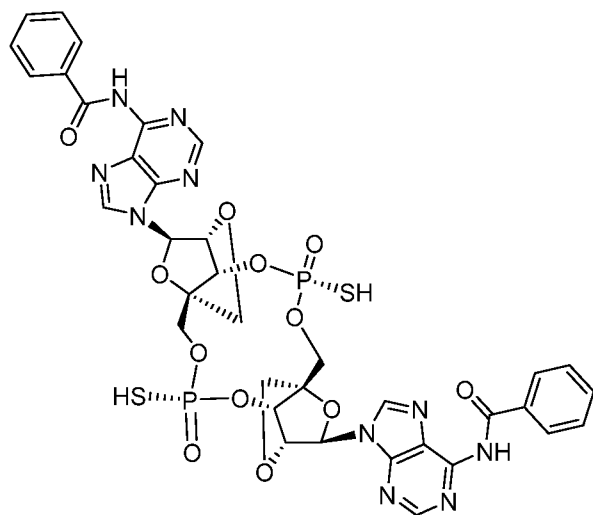
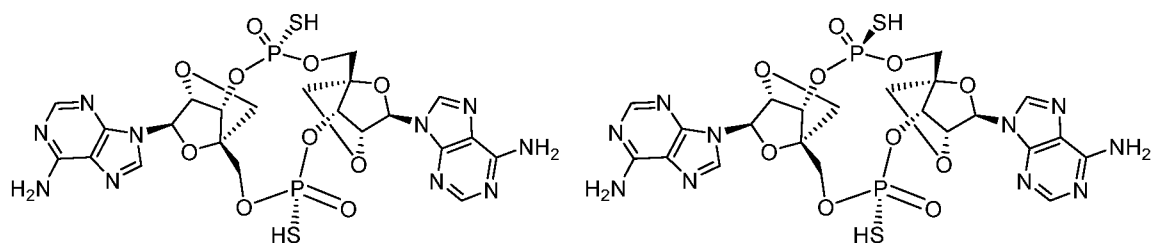


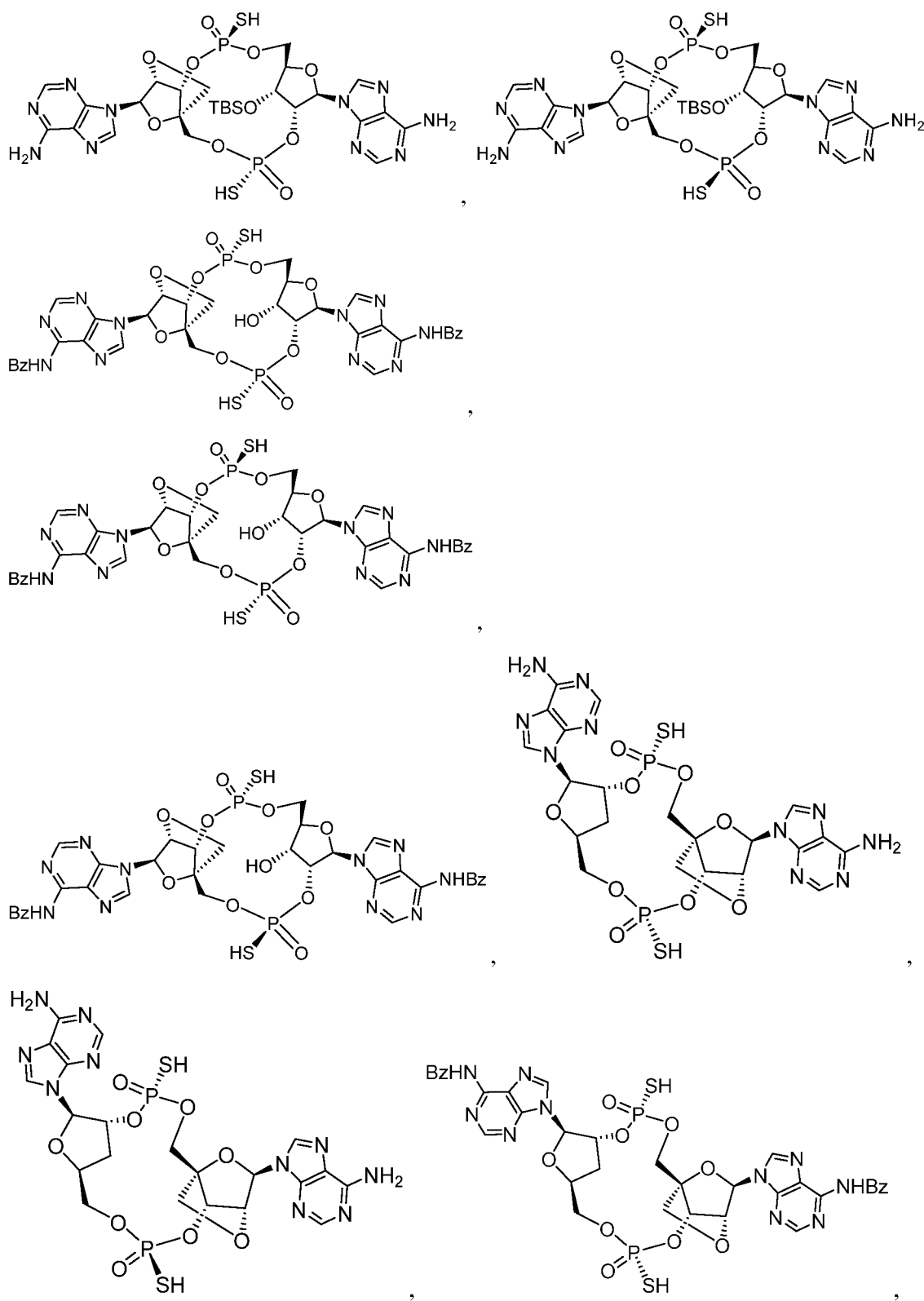


and

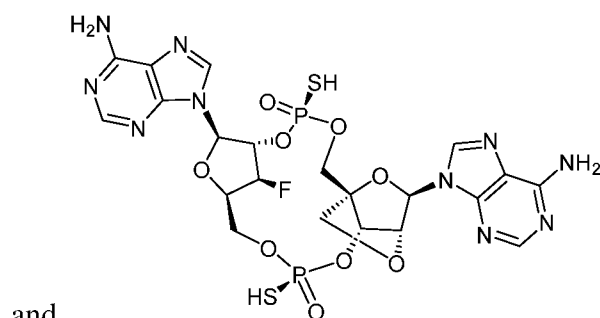
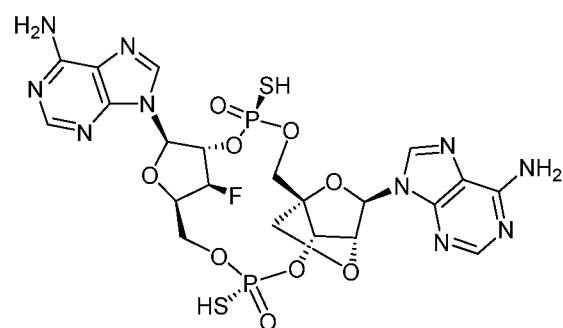
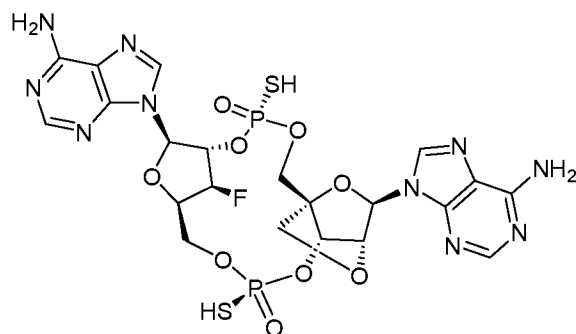
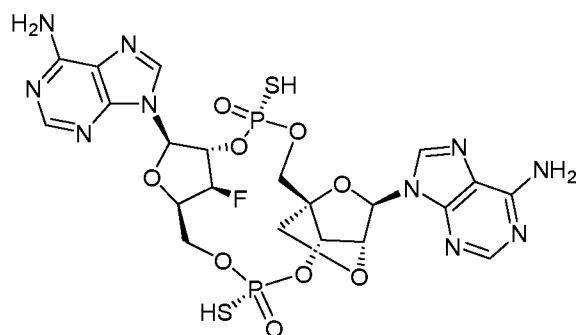
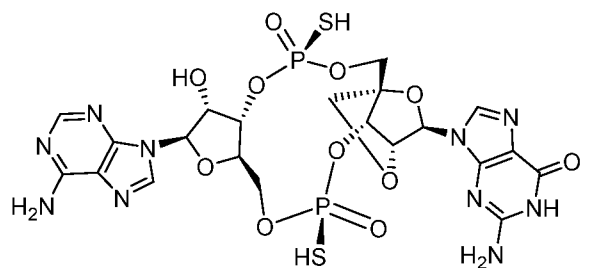
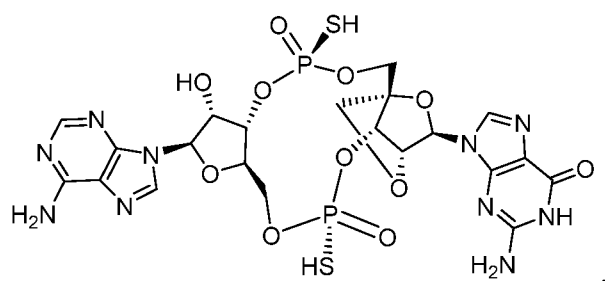
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0064] In some embodiments of any of the above aspects and embodiments, the LNA-CDN compound is selected from the group consisting of:





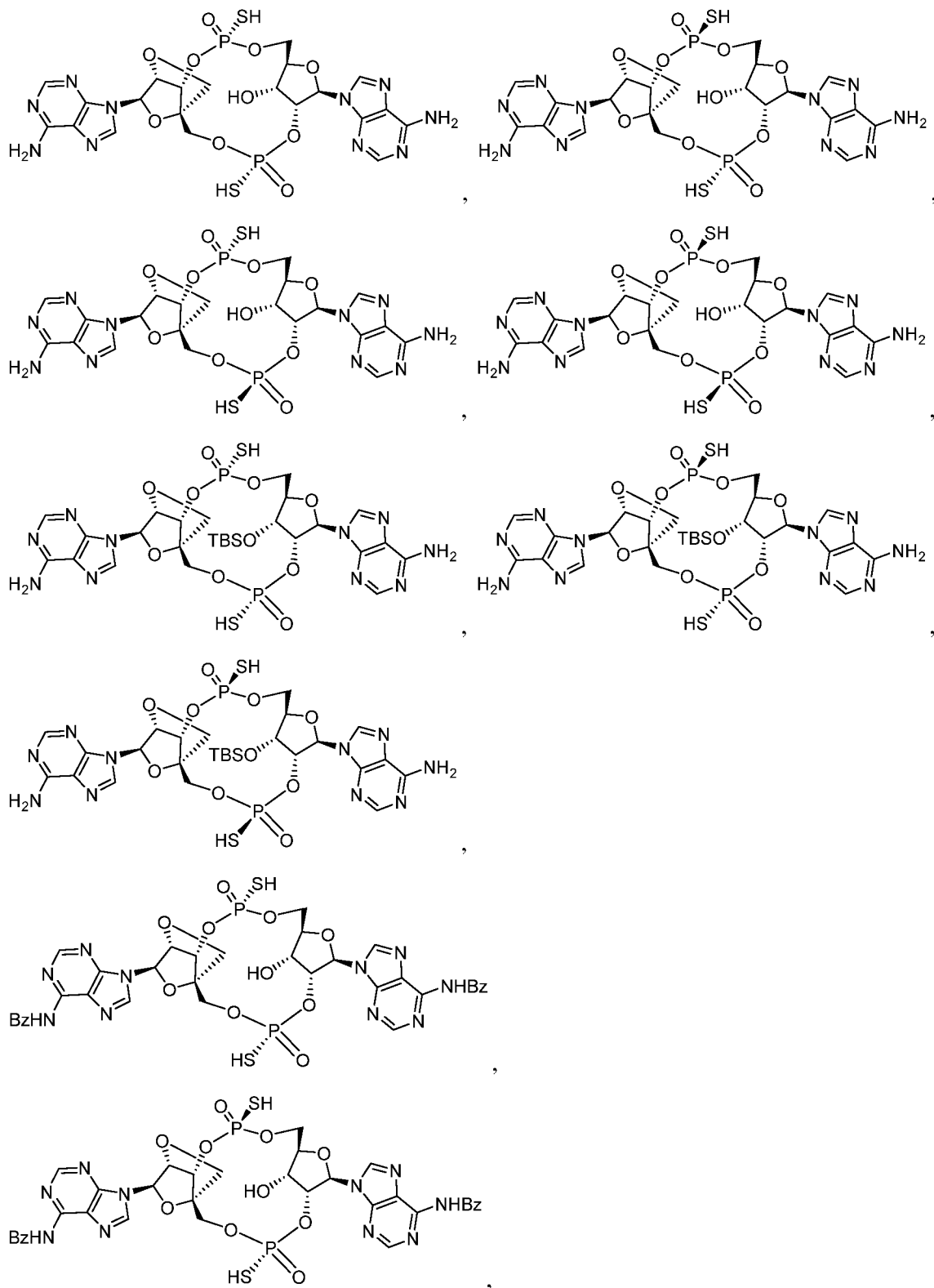


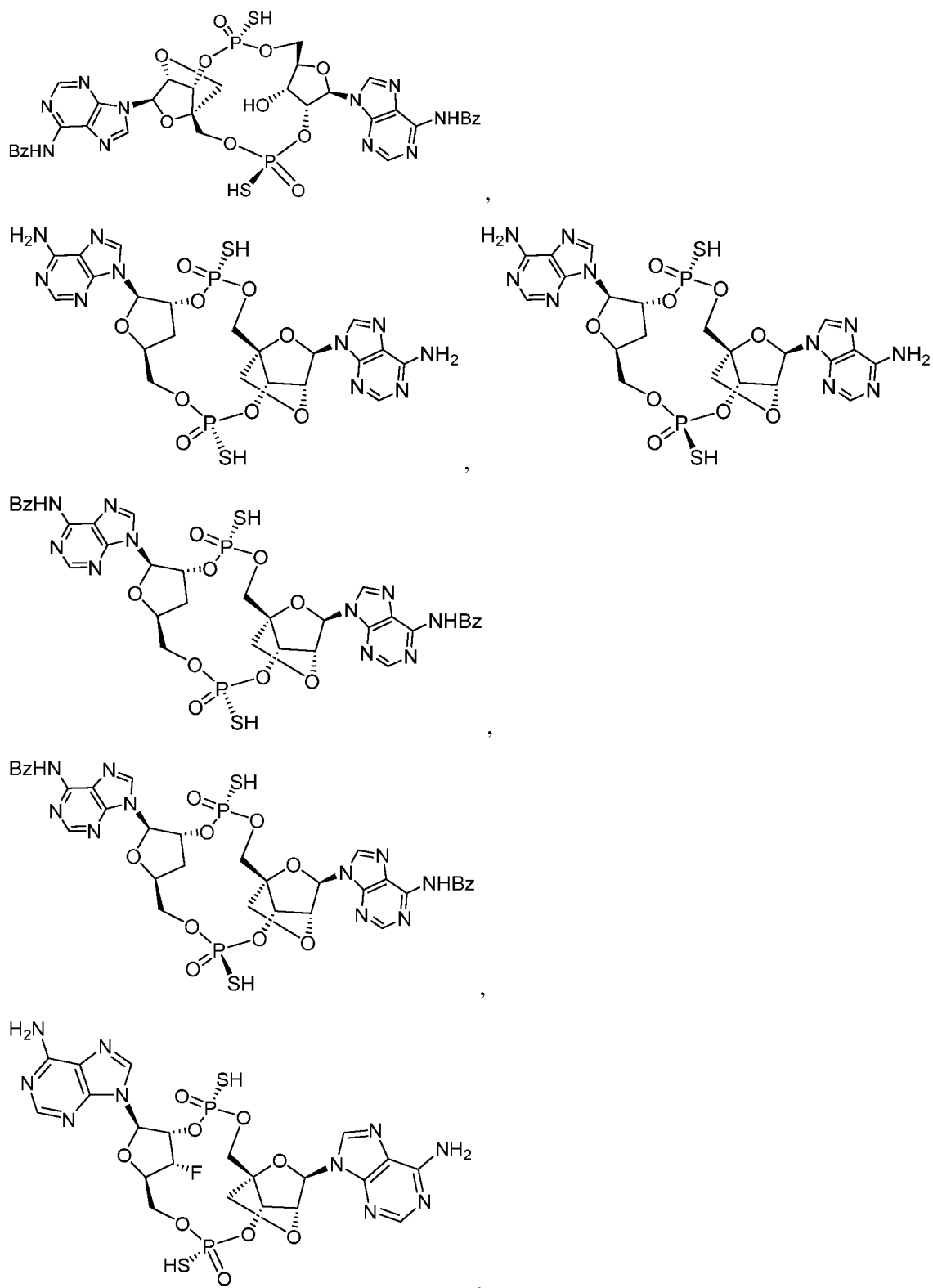


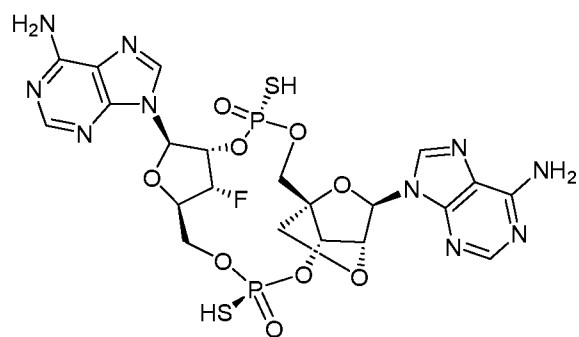
and

or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

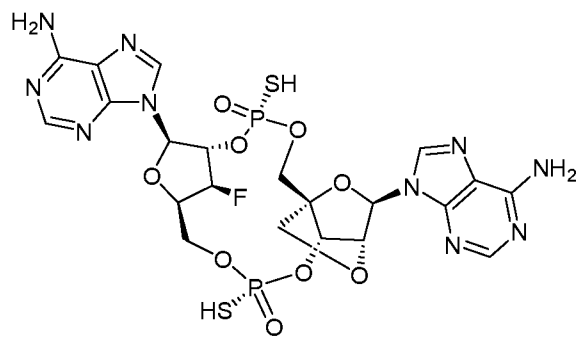
[0065] In some embodiments of any of the above aspects and embodiments, the LNA-CDN compound is selected from the group consisting of:



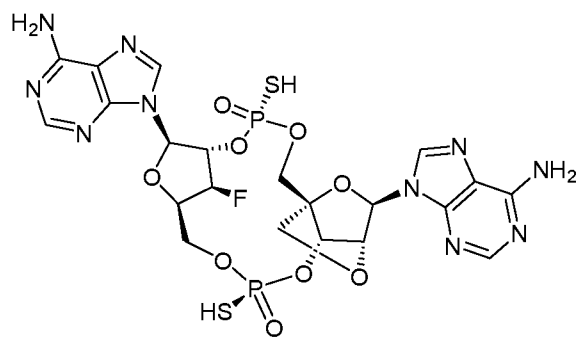




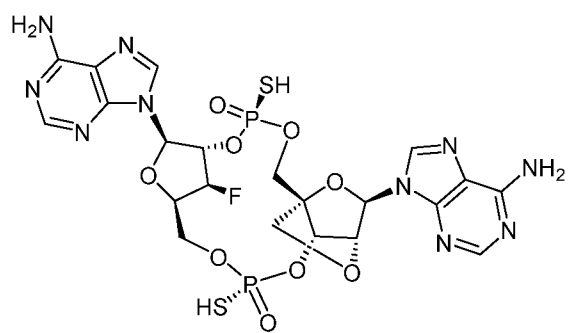
,



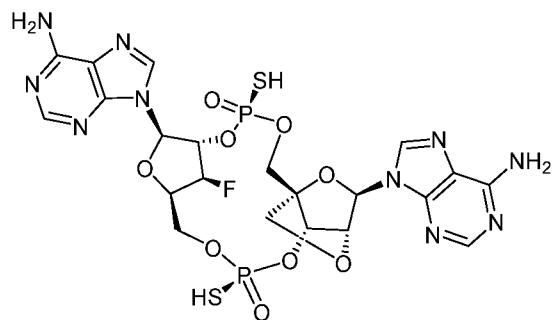
,



,



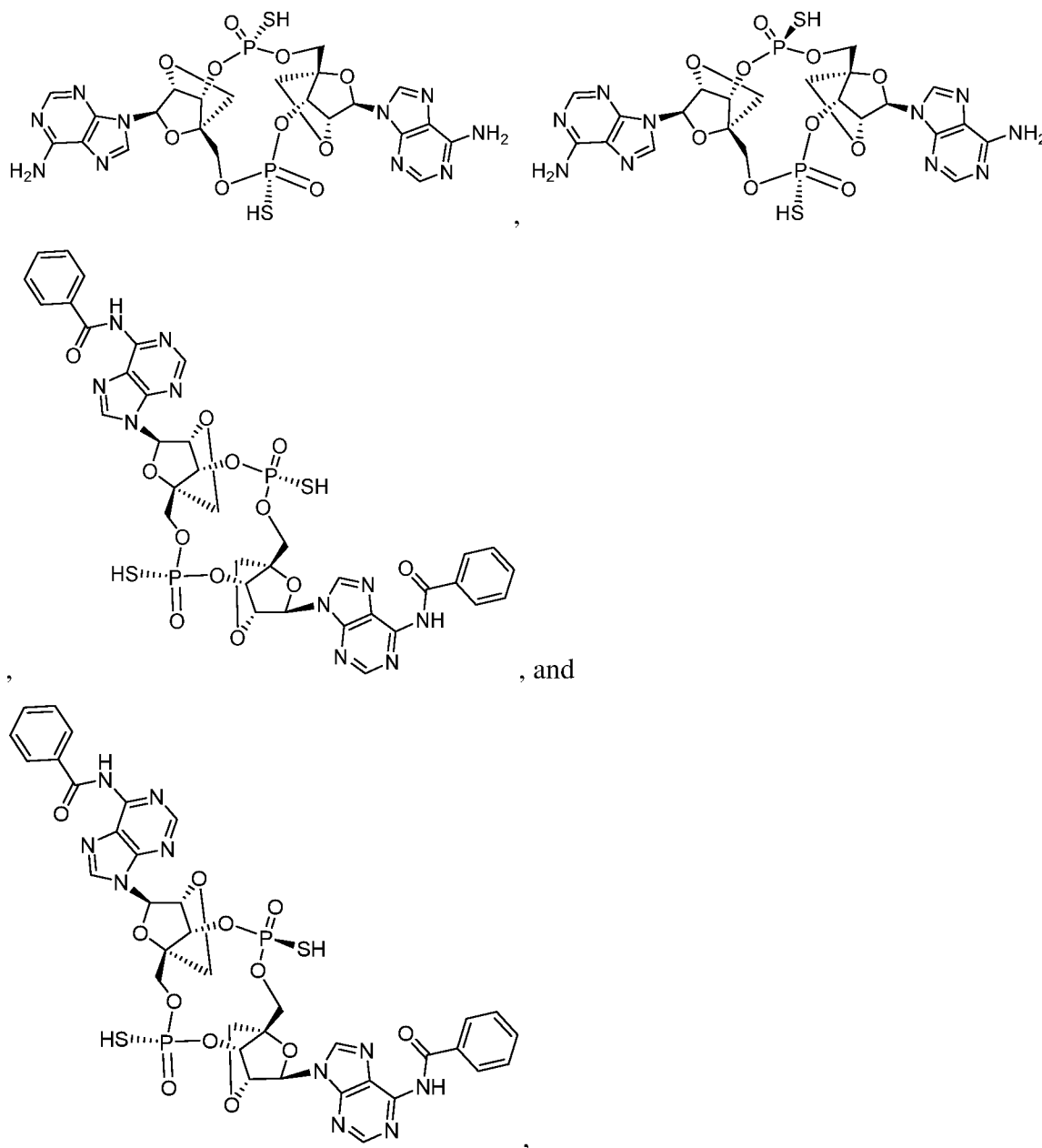
, and



,

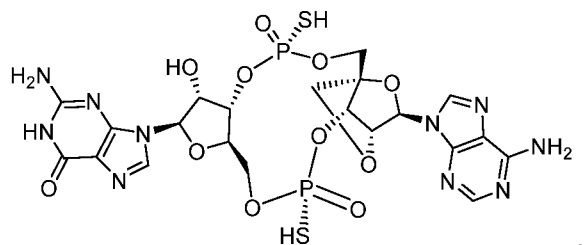
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0066] In some embodiments of any of the above aspects and embodiments, the LNA-CDN compound is selected from the group consisting of:

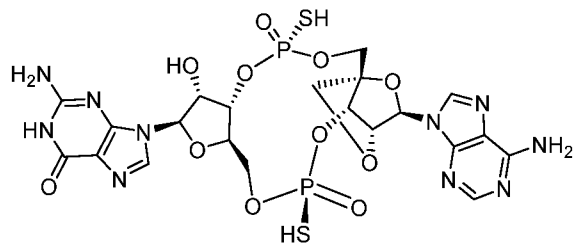


or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

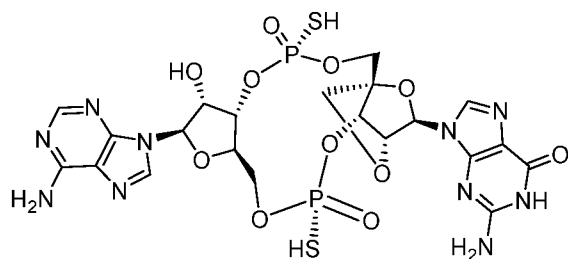
[0067] In some embodiments of any of the above aspects and embodiments, the LNA-CDN compound is selected from the group consisting of:



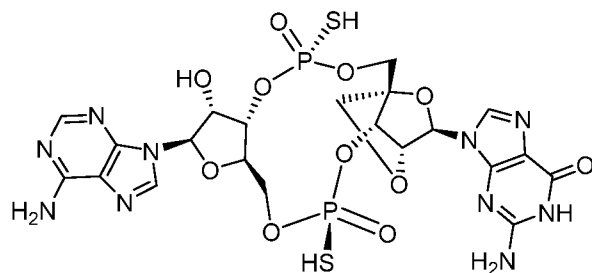
,



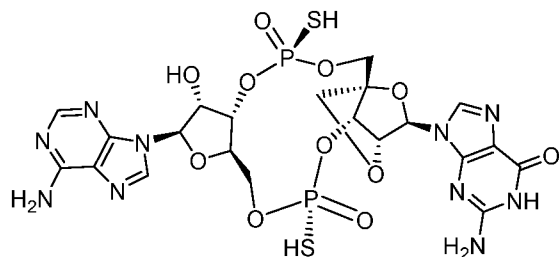
,



,

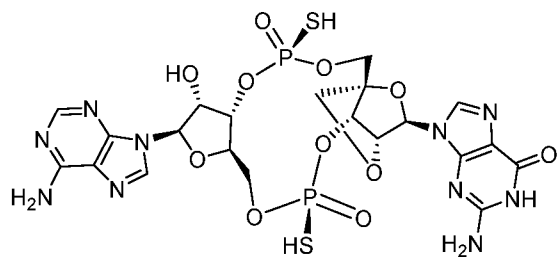


,



,

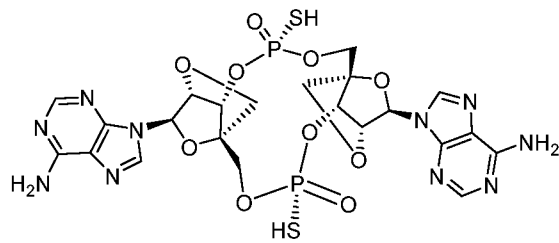
, and



,

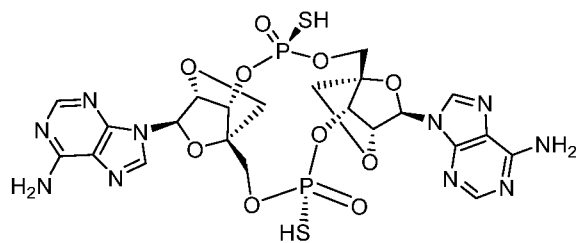
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0068] In a fifth aspect, a compound 3'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) is provided, having the structure:



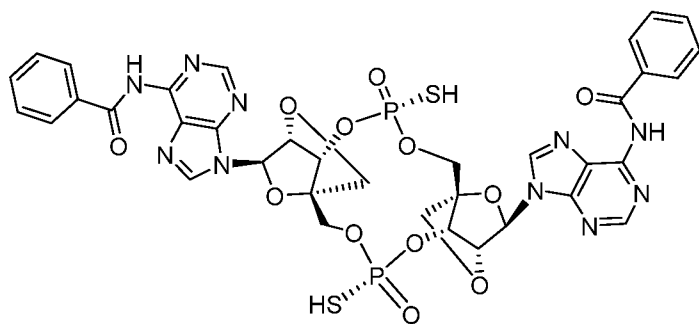
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0069] In a sixth aspect, a compound 3'3'-RS-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) is provided, having the structure:



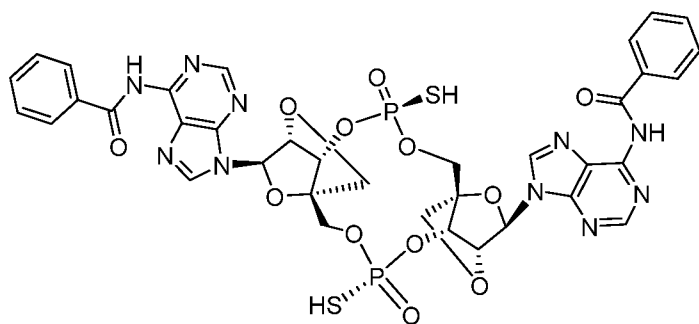
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0070] In a seventh aspect, a compound 3'3'-RR-(2'O,4'C-LNA-BzA)(2'O,4'C-LNA-BzA) is provided, having the structure:



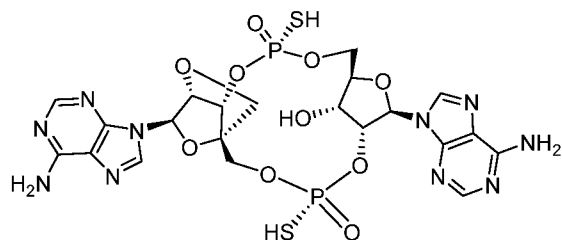
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0071] In an eighth aspect, a compound 3'3'-RS-(2'O,4'C-LNA-BzA)(2'O,4'C-LNA-BzA) is provided, having the structure:



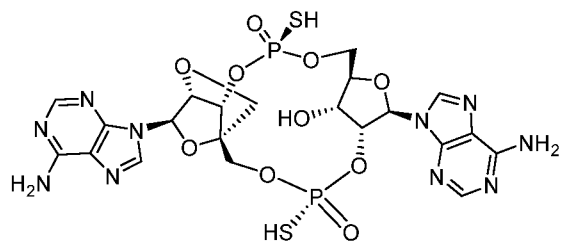
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0072] In a ninth aspect, a compound 2'3'-RR-(A)(2'O,4'C-LNA-A) is provided, having the structure:



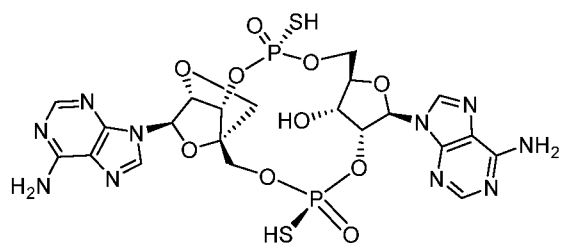
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0073] In a tenth aspect, a compound 2'3'-RS-(A)(2'O,4'C-LNA-A) is provided, having the structure:



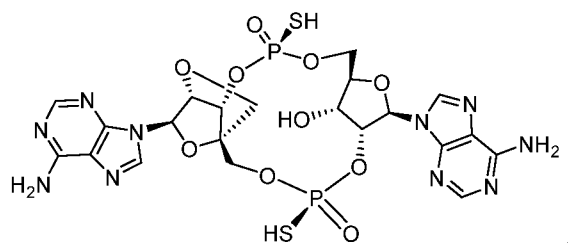
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0074] In an eleventh aspect, a compound 2'3'-SR-(A)(2'O,4'C-LNA-A) is provided, having the structure:



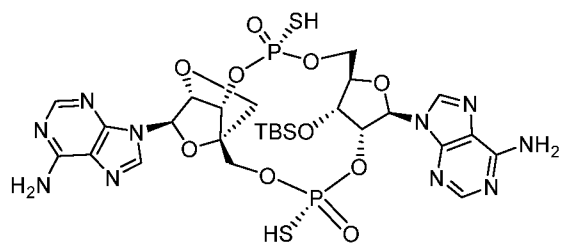
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0075] In a twelfth aspect, a compound 2'3'-SS-(A)(2'O,4'C-LNA-A) is provided, having the structure:



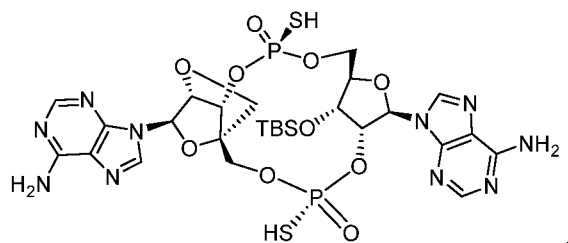
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0076] In a thirteenth aspect, a compound 2'3'-RR-(3'-OTBS-A)(2'O,4'C-LNA-A) is provided, having the structure:



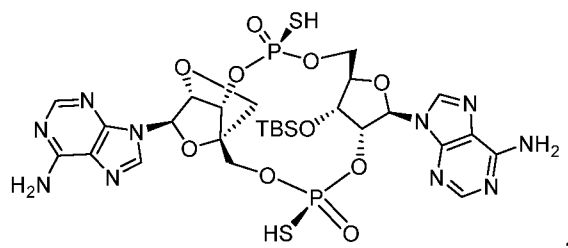
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0077] In a fourteenth aspect, a compound 2'3'-RS-(3'-OTBS-A)(2'O,4'C-LNA-A) is provided, having the structure:



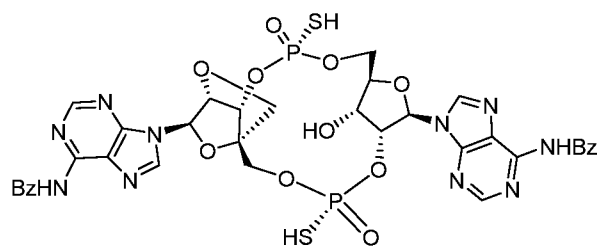
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0078] In a fifteenth aspect, a compound 2'3'-SS-(3'-OTBS-A)(2'O,4'C-LNA-A) is provided, having the structure:



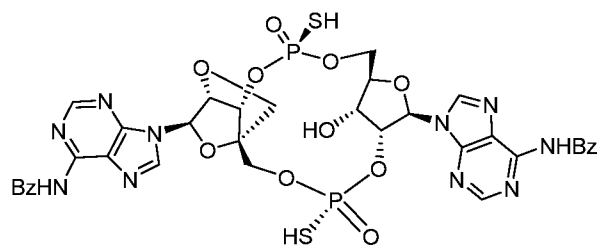
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0079] In a sixteenth aspect, a compound 2'3'-RR-(BzA)(2'O,4'C-LNA-BzA) is provided, having the structure:



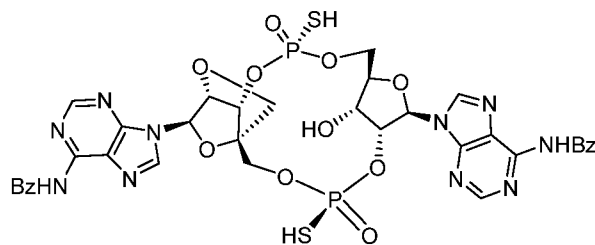
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0080] In a seventeenth aspect, a compound 2'3'-RS-(BzA)(2'O,4'C-LNA-BzA) is provided, having the structure:



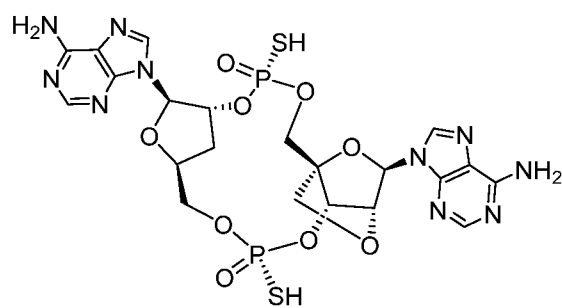
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0081] In an eighteenth aspect, a compound 2'3'-SR-(BzA)(2'O,4'C-LNA-BzA) is provided, having the structure:



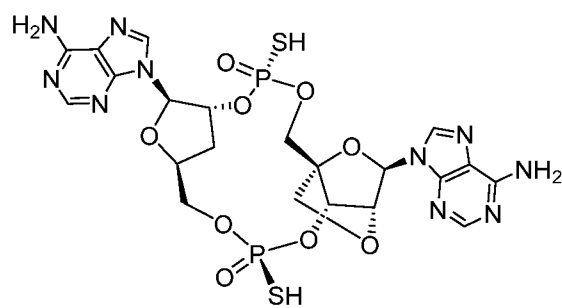
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0082] In a nineteenth aspect, a compound 2'3'-RR-(3'H-A)(2'O,4'C-LNA-A) is provided, having the structure:



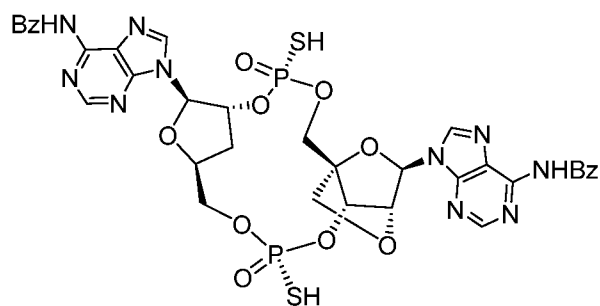
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0083] In a twentieth aspect, a compound 2'3'-RS-(3'H-A)(2'O,4'C-LNA-A) is provided, having the structure:



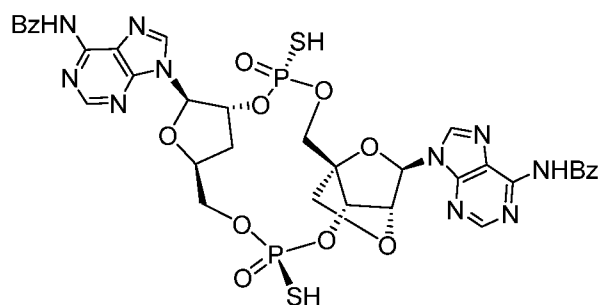
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0084] In a twenty-first aspect, a compound 2'3'-RR-(3'H-BzA)(2'O,4'C-LNA-BzA) is provided, having the structure:



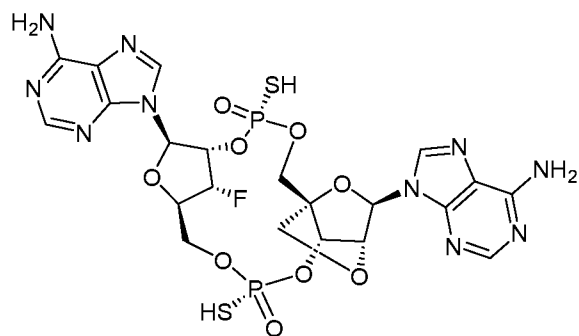
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0085] In a twenty-second aspect, a compound 2'3'-RS-(3'H-BzA)(2'O,4'C-LNA-BzA) is provided, having the structure:



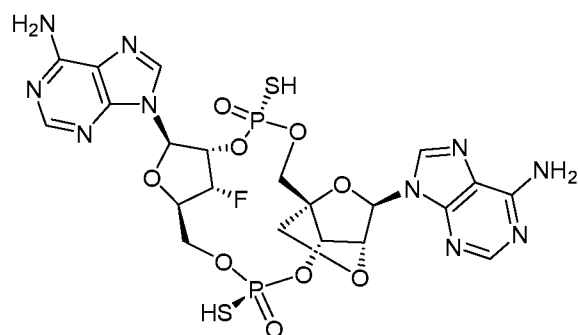
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0086] In a twenty-third aspect, a compound 2'3'-RR-(3'F-A)(2'O,4'C-LNA-A) is provided, having the structure:



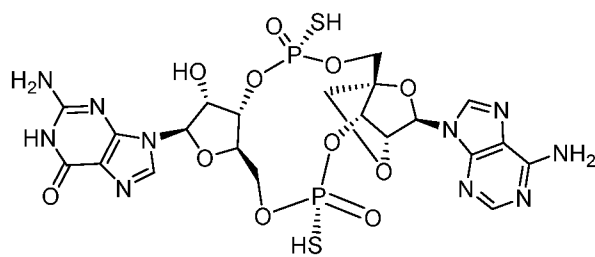
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0087] In a twenty-fourth aspect, a compound 2'3'-RS-(3'F-A)(2'O,4'C-LNA-A) is provided, having the structure:



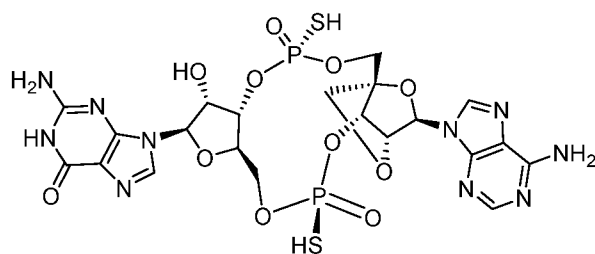
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0088] In a twenty-fifth aspect, a compound 3'3'-RR-(G)(2'O,4'C-LNA-A) is provided, having the structure:



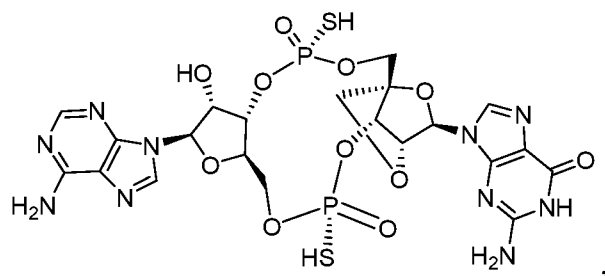
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0089] In a twenty-sixth aspect, a compound 3'3'-RS-(G)(2'O,4'C-LNA-A) is provided, having the structure:



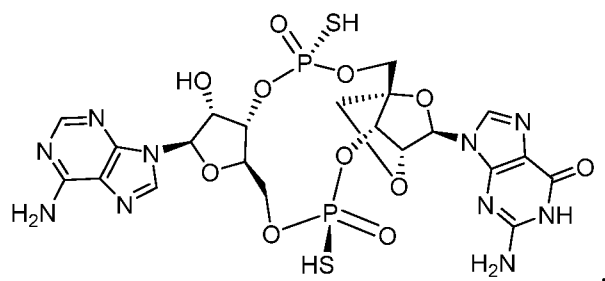
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0090] In a twenty-seventh aspect, a compound 3'3'-RR-(A)(2'O,4'C-LNA-G) is provided, having the structure:



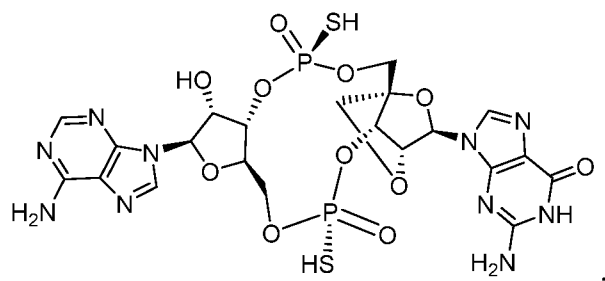
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0091] In a twenty-eighth aspect, a compound 3'3'-RS-(A)(2'O,4'C-LNA-G) is provided, having the structure:



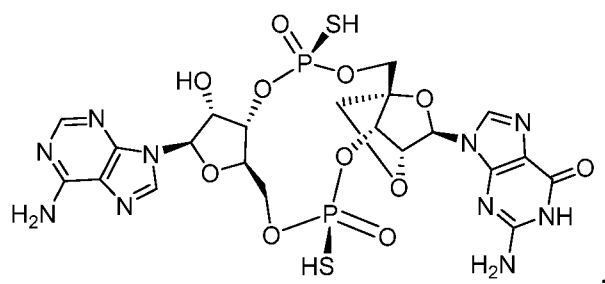
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0092] In a twenty-ninth aspect, a compound 3'3'-SR-(A)(2'O,4'C-LNA-G) is provided, having the structure:



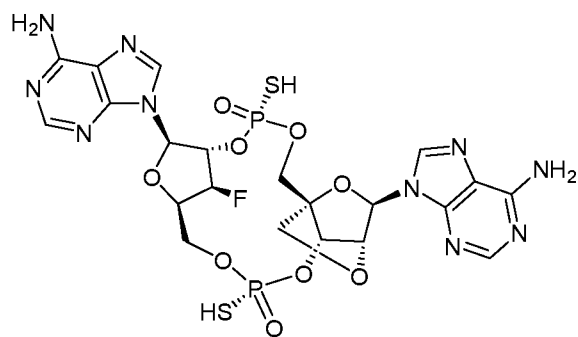
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0093] In a thirtieth aspect, a compound 3'3'-SS-(A)(2'O,4'C-LNA-G) is provided, having the structure:



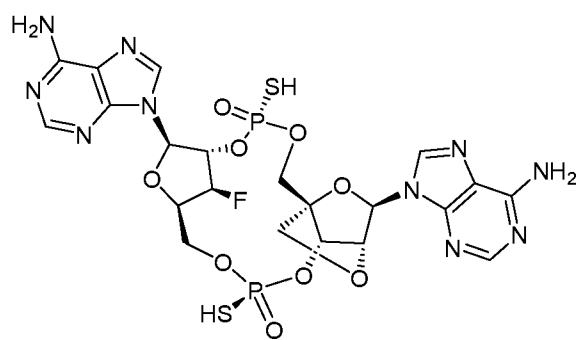
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0094] In a thirty-first aspect, a compound 2'3'-RR-(3'βF-A)(2'O,4'LNA-A) is provided, having the structure:



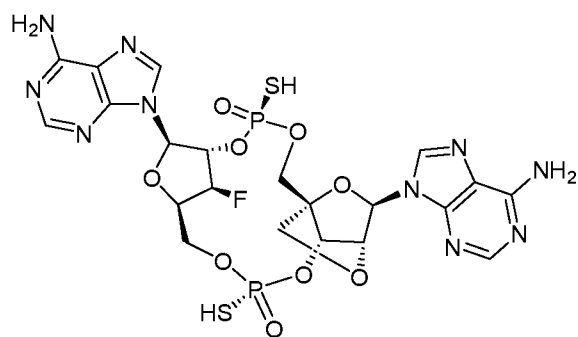
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0095] In a thirty-second aspect, a compound 2'3'-RS-(3'βF-A)(2'O,4'LNA-A) is provided, having the structure:



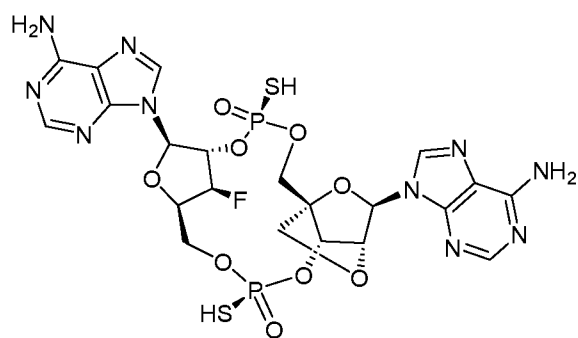
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0096] In a thirty-third aspect, a compound 2'3'-SR-(3'βF-A)(2'O,4'LNA-A) is provided, having the structure:



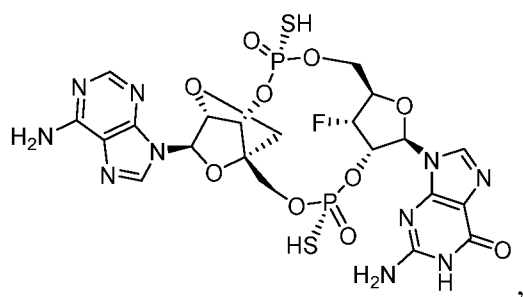
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0097] In a thirty-fourth aspect, a compound 2'3'-SS-(3'βF-A)(2'O,4'LNA-A) is provided, having the structure:



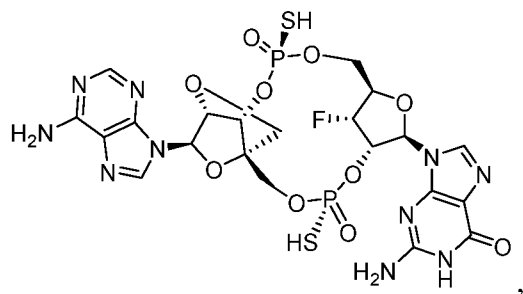
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0098] In a thirty-fifth aspect, a compound 2'3'-RR-(3'F-G)(2'O,4'C-LNA-A) is provided, having the structure:



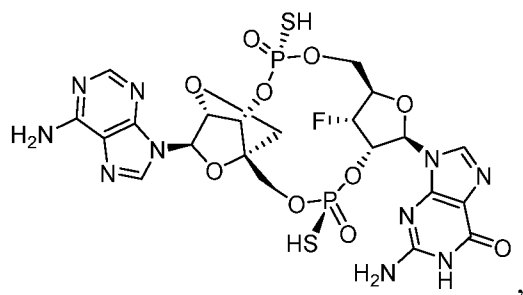
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[0099] In a thirty-sixth aspect, a compound 2'3'-RS-(3'F-G)(2'O,4'C-LNA-A) is provided, having the structure:



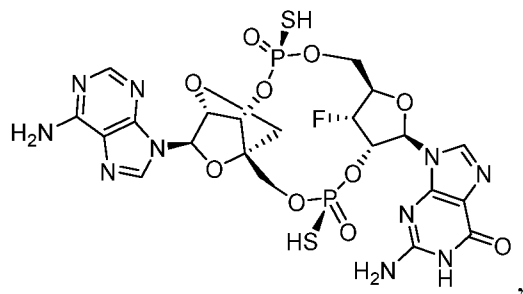
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[00100] In a thirty-seventh aspect, a compound 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A) is provided, having the structure:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[00101] In a thirty-eighth aspect, a compound 2'3'-SS-(3'F-G)(2'O,4'C-LNA-A) is provided, having the structure:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[00102] In some embodiments of any of the above aspects and embodiments thereof, the LNA-CDN compounds include prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates or pharmaceutically acceptable hydrates thereof, including pharmaceutically acceptable salts, pharmaceutically acceptable solvates or pharmaceutically acceptable hydrates of any prodrugs thereof, and including any

pharmaceutically acceptable solvates or pharmaceutically acceptable hydrates of any pharmaceutically acceptable salts thereof. In some embodiments, the LNA-CDN compounds include pharmaceutically acceptable solvates, pharmaceutically acceptable hydrates or pharmaceutically acceptable salts thereof. In some embodiments, the LNA-CDN compound is a pharmaceutically acceptable salt, or a pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof. In some embodiments, the LNA-CDN compound is a pharmaceutically acceptable salt.

[00103] In some embodiments of any of the above aspects and embodiments thereof, the LNA-CDN compounds include pharmaceutically acceptable salts thereof. In some embodiments, the pharmaceutically acceptable salt is selected from the group consisting of the sodium, potassium, calcium, magnesium, zinc, aluminum, ammonium, diethylamine, isopropylamine, olamine, benzathine, benethamine, tromethamine (2-amino-2-(hydroxymethyl)propane-1,3-diol), morpholine, epolamine, piperidine, piperazine, picoline, dicyclohexylamine, N,N'-dibenzylethylenediamine, 2-hydroxyethylamine, tri-(2-hydroxyethyl)amine, chloroprocaine, choline, deanol, imidazole, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), procaine, dibenzylpiperidine, dehydroabietylamine, glucamine, collidine, quinine, quinolone, erbumine, lysine and arginine salt.

[00104] In one embodiment of any of the above aspects or embodiments thereof, the LNA-CDN compounds are provided as the disodium salt thereof. In some embodiments, the LNA-CDN compounds are provided as the disodium salt thereof, or a pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

[00105] In some embodiments of any of the above aspects and embodiments thereof, the LNA-CDN compound is a STING agonist. In some embodiments, the LNA-CDN compound is more active in a cellular assay that measures the induction of human STING dependent IFN- β production as compared to one or more STING agonist reference compounds. In some embodiments, the one or more reference compounds are selected from the group consisting of 2'3'-(A)(A), 2'3'-(G)(A), 3'2'-(G)(A), 2'3'-RR-(A)(A) and 2'3'-RR-(G)(A). In some embodiments, the LNA-CDN compound is a LNA-RR-CDN compound and the one or more reference compounds are selected from the group consisting of 2'3'-RR-(A)(A) and 2'3'-(G)(A). In some embodiments, the cellular assay is an hPBMC assay, for example the assay as described in Example 11. In some

embodiments, the cellular assay is a THP1 assay, for example the assay as described in Example 12. In a preferred embodiment, the cellular assay is performed without the addition of an agent that enhances uptake of the LNA-CDN compound or reference compound by the assay cells or an agent that enhances the permeability of the LNA-CDN compound or reference compound to the assay cells. In some embodiments, the cellular assay is a THP1 cellular assay performed without the addition of an agent that enhances uptake of the LNA-CDN compound or reference compound by the assay cells or an agent that enhances the permeability of the LNA-CDN compound or reference compound to the assay cells, in which the LNA-CDN compound has an EC₅₀ of less than 40 μ M, less than 30 μ M, less than 20 μ M, less than 15 μ M, less than 10 μ M or less than 5 μ M. In some embodiments the LNA-CDN compound has an EC₅₀ of less than 40 μ M, less than 30 μ M, less than 20 μ M, less than 15 μ M, less than 10 μ M or less than 5 μ M in a cellular assay that measures induction of human STING dependent IFN- β production, wherein the cellular assay is performed without the addition of an agent that enhances uptake of the LNA-CDN compound by the assay cells or an agent that enhances the permeability of the LNA-CDN compound to the assay cells. In one embodiment the cellular assay is a THP1 cellular assay as described in Example 12, wherein the assay is performed without addition of digitonin. In some embodiments, the LNA-CDN compound has an EC₅₀ that is less than the EC₅₀ of one or more reference compounds selected from the group consisting of 2'3'-(A)(A), 2'3'-(G)(A), 3'2'-(G)(A), 2'3'-RR-(A)(A), and 2'3'-RR-(G)(A) in a cellular assay that measures induction of human STING dependent IFN- β production, preferably wherein the cellular assay is performed without the addition of an agent that enhances uptake of the LNA-CDN compound or reference compound by the assay cells or an agent that enhances the permeability of the LNA-CDN compound or reference compound to the assay cells. In some embodiments, the LNA-CDN compound has an EC₅₀ that is less than the EC₅₀ of a reference compound in a THP1 cellular assay as described in Example 12, preferably wherein the assay is performed without addition of digitonin. In some embodiments, the LNA-CDN compound has an EC₅₀ that is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, or at least 8-fold lower than the EC₅₀ of the reference compound in the THP1 cellular assay as described in Example 12, wherein the assay is performed without addition of digitonin.

[00106] In some embodiments of any of the above aspects and embodiments thereof, the LNA-CDN compound binds at least one human STING allelic protein product

(including any one of WT, REF, HAQ, AQ and Q alleles) with a greater affinity than one or more reference compounds selected from the group consisting of 2'3'-(A)(A), 2'3'-(G)(A), 3'2'-(G)(A), 2'3'-RR-(A)(A), and 2'3'-RR-(G)(A) when measured using at least one human STING protein. Preferably, this is measured using the isolated protein encoded by the hSTING (WT), hSTING (HAQ) or hSTING (REF) allele (*Ishikawa, H., and Barber, G.N. (2008). Nature 455, 674–678; Yi et al., 2013, PLoS One 2013 Oct 21, 8(10):e77846; the protein sequence of the REF allele is NCBI Reference Sequence NP_938023) using a method such as differential scanning fluorometry (DSF) as described hereinafter and in Example 10.*

[00107] In a thirty-ninth aspect, the present invention provides pharmaceutical compositions comprising one or more LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, and a delivery vehicle which enhances cellular uptake and/or stability of the compound. In some embodiments, the delivery vehicle comprises one or more agents selected from the group consisting of adjuvants, lipids, liposomes, interbilayer crosslinked multilamellar vesicles, biodegradable poly(D,L-lactic-co-glycolic acid) [PLGA]-based or poly anhydride-based nanoparticles or microparticles, and nanoporous particle-supported lipid bilayers.

[00108] In a fortieth aspect, the present invention provides pharmaceutical compositions comprising one or more LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, and a pharmaceutically acceptable excipient.

[00109] In a first embodiment of the fortieth aspect, the pharmaceutical composition does not include an agent that enhances cellular permeability of the one or more LNA-CDN compounds.

[00110] In a second embodiment of the fortieth aspect, the pharmaceutical composition does not include an agent that enhances cellular uptake of the one or more LNA-CDN compounds.

[00111] In a third embodiment of the fortieth aspect, the pharmaceutical composition further comprises a delivery vehicle which enhances cellular uptake and/or stability of the compound. In some embodiments, the delivery vehicle comprises one or more agents selected from the group consisting of adjuvants, lipids, liposomes, interbilayer crosslinked multilamellar vesicles, biodegradable poly(D,L-lactic-co-glycolic acid) [PLGA]-based or poly anhydride-based nanoparticles or microparticles, and nanoporous particle-supported lipid bilayers.

[00112] In some embodiments of the thirty-ninth aspect or fortieth aspect and any of the above embodiments thereof, the pharmaceutical composition further comprises one or more additional pharmaceutically active components selected from the group consisting of an immune checkpoint inhibitor (e.g. CTLA-4, PD-1, Tim-3, Vista, BTLA, LAG-3 and TIGIT pathway antagonists; PD-1 pathway blocking agents; PD-L1 inhibitors; including without limitation anti-PD-1 antibodies nivolumab, pembrolizumab or pidilizumab; PD-1 inhibitor AMP-224; anti-CTLA-4 antibody ipilimumab; and anti-PD-L1 antibodies BMS-936559, MPDL3280A, MEDI4736, or avelumab); a TLR agonist (e.g. CpG or monophosphoryl lipid A); an inactivated or attenuated bacteria which induce innate immunity (e.g., inactivated or attenuated *Listeria monocytogenes*); a composition that mediates innate immune activation via Toll-like Receptors (TLRs), via (NOD)-like receptors (NLRs), via Retinoic acid inducible gene-based (RIG)-I-like receptors (RLRs), via C-type lectin receptors (CLRs), or via pathogen-associated molecular patterns (PAMPs); and a chemotherapeutic agent. In some embodiments, the immune checkpoint inhibitor is selected from the group consisting of a CTLA-4 pathway antagonist, a PD-1 pathway antagonist, a Tim-3 pathway antagonist, a Vista pathway antagonist, a BTLA pathway antagonist, a LAG-3 pathway antagonist, and a TIGIT pathway antagonist. In some embodiments, the immune checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-CTLA-4 antibody, an anti-TIM-3 antibody, an anti-BTLA antibody, an anti-B7-H3 antibody, an anti-CD70 antibody, an anti-CD40 antibody, an anti-CD137 antibody, an anti-GITR antibody, an anti-OX40 antibody, an anti-KIR antibody or an anti-LAG-3 antibody. In some embodiments, the immune checkpoint inhibitor is selected from the group consisting of nivolumab, pembrolizumab, pidilizumab, PDR001, MEDI0680, REGN2810, AMP-224, ipilimumab, BMS-936559, MPDL3280A, MEDI4736, and avelumab. In some embodiments, the TLR agonist is CpG or monophosphoryl lipid A.

[00113] In some embodiments of the thirty-ninth aspect or fortieth aspect and any of the above embodiments thereof, the pharmaceutical composition further comprises an inactivated tumor cell which expresses and secretes one or more cytokines which stimulate dendritic cell induction, recruitment and/or maturation, or which expresses and secretes one or more heat shock proteins, including gp96-Ig fusion proteins. In some embodiments, the one or more cytokines is selected from the group consisting of GM-CSF, CCL20, CCL3, IL-12p70 and FLT-3 ligand. In some embodiments, the tumor cell is inactivated by treatment with radiation. In some embodiments, the one or more cytokines is selected from the group consisting of GM-CSF, CCL20, CCL3, IL-12p70 and FLT-3 ligand, and the tumor cell is inactivated by treatment with radiation. In some embodiments, the inactivated tumor cell expresses and secretes a gp96-Ig fusion protein.

[00114] In some embodiments of the thirty-ninth aspect or fortieth aspect and any of the above embodiments thereof, the pharmaceutical composition further comprises one or more antigens selected for the purposes of inducing an immune response against said one or more antigen(s) when the composition is administered to an individual. In some embodiments, the antigen is a recombinant protein antigen. In some embodiments, the antigen is a recombinant protein antigen related to an infectious disease, a malignancy, or an allergan. In some embodiments, the one or more antigens is one or more antigens in Table 1.

[00115] In some embodiments of the thirty-ninth aspect or fortieth aspect and any of the above embodiments thereof, the pharmaceutical compositions are formulated as aqueous or oil-in-water emulsions.

[00116] In a forty-first aspect, the invention provides a method for treating an individual suffering from a cancer, wherein the method comprises administering to the individual in need thereof an effective amount of one or more LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, or compositions thereof as described in the thirty-ninth and fortieth aspects and any embodiments thereof as described herein above. In some embodiments, the one or more LNA-CDN compounds or composition thereof is administered non-parenterally or parenterally. In some embodiments, the administration is subcutaneous, intramuscular, intradermal,

mucosal, vaginal, cervical, peri-tumoral, intra-tumoral, or directly into the tumor-draining lymph node(s). In some embodiments, the administration is mucosal, preferably oral.

[00117] In a first embodiment of the forty-first aspect, the individual receiving such treatment may be suffering from a cancer selected from the group consisting of a colorectal cancer, an aero-digestive squamous cancer, a lung cancer (small cell lung cancer, non-small cell lung cancer), a brain cancer, a liver cancer (e.g. hepatocellular carcinoma), a stomach cancer, a bladder cancer, a thyroid cancer, an adrenal cancer, a gastrointestinal cancer, an oropharyngeal cancer, an esophageal cancer, a head and neck cancer, an ovarian cancer, a uterine cancer, a cervical cancer, an endometrial cancer, a breast cancer, a melanoma, a prostate cancer, a pancreatic carcinoma, a renal carcinoma, a sarcoma, a leukemia, a Merkel-cell carcinoma, a lymphoma (e.g. Hodgkin lymphoma, non-Hodgkin lymphoma), and a multiple myeloma.

[00118] In a second embodiment of the forty-first aspect and of the first embodiment thereof, the method for treating an individual suffering from a cancer further comprises administering one or more additional cancer therapies. In some embodiments, the one or more additional cancer therapies comprises radiation therapy, surgery, a chemotherapy, or an immunotherapy (for example, without limitation, an immunomodulator, an immune checkpoint inhibitor, a cellular immunotherapy, or a cancer vaccine). In some embodiments, the one or more additional cancer therapies comprises an inactivated tumor cell that expresses and secretes one or more cytokines or one or more heat shock proteins. In some embodiments, the cytokine is selected from the group consisting of GM-CSF, CCL20, CCL3, IL-12p70, and FLT-3 ligand. In some embodiments the heat shock protein is a gp96-Ig protein. In some embodiments, the method comprises administering one or more additional cancer therapies selected from the group consisting of a chemotherapeutic agent; an immune checkpoint inhibitor; a TLR agonist; a vaccine selected to stimulate an immune response to one or more cancer antigens, a therapeutic antibody that induces antibody-dependent cellular cytotoxicity; an immunomodulatory cell line; an inactivated or attenuated bacteria that induces innate immunity; an antigen selected for the purpose of inducing an immune response, and a composition that mediates innate immune activation via Toll-like Receptors (TLRs), (NOD)-like receptors (NLRs), Retinoic acid inducible gene-based (RIG)-I-like receptors (RLRs), C-type lectin receptors (CLRs) or pathogen-associated molecular patterns ("PAMPs"). In some embodiments, the

immune checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-CTLA-4 antibody, an anti-TIM-3 antibody, an anti-BTLA antibody or an anti-LAG-3 antibody. In some embodiments, the TLR agonist is CpG or monophosphoryl lipid A. In some embodiments, the therapeutic antibody that induces antibody-dependent cellular cytotoxicity is rituximab, ibritumomab, tositumomab, cetuximab, trastuzumab, brentuximab vedotin, alemtuzumab, oncolym, ibilimumab, vitaxin, or bevacizumab.

[00119] In some embodiments of the forty-first aspect and first and second embodiments thereof, the individual suffers from a cancer expressing a cancer antigen, and the method for treating said individual further comprises administering to the individual a primary therapy to remove or kill cancer cells expressing the cancer antigen, wherein the administration of the primary therapy is simultaneously with, prior to or following administration of the LNA-CDN compound or composition thereof. In some embodiments, the LNA-CDN compound or composition thereof is administered as a neoadjuvant therapy to the primary therapy. In preferred embodiments, the LNA-CDN compound or composition thereof is administered following the primary therapy. In some embodiments, the primary therapy comprises surgery to remove the cancer cells from the mammal, radiation therapy to kill the cancer cells in the mammal, or both surgery and radiation therapy.

[00120] In a forty-second aspect, the invention provides a method of treating a disease in an individual, comprising administering to the individual in need thereof i) an effective amount of one or more LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, or compositions thereof as described in the thirty-ninth and fortieth aspects and any embodiments thereof as described herein above; and ii) an effective amount of one or more therapeutic antibodies that induce antibody-dependent cellular cytotoxicity, wherein the disease is selected from the group consisting of a cancer, acute rejection of an organ transplant, Type I diabetes mellitus, rheumatoid arthritis, psoriasis, Crohn's disease, restenosis and allergic asthma. In some embodiments, the cancer is selected from the group consisting of lymphoma (e.g. B-cell lymphoma), breasts cancer, chronic lymphocytic leukemia, colorectal cancer, melanoma, non-small cell lung carcinoma, small cell lung cancer, bladder cancer, prostate cancer and

other solid tumors. In some embodiments, the therapeutic antibody is selected from the group consisting of muromonab-CD3, infliximab, daclizumab, omalizumab, abciximab, rituximab, ibritumomab, tositumomab, cetuximab, trastuzumab, brentuximab vedotin, alemtuzumab, oncolym, ibilimumab, vitaxin, and bevacizumab.

[00121] In a forty-third aspect, the invention provides a method for the treatment of disorders in which shifting of Th1 to Th2 immunity confers clinical benefit, wherein the method comprises administering to the individual in need thereof one or more LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, or compositions thereof as described in the thirty-ninth and fortieth aspects and any embodiments thereof as described herein above. Cell-mediated immunity (CMI) is associated with TH1 CD4+ T lymphocytes producing cytokines IL-2, interferon (IFN)- γ and tumor necrosis factor (TNF)- α . In contrast, humoral immunity is associated with TH2 CD4+ T lymphocytes producing IL-4, IL-6 and IL-10. Immune deviation towards TH1 responses typically produces activation of cytotoxic T-cell lymphocytes (CTL), natural killer (NK) cells, macrophages and monocytes. Generally, Th1 responses are more effective against intracellular pathogens (viruses and bacteria that are inside host cells) and tumors, while Th2 responses are more effective against extracellular bacteria, parasites including helminths and toxins. In addition, the activation of innate immunity is expected to normalize the T-helper type 1 and 2 (Th1/Th2) immune system balance and to suppress the excessive reaction of Th2 type responses that cause immunoglobulin (Ig) E-dependent allergies and allergic asthma.

[00122] In a forty-fourth aspect, the invention provides a method for treating an individual suffering from a chronic infectious disease, wherein the method comprises administering to the individual in need thereof an effective amount of one or more LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, or compositions thereof as described in the thirty-ninth and fortieth aspects and any embodiments thereof as described herein above. In some embodiments, the one or more LNA-CDN compounds or composition

thereof is administered in combination with another agent for use in treating the chronic infectious disease. In some embodiments, the chronic infectious disease is selected from the group consisting of HBV infection, HCV infection, HPV infection, HSV infection and hepatocellular cancer.

[00123] The LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, or compositions thereof as described in the thirty-ninth and fortieth aspects and any embodiments thereof as described herein above, may be administered to individuals in need thereof, as described in the methods of the forty-first through forty-fourth aspects and any embodiments thereof as described herein above, by a variety of parenteral and non-parenteral routes in formulations containing pharmaceutically acceptable excipients (e.g. carriers, adjuvants, vehicles and the like). Preferred non-parenteral routes include mucosal (e.g., oral, vaginal, nasal, cervical, etc.) routes. Preferred parenteral routes include but, are not limited to, one or more of subcutaneous, intravenous, intramuscular, intraarterial, intradermal, intrathecal and epidural administrations. Preferably administration is by subcutaneous, intra-tumoral or peri-tumoral routes.

[00124] The LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, or compositions thereof as described in the thirty-ninth and fortieth aspects and any embodiments thereof as described herein may be co-administered to individuals in need thereof, as described in the methods of the forty-first through forty-fourth aspects and any embodiments thereof as described herein above, with one or more additional pharmaceutically active components such as adjuvants, lipids, interbilayer crosslinked multilamellar vesicles, biodegradable poly(D,L-lactic-co-glycolic acid) [PLGA]-based or poly anhydride-based nanoparticles or microparticles, and nanoporous particle-supported lipid bilayers, immune checkpoint inhibitors (e.g. CTLA-4, PD-1, Tim-3, VISTA, BTLA, LAG-3 and TIGIT pathway antagonists; PD-1 pathway blocking agents; PD-L1 inhibitors; including without limitation anti-PD-1 antibodies nivolumab, pembrolizumab or pidilizumab; PD-1 inhibitor AMP-224; anti-

CTLA-4 antibody ipilimumab; and anti-PD-L1 antibodies BMS-936559, MPDL3280A, MEDI4736, or avelumab), inactivated or attenuated bacteria which induce innate immunity (e.g., inactivated or attenuated *Listeria monocytogenes*), compositions which mediate innate immune activation via Toll-like Receptors (TLRs), (NOD)-like receptors (NLRs), Retinoic acid inducible gene-based (RIG)-I-like receptors (RLRs), C-type lectin receptors (CLRs), or pathogen-associated molecular patterns ("PAMPs"), or chemotherapeutic agents.

[00125] In a forty-fifth aspect, the invention provides one or more LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, or compositions thereof as described in the thirty-ninth and fortieth aspects and any embodiments thereof as described herein above, for use as adjuvants in combination with a therapeutic or prophylactic vaccine. In some embodiments, the vaccine is selected to stimulate an immune response to one or more predetermined antigens. In some embodiments, the vaccine comprises one or more antigens, including a recombinant protein antigen related to an infectious disease, a malignancy, or an allergan. In some embodiments, the one or more LNA-CDN compound or composition thereof is used simultaneously with, prior to or following the vaccine. In some embodiments, the one or more LNA-CDN compound or composition thereof is formulated in the same composition as the vaccine.

[00126] In a first embodiment of the forty-fifth aspect, the vaccine comprises an inactivated or attenuated bacteria or virus comprising the one or more antigens of interest, one or more purified antigens, live viral or bacterial delivery vectors recombinantly engineered to express and/or secrete the one or more antigens, antigen presenting cell (APC) vectors comprising cells that are loaded with the one or more antigens or transfected with a composition comprising a nucleic acid encoding the one or more antigens, liposomal antigen delivery vehicles, or naked nucleic acid vectors encoding the one or more antigens. In some embodiments, the vaccine is an anti-bacterial, anti-viral, or anti-cancer therapeutic or prophylactic vaccine. In some embodiments, the one or more antigens is one or more antigens selected from the group consisting of a viral antigen, a bacterial antigen and a cancer antigen.

[00127] In some embodiments of the forty-fifth aspect and first embodiment thereof, the vaccine comprises an inactivated tumor cell that expresses and secretes one or more cytokines. In some embodiments, the cytokine is selected from the group consisting of GM-CSF, CCL20, CCL3, IL-12p70, and FLT-3 ligand.

[00128] In some embodiments of the forty-fifth aspect and first embodiment thereof, the vaccine comprises an inactivated tumor cell that expresses and secretes one or more heat shock proteins. In some embodiments, the heat shock protein is gp96-Ig fusion protein.

[00129] In a forty-sixth aspect, the invention provides one or more LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, or compositions thereof as described in the thirty-ninth through fortieth aspects and any embodiments thereof as described herein above, for use in treating a disease or indication as described in any of the forty-first through forty-fifth aspects and any embodiments thereof as described herein above. In a preferred embodiment, the one or more LNA-CDN compounds are for use in treating a cancer. In some embodiments, the cancer is selected from the group consisting of a colorectal cancer, an aero-digestive squamous cancer, a lung cancer, a brain cancer, a liver cancer, a stomach cancer, a bladder cancer, a thyroid cancer, an adrenal cancer, a gastrointestinal cancer, an oropharyngeal cancer, an esophageal cancer, a head and neck cancer, an ovarian cancer, a uterine cancer, a cervical cancer, an endometrial cancer, a breast cancer, a melanoma, a prostate cancer, a pancreatic carcinoma, a renal carcinoma, a sarcoma, a leukemia, a Merkel-cell carcinoma, a lymphoma and a multiple myeloma.

[00130] In a forty-seventh aspect, the invention provides one or more LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, or compositions thereof as described in the thirty-ninth and fortieth aspects and any embodiments thereof as described herein above, for use the preparation of a medicament for the treatment of a disease or indication as described in any of the forty-first through forty-fifth aspects and any embodiments thereof as described herein above. In a preferred embodiment, the one or more LNA-CDN compounds are for use in

preparation of a medicament for the treatment of a cancer. In some embodiments, the cancer is selected from the group consisting of a colorectal cancer, an aero-digestive squamous cancer, a lung cancer, a brain cancer, a liver cancer, a stomach cancer, a bladder cancer, a thyroid cancer, an adrenal cancer, a gastrointestinal cancer, an oropharyngeal cancer, an esophageal cancer, a head and neck cancer, an ovarian cancer, a uterine cancer, a cervical cancer, an endometrial cancer, a breast cancer, a melanoma, a prostate cancer, a pancreatic carcinoma, a renal carcinoma, a sarcoma, a leukemia, a Merkel-cell carcinoma, a lymphoma and a multiple myeloma.

[00131] In a forty-eighth aspect, the invention provides a kit that includes one or more LNA-CDN compounds, as described in the first through thirty-eighth aspects and any embodiments thereof as described herein above, including any prodrugs, pharmaceutically acceptable salts, pharmaceutically acceptable solvates, or pharmaceutically acceptable hydrates thereof, or compositions thereof as described in the thirty-ninth and fortieth aspects and any embodiments thereof as described herein above. In some embodiments, one or more LNA-CDN compounds or compositions thereof is packaged, e.g., in a vial, bottle or similar container, which may be further packaged, e.g., within a box, envelope, or similar container. In some embodiments, one or more LNA-CDN compounds or compositions thereof is approved by the U.S. Food and Drug Administration or similar regulatory agency for administration to a mammal, e.g., a human. In one embodiment, such a kit includes written instructions for use and/or other indication that the one or more LNA-CDN compounds or compositions thereof is suitable or approved for administration to a mammal, e.g., a human, for a suitable disease or condition. In some embodiments, the compound or composition is packaged in unit dose or single dose form, e.g., single dose pills, capsules, or the like.

BRIEF DESCRIPTION OF THE FIGURES

[00132] Fig. 1A-C depicts relative expression of IFN β by STING^{WT/WT} (1A), STING^{HAQ/HAQ} (1B), and STING^{REF/REF} (1C) in human PBMCs at 2 hours following stimulation with 10 μ M or 100 μ M of LNA-CDN Compounds 5a and 5b.

[00133] Fig. 2A-C depicts relative expression of TNF α by STING^{WT/WT} (2A), STING^{HAQ/HAQ} (2B), and STING^{REF/REF} (2C) in human PBMCs at 2 hours following stimulation with 10 μ M or 100 μ M of LNA-CDN Compounds 5a and 5b.

[00134] Fig. 3A-C depicts relative expression of IFN γ by STING^{WT/WT} (3A), STING^{HAQ/HAQ} (3B), and STING^{REF/REF} (3C) in human PBMCs at 2 hours following stimulation with 10 μ M or 100 μ M of LNA-CDN Compounds 5a and 5b.

[00135] Fig. 4A-C depicts relative expression of IL-6 by STING^{WT/WT} (4A), STING^{HAQ/HAQ} (4B), and STING^{REF/REF} (4C) in human PBMCs at 2 hours following stimulation with 10 μ M or 100 μ M of LNA-CDN Compounds 5a and 5b.

[00136] Fig. 5A-C depicts relative expression of IL-12p35 by STING^{WT/WT} (5A), STING^{HAQ/HAQ} (5B), and STING^{REF/REF} (5C) in human PBMCs at 2 hours following stimulation with 10 μ M or 100 μ M of LNA-CDN Compounds 5a and 5b.

[00137] Fig. 6A-C depicts relative expression of IFN β by STING^{WT/WT} (6A), STING^{HAQ/HAQ} (6B), and STING^{REF/REF} (6C) in human PBMCs at 2 and 6 hours following stimulation with 10 μ M or 100 μ M of LNA-CDN Compounds 5a and 15a.

[00138] Fig. 7A-C depicts relative expression of TNF α by STING^{WT/WT} (7A), STING^{HAQ/HAQ} (7B), and STING^{REF/REF} (7C) in human PBMCs at 2 and 6 hours following stimulation with 10 μ M or 100 μ M of LNA-CDN Compounds 5a and 15a.

[00139] Fig. 8A-C depicts relative expression of IFN γ by STING^{WT/WT} (8A), STING^{HAQ/HAQ} (8B), and STING^{REF/REF} (8C) in human PBMCs at 2 and 6 hours following stimulation with 10 μ M or 100 μ M of LNA-CDN Compounds 5a and 15a.

[00140] Fig. 9A-C depicts relative expression of IL-6 by STING^{WT/WT} (9A), STING^{HAQ/HAQ} (9B), and STING^{REF/REF} (9C) in human PBMCs at 2 and 6 hours following stimulation with 10 μ M or 100 μ M of LNA-CDN Compounds 5a and 15a.

[00141] Fig. 10A-C depicts relative expression of IL-12p35 by STING^{WT/WT} (10A), STING^{HAQ/HAQ} (10B), and STING^{REF/REF} (10C) in human PBMCs at 2 and 6 hours following stimulation with 10 μ M or 100 μ M of LNA-CDN Compounds 5a and 15a.

[00142] Fig. 11A-F depicts the tumor volume in a B16.SIY melanoma mouse model, following intra-tumoral injection of HBSS vehicle (11A), reference compound 2'3'-RR-(A)(A) at 1 μ g (11B) and LNA-CDN Compound 5a at 0.1 μ g (11C), 1 μ g (11D), 10 μ g (11E), or 100 μ g (11F).

[00143] Fig. 12A-F depicts the tumor volume in a B16.SIY melanoma mouse model, following intra-tumoral injection of HBSS vehicle (12A), reference compound 2'3'-RR-(A)(A) at 1 μ g (12B) and LNA-CDN Compound 10c at 0.1 μ g (12C), 1 μ g (12D), 10 μ g (12E), or 100 μ g (12F).

[00144] Fig. 13A-D depicts the tumor volume in a B16.SIY melanoma mouse model, using WT or STING-deficient *Goldenticket* (GT) mice, following intra-tumoral injection of HBSS vehicle into WT (13A) or GT (13C); or 100 μ g of LNA-CDN Compound 15c into WT (13B) or GT (13D).

[00145] Fig. 14A-D depicts serum concentrations of pro-inflammatory cytokines TNF- α (14A), IFN- γ (14B), IL-6 (14C), and MCP-1 (14D) in a B16.SIY melanoma mouse model, 6 hours post intra-tumoral injection of HBSS vehicle control, reference compound 2'3'-RR-(A)(A) at 1 μ g, or LNA-CDN Compound 5a at 0.1 μ g, 1 μ g, 10 μ g or 100 μ g.

[00146] Fig. 15A-D depicts serum concentrations of pro-inflammatory cytokines TNF- α (15A), IFN- γ (15B), IL-6 (15C), and MCP-1 (15D) in a B16.SIY melanoma mouse model, 6 hours post intra-tumoral injection of HBSS vehicle control, reference compound 2'3'-RR-(A)(A) at 1 μ g, or LNA-CDN Compound 10c at 0.1 μ g, 1 μ g, 10 μ g or 100 μ g.

[00147] Fig. 16A-B depicts SIY⁺ CD8⁺ T-cells in splenocytes in a B16.SIY melanoma mouse model, measured in an IFN γ ELISPOT assay 7 days post intra-tumoral injection of HBSS vehicle control, reference compound 2'3'-RR-(A)(A) at 1 μ g, or LNA-CDN Compound 5a (16A) or LNA-CDN Compound 10c (16B) at 0.1 μ g, 1 μ g, 10 μ g or 100 μ g.

[00148] Fig. 17A-B depicts SIY⁺ CD8⁺ T-cells in splenocytes in a B16.SIY melanoma mouse model, measured in a FACS assay 7 days post intra-tumoral injection of HBSS vehicle control, reference compound 2'3'-RR-(A)(A) at 1 μ g, or LNA-CDN Compound 5a (17A) or LNA-CDN Compound 10c (17B) at 0.1 μ g, 1 μ g, 10 μ g or 100 μ g.

DETAILED DESCRIPTION OF THE INVENTION

[00149] The present invention relates to the production and use of locked nucleic acid cyclic-di-nucleotide (LNA-CDN) immune stimulators that activate DCs via a cytoplasmic receptor known as STING (Stimulator of Interferon Genes).

[00150] Conserved microbial structures known as Pathogen-Associated Molecular Patterns (PAMPs) are sensed by host cell Pattern Recognition Receptors (PRRs with germ-line encoded specificity), triggering a downstream signaling cascade resulting in the induction of cytokines and chemokines, and initiation of a specific adaptive immune response (Iwasaki and Medzhitov, *Science* 327, 291-5, 2010). How the innate immune system is engaged by PAMPs presented from an infectious agent shapes the development of an adaptive response appropriate to combat the invading pathogen from causing disease.

[00151] One objective in the design of immune modulators and adjuvants is to select defined PAMPs or synthetic molecules which activate designated PRRs and initiate a desired response. Adjuvants such as monophosphoryl lipid A (MPL) and CpG are microbial-derived PAMPs recognized by Toll-like receptors (TLRs), a class of PRRs that signal through MyD88 and TRIF adaptor molecules and mediate induction of NF- κ B dependent proinflammatory cytokines (Pandey et. al., *Cold Spring Harb Perspect Biol* 2015;7: a016246). MPL (TLR-4 agonist) and CpG (TLR-9 agonist) are the most clinically advanced adjuvants, and are components of vaccines that are approved or pending approval by the FDA (Einstein et al., *Human Vaccines*, 5: 705-19, 2009; Ahmed et al., *Nature Immunol.* 12: 509-17, 2011). While TLRs present on the cell surface (e.g., TLR-4) and endosomes (e.g., TLR-9) sense extracellular and vacuolar pathogens, the productive growth cycle of multiple pathogens including viruses and intracellular bacteria occurs in the cytosol. The compartmentalization of extracellular, vacuolar, and cytosolic PRRs has led to the hypothesis that the innate immune system can sense productively replicating pathogenic microbes by monitoring the cytosol (Vance et al., *Cell Host & Microbe* 6: 10-21, 2009). This provides a rationale for the use of agonists that activate PRRs comprising the cytosolic surveillance pathway and may be an effective strategy for the design of effective vaccines for eliciting cellular immunity, an immune correlate of protection against intracellular pathogens and therapeutic benefit in cancer.

[00152] Type I interferons (IFN- α , IFN- β) are the signature cytokines induced by two distinct TLR-independent cytosolic signaling pathways. In the first pathway, various forms of single-stranded and double-stranded (ds) RNA are sensed by RNA helicases, including retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5), and through the IFN- β promoter stimulator 1 (IPS-1) adaptor protein mediate phosphorylation of the IRF-3 transcription factor, leading to induction of IFN- β (Ireton and Gale, *Viruses* 3: 906-19, 2011). IPS-1^{-/-} deficient mice have increased susceptibility to infection with RNA viruses. Sensors that signal through the IPS-1 pathway are directly targeted for inactivation by various viral proteins, demonstrating a requirement of this cytosolic host defense pathway to control productive virus infection. Synthetic dsRNA, such as polyinosinic:polycytidylic acid (poly (I:C) and poly ICLC, an analog that is formulated with poly L lysine to resist RNase digestion, is an agonist for both TLR3 and MDA5 pathways, is a powerful inducer of IFN- β , and is currently being evaluated in several diverse clinical settings (Caskey et al., *J. Exp. Med.* 208: 2357-77, 2011).

[00153] STING (Stimulator of Interferon Genes) is a central mediator for the second cytosolic pathway that triggers type 1 interferon, in response to sensing cytosolic double-stranded (ds) DNA from infectious pathogens or aberrant host cells (Danger Associated Molecular Patterns, DAMPS) (Barber, *Immunol. Rev* 243: 99-108, 2011). Alternatively known as TMEM173, MITA, ERIS, and MPYS, STING was discovered using cDNA expression cloning methods as a MyD88-independent host cell defense factor expressed in macrophages, dendritic cells (DCs) and fibroblasts was found to induce expression of IFN- β and NF- κ B dependent pro-inflammatory cytokines in response to sensing cytoplasmic DNA, in response to infection with herpes simplex virus (Ishikawa and Barber, *Nature* 455: 674-79, 2008).

[00154] Cyclic dinucleotides (CDNs) have been studied as ubiquitous small molecule second messengers synthesized by bacteria which regulate diverse processes including motility and formation of biofilms (Romling et al., *Microb. Mol. Biol. Rev.*, 77: 1-52, 2013). CDNs are also a ligand for STING (Burdette et al., *Nature* 478: 515-18, 2011). In response to binding CDNs, STING activates signaling through the TBK-1/IRF-3 axis and NF- κ B axis and induces the expression of IFN- β and other co-regulated genes (Burdette and Vance, *Nat Immunol.* 14: 19-26, 2013; McWhirter et al., *J. Exp. Med.* 206: 1899-

1911, 2009). Cyclic (c)-di-AMP is secreted by multidrug resistance efflux pumps from the intracellular bacterium *Listeria monocytogenes* into the cytosol of infected host antigen presenting cells, and is correlated with CD4⁺ and CD8⁺ T cell-mediated protection in the mouse listeriosis model (Woodward et al., Science 328, 1703-05, 2010; Crimmins et al., Proc. Natl. Acad. Sci. USA 105: 10191-10196, 2008). Induction of IFN- β in Lm-infected macrophages is dependent upon activation of the STING signaling pathway, and the level of type I IFN induced by c-di-AMP in macrophages from MyD88^{-/-} Trif^{-/-} or C57BL/6 parental mice is indistinguishable (Leber et al., PLoS Pathog 4(1): e6. doi:10.1371, 2008; Witte et al., mBio 4: e00282-13, 2012). In contrast, IFN- β is not induced by CDNs in macrophages derived from goldenticket (gt) mice encoding a nonfunctional mutant STING protein (Sauer et al., Infect. Immun. 79: 688-94, 2011). The extracellular bacterium, *Vibrio cholera*, produces a hybrid c-GMP-AMP (cGAMP) molecule, which also induces the STING pathway (Davies et al., Cell 149: 358–70, 2012). The activation of innate immunity with these ubiquitous second messengers suggests that sensing CDNs may be integral to host defense against bacterial infection.

[00155] While STING was discovered as being the critical sensor for inducing the production of IFN- β in response to infection with herpes simplex virus, how the DNA from this viral pathogen was detected in the cytoplasm initially remained elusive. This conundrum was solved with the discovery of cyclic GMP-AMP synthase (cGAS), a host cell nucleotidyl transferase that directly binds dsDNA, and in response synthesizes a second messenger, cyclic GMP-AMP (cGAMP), which activates the STING pathway and induces IFN- β expression (Sun et al., Science 339: 786-91, 2013; Wu et al., Science 339: 826-30, 2013). Cells without a functional cGAS are unable to express IFN- β in response to stimulation with cytosolic DNA. It was later shown that cells expressing a particular STING allele were non-responsive to stimulation by CDNs, but responsive to stimulation with dsDNA in a cGAS-dependent and TLR9 (MyD88)-independent manner (Diner et al., 2013). This observation was incompatible with a mechanism defined by cGAS synthesizing STING-activating CDN ligands in response to sensing cytosolic dsDNA. This apparent paradox was resolved by several independent investigators, who demonstrated that cGAS produces a non-canonical CDN (c-GMP-AMP; cGAMP) that activates STING alleles that are non-responsive to canonical CDNs (Civril et al., Nature 498: 332-37, 2013, Diner et al., 2013, Gao et al., 2013, Ablasser et al., Nature 498: 380–84, 2013, Kranzusch et al., Cell Reports 3: 1362-68, 2013, Zhang et al., Mol. Cell. 51:

226-35, 2013). cGAMP thus functions as a second messenger that binds to and activates STING. Unlike the CDN second messengers produced by bacteria, in which the two purine nucleosides are joined by a phosphate bridge with bis-(3', 5') linkages, the internucleotide phosphate bridge in the cyclic-GMP-AMP synthesized by cGAS is joined by non-canonical 2',5' and 3',5' linkages (alternatively termed "mixed" linkages or ML), represented c[G(2',5')pA(3',5')p]. These 2',5'-3',5' molecules bind STING with nM affinity, some 300-fold better than bacterial c-di-GMP. Thus, it has been suggested that the 2',5'-3',5' molecules represent much more potent physiological ligands in terms of STING targeting. Zhang et al., 2013; see also, Xiao and Fitzgerald, Mol. Cell 51: 135-39, 2013. The differences in internucleotide phosphate bridge structures between CDNs produced by bacteria [canonical bis-(3', 5') linkages] and by host cell cGAS (non-canonical 2',5' and 3',5' linkages) indicates that the STING receptor evolved to distinguish between CDNs produced by bacteria or by host cell cGAS. The LNA-CDN compounds as described herein demonstrate high potency, in some instances even more potent than the natural 2'5'-3'5' cGAMP ligand, or other known mixed linkage CDNs such as 2'3'-RR-(A)(A), and provide STING activating compounds for therapeutic use.

[00156] Human STING has known polymorphisms, including alleles encoding histidine at position 232, which are refractory to canonical CDNs, but not non-canonical CDNs (Diner et al., 2013, Jin et al., 2011). Single nucleotide polymorphisms in the hSTING gene have been shown to affect the responsiveness to bacterial-derived canonical CDNs (Diner et al., 2013; Gao et al., 2013; Conlon et. al., 2013). Five haplotypes of hSTING have been identified (WT, REF, HAQ, AQ and Q alleles), which vary at amino acid positions 71, 230, 232 and 293 (Jin et al., 2011; Yi et al., 2013). Cells expressing hSTING reportedly respond poorly to stimulation with bacterial CDNs cGAMP, c-di-AMP and c-di-GMP having bis-(3', 5') linkages, but are responsive to the endogenously produced cGAS product, ML cGAMP (Diner et al., 2013). Therefore, the published literature indicates that for broad activation of all human STING alleles it is desirable that the CDN internucleotide phosphate bridge have a non-canonical, mixed linkage c[G(2',5')pA(3',5')p] structure. Examples of cyclic purine dinucleotides are described in some detail in, e.g., U.S. Patent Nos. 7,709,458 and 7,592,326; WO2007/054279, WO2014/093936, and WO2014/189805; and Yan et al., Bioorg. Med. Chem Lett. 18: 5631 (2008).

[00157] Native CDN molecules are sensitive to degradation by phosphodiesterases that are present in host cells, for example in antigen presenting cells, which take up vaccine formulations that contain said native CDN molecules. The potency of a defined adjuvant may be diminished by such degradation, as the adjuvant would be unable to bind and activate its defined PRR target. Lower adjuvant potency could be measured, for example by a lower amount of induced expression of a signature molecule of innate immunity (e.g., IFN- β), correlated with weaker vaccine potency, as defined by the magnitude of a measured antigen-specific immune response.

Definitions

[00158] Compounds of this invention include those described generally above, and are further illustrated by the classes, subclasses, and species disclosed herein. As used herein, the following definitions shall apply unless otherwise indicated. The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[00159] Unless otherwise stated or depicted, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, Z and E double bond isomers, and Z and E conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the compounds of the invention are within the scope of the invention. Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures including the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ^{13}C - or ^{14}C -enriched carbon are within the scope of this invention. Such compounds are useful, for example, as analytical tools, as probes in biological assays, or as therapeutic agents in accordance with the present invention.

[00160] The term “alkyl” as used herein, by itself or as part of another substituent, refers to, unless otherwise indicated, a saturated straight or branched chain hydrocarbon

radical having from 1 to 24 carbon atoms (i.e. C₁₋₂₄alkyl), from 1 to 20 carbon atoms (i.e. C₁₋₂₀alkyl), from 1 to 14 carbon atoms (i.e. C₁₋₁₄alkyl), from 1 to 12 carbon atoms (i.e. C₁₋₁₂alkyl), from 1 to 10 carbon atoms (i.e. C₁₋₁₀alkyl), from 1 to 8 carbon atoms (i.e. C₁₋₈alkyl), from 1 to 6 carbon atoms (i.e. C₁₋₆alkyl), or from 1 to 4 carbon atoms (i.e. C₁₋₄alkyl). Unless indicated otherwise, more preferred alkyl groups are C₁₋₆ alkyl. Examples of alkyl radicals include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, tert-butyl, iso-butyl, sec-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, n-dodecyl, and the like, including any isomers thereof. Where it is indicated that alkyl is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4, or 5; 1, 2, 3, or 4; 1, 2, or 3; 1 or 2; or 1, where multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of alkyl, or alkyl substituted on another moiety, are attached at any available atom to provide a stable compound.

[00161] The term “alkenyl” as used herein, by itself or as part of another substituent, refers to a straight or branched chain hydrocarbon radical that is unsaturated or polyunsaturated so as to have at least one double bond, for example one, two, three or more double bonds, and having from 2 to 24 carbon atoms (i.e. C₂₋₂₄alkenyl). In some embodiments, alkenyl includes from 2 to 24 carbon atoms (C₂₋₂₄alkenyl), from 2 to 20 carbon atoms (i.e. C₂₋₂₀alkenyl), from 2 to 14 carbon atoms (i.e. C₂₋₁₄alkenyl), from 2 to 12 carbon atoms (i.e. C₂₋₁₂alkenyl), from 2 to 10 carbon atoms (i.e. C₂₋₁₀alkenyl), from 2 to 8 carbon atoms (i.e. C₂₋₈alkenyl), or from 2 to 6 carbon atoms (i.e. C₂₋₆alkenyl) having 1 to 3 double bonds, or from 2 to 4 carbon atoms (i.e. C₂₋₄alkenyl) having 1 or 2 double bonds. Unless indicated otherwise, more preferred alkenyl groups are C₂₋₆alkenyl, and having 1 to 3 double bonds, 1 to 2 double bonds, or preferably 1 double bond. Exemplary "alkenyl" groups include vinyl, propenyl, (*E*)-but-1-enyl, (*Z*)-but-1-enyl, (*E*)-but-2-enyl, but-3-enyl, (*E*)-buta-1,3-dienyl, (*E*)-penta-2,4-dienyl, (*2E,4E,6E*)-octa-2,4,6-trienyl and the like, including any isomers thereof. Where it is indicated that alkenyl is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4, or 5; 1, 2, 3, or 4; 1, 2, or 3; 1 or 2; or 1, where multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of alkenyl, or alkenyl substituted on another moiety, are attached at any available atom to provide a stable compound.

[00162] The term “alkynyl” as used herein, by itself or as part of another substituent, refers to a straight or branched chain hydrocarbon radical that is unsaturated or polyunsaturated so as to have at least one triple bond, for example one, two, three or more triple bonds, and having from 2 to 24 carbon atoms (i.e. C₂₋₂₄alkynyl). In some embodiments, alkynyl includes from 2 to 24 carbon atoms (C₂₋₂₄alkynyl), from 2 to 20 carbon atoms (i.e. C₂₋₂₀alkynyl), from 2 to 14 carbon atoms (i.e. C₂₋₁₄alkynyl), from 2 to 12 carbon atoms (i.e. C₂₋₁₂alkynyl), from 2 to 10 carbon atoms (i.e. C₂₋₁₀alkynyl), from 2 to 8 carbon atoms (i.e. C₂₋₈alkynyl), or from 2 to 6 carbon atoms (i.e. C₂₋₆alkynyl) having 1 to 3 triple bonds, or from 2 to 4 carbon atoms (i.e. C₂₋₄alkynyl) having 1 or 2 triple bonds. Unless indicated otherwise, more preferred alkynyl groups are C₂₋₆alkynyl, and having 1 to 3 triple bonds, 1 to 2 triple bonds, or preferably 1 triple bond. Exemplary “alkynyl” groups include prop-1-ynyl, prop-2-ynyl (propargyl), ethynyl and 3-butynyl and the like, including any isomers thereof. Where it is indicated that alkynyl is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4, or 5; 1, 2, 3, or 4; 1, 2, or 3; 1 or 2; or 1, where multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of alkynyl, or alkynyl substituted on another moiety, are attached at any available atom to provide a stable compound.

[00163] The term “C₁₋₆alkoxy” represents a substituent -O-C₁₋₆alkyl, wherein C₁₋₆alkyl is as defined herein, and is optionally substituted where indicated. Where it is indicated that the C₁₋₆alkyl group within C₁₋₆alkoxy is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4, or 5, also 1, 2, 3, or 4, also 1, 2, or 3, also 1 or 2, or one, where multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of C₁₋₆alkoxy, or any C₁₋₆alkoxy substituted on another moiety, are attached at any available atom to provide a stable compound.

[00164] The terms “C₁₋₆alkylthio”, “C₁₋₆alkylsulfinyl” or “C₁₋₆alkylsulfonyl” represent a substituent -S-C₁₋₆alkyl, -S(=O)-C₁₋₆alkyl or -S(=O)₂-C₁₋₆alkyl, respectively, wherein C₁₋₆alkyl is as defined herein, and is optionally substituted where indicated. Where it is indicated that the C₁₋₆alkyl group within C₁₋₆alkylthio, C₁₋₆alkylsulfinyl or C₁₋₆alkylsulfonyl is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4, or 5, also 1, 2, 3, or 4, also 1, 2, or 3, also 1 or 2, or one, where

multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of C₁₋₆alkylthio, C₁₋₆alkylsulfinyl or C₁₋₆alkylsulfonyl, or any C₁₋₆alkylthio, C₁₋₆alkylsulfinyl or C₁₋₆alkylsulfonyl substituted on another moiety, are attached at any available atom to provide a stable compound.

[00165] The terms "C₁₋₆alkylamino" or "C₁₋₆dialkylamino" represent a substituent -NH-C₁₋₆alkyl or -N-(C₁₋₆alkyl)(C₁₋₆alkyl), respectively, wherein C₁₋₆alkyl is as defined herein, and is optionally substituted where indicated. The two C₁₋₆alkyl groups of C₁₋₆dialkylamino may be the same or different. Where it is indicated that the C₁₋₆alkyl group within C₁₋₆alkylamino or C₁₋₆dialkylamino is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4, or 5, also 1, 2, 3, or 4, also 1, 2, or 3, also 1 or 2, or one, where multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of C₁₋₆alkylamino or C₁₋₆dialkylamino, or any C₁₋₆alkylamino or C₁₋₆dialkylamino substituted on another moiety, are attached at any available atom to provide a stable compound.

[00166] The terms "halo" or "halogen," by themselves or as part of another substituent, mean at least one of fluorine, chlorine, bromine and iodine. For example the terms "C₁₋₆haloalkoxy", "C₁₋₆haloalkylthio", "C₁₋₆haloalkylsulfinyl", and "C₁₋₆haloalkylsulfonyl", mean that the alkyl portion of these substituents is substituted with at least one halogen, such as 1, 2 or 3 halogen. For example, C₁₋₆haloalkoxy includes -OCH₂CH₂F, -OCH₂CH₂Cl, -OCF₃, and the like.

[00167] The term "N-linked heterocycloalkyl" by itself or as part of another substituent, means the group -NR₁'R₁", where R₁' and R₁" combine with the nitrogen to form a nitrogen linked 5-7 membered heterocycloalkyl monocycle, where the heterocycloalkyl may contain an additional heteroatom within the ring, such as O, N, or S, and is optionally substituted with C₁₋₆alkyl. The ring is bound to the group it is a substituent of via the nitrogen. Examples of N-linked-heterocycloalkyl include, but are not limited to, piperidine, piperazine, 4-methylpiperazine, morpholine, and thiomorpholine. Where it is indicated that N-linked-heterocycloalkyl is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4 or 5, also 1, 2, 3, or 4, also 1, 2, or 3, also 1 or 2, or one, where multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of N-linked-

heterocycloalkyl, or N-linked-heterocycloalkyl substituted on another moiety, are attached at any available atom to provide a stable compound.

[00168] The term “heteroalkyl” as used herein, by itself or as part of another substituent, refers to a stable, straight or branched chain radical consisting of a number of carbon atoms (e.g., C₂₋₂₄, C₂₋₂₀, C₂₋₁₄, C₂₋₁₂, C₂₋₁₀, C₂₋₈, C₂₋₆ or C₂₋₄) and at least one heteroatom, e.g. 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, or 1 heteroatom independently selected from the group consisting of N, O, S, Si, B and P (preferably N, O and S), wherein the nitrogen, sulfur and phosphorus atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. Typically, a heteroalkyl group will have from 3 to 24 carbon and heteroatoms in a straight or branched chain, excluding hydrogen (3- to 24-membered heteroalkyl), 3 to 12 carbon and heteroatoms (3- to 12-membered heteroalkyl), 3 to 10 carbon and heteroatoms (3- to 10-membered heteroalkyl) or from 3 to 8 carbon and heteroatoms (3- to 8-membered heteroalkyl). The heteroatom(s) is/are placed at any position of the heteroalkyl group. In some embodiments, where more than one heteroatoms are in the heteroalkyl group, the more than one heteroatoms are the same. The heteroalkyl group can be unsaturated or poly unsaturated, i.e. can include at least one, e.g. one, two or three, carbon carbon or carbon nitrogen double bond, or a carbon carbon triple bond. Examples of heteroalkyl groups include, but are not limited to, -CH₂-CH₂-O-CH₃, -CH₂-CH₂-NH-CH₃, -CH₂-CH₂-N(CH₃)-CH₃, -CH₂-S-CH₂-CH₃, -CH₂-CH₂-S(=O)-CH₃, -CH₂-CH₂-S(=O)₂-CH₃, -CH=CH-O-CH₃, -CH₂-Si(CH₃)₃, -CH₂-CH=N-OCH₃, and -CH=CH-N(CH₃)-CH₃. Up to two heteroatoms within heteroalkyl can be consecutive, such as, for example, -CH₂-NH-OCH₃ and -CH₂-O-Si(CH₃)₃. Heteroalkyl also includes, without limitation, a group comprising polyalkoxy (e.g. polyalkylene glycols), polyamines or polyimines, for example -C₀₋₄alkyl-O-(C₂₋₃alkyl-O)_a-H, -C₀₋₄alkyl-O-(C₂₋₃alkyl-O)_a-C₁₋₄alkyl, -C₀₋₄alkyl-NH-(C₂₋₃alkyl-NH)_a-H or -C₀₋₄alkyl-NH-(C₂₋₃alkyl-NH)_a-C₁₋₄alkyl, where a is e.g. 2-7, such as -O-(CH₂CH(CH₃)O)_a-H, -O-(CH₂CH(CH₃)O)_a-CH₃, -O-(CH₂CH(CH₃)O)_a-CH₂CH₃, -O-(CH₂CH₂O)_a-H, -O-(CH₂CH₂O)_a-CH₃, -O-(CH₂CH₂O)_a-CH₂CH₃ or the like, -NH-(CH₂CH₂NH)_a-H, -NH-(CH₂CH₂NH)_a-CH₃, -NH-(CH₂CH₂NH)_a-CH₂CH₃ or the like, wherein a is 2-7, 2-6, 2-5, 2-4 or 2-3. Where it is indicated that heteroalkyl is optionally substituted with one or more substituents, one or

more is typically 1, 2, 3, 4, or 5; 1, 2, 3, or 4; 1, 2, or 3; 1 or 2; or 1, where multiple substituents are independently selected unless indicated otherwise. Substituents may be bound to any substitutable atom, i.e. carbon or heteroatom. It is understood that any substitutions of heteroalkyl, or heteroalkyl substituted on another moiety, are attached at any available atom to provide a stable compound.

[00169] The term “aryl” as used herein, by itself or as part of another substituent, refers to a fully aromatic monocyclic (i.e. phenyl) or polycyclic (e.g. naphthalene, anthracene) carbocyclic ring system having 6 to 14 carbon atoms (C_{6-14} aryl), or 6 to 10 carbon atoms (C_{6-10} aryl), or includes a polycyclic ring system wherein at least one ring is aromatic, e.g. aryl fused to 1 or 2 additional rings. Exemplary polycyclic aryl having at least one aromatic ring include a phenyl ring fused to a carbocyclyl, heterocycloalkyl or heteroaryl ring (e.g., 1 or 2 other rings), wherein such polycyclic aryl ring system is attached to the group it is a substituent of via the aryl (e.g. phenyl) ring portion. In a preferred embodiment, aryl is phenyl. Where it is indicated that aryl is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4, or 5; 1, 2, 3, or 4; 1, 2, or 3; 1 or 2; or 1, where multiple substituents are independently selected unless indicated otherwise. Non-limiting examples of aryl groups include phenyl, 1-naphthyl, 2-naphthyl, indanyl, indenyl, dihydronaphthyl, fluorenyl, tetralinyl, chromanyl, isochromanyl, benzo[d][1,3]dioxolyl, 2,3-dihydrobenzo[b][1,4]dioxinyl, and the like. Where it is indicated that aryl is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4 or 5, also 1, 2, 3, or 4, also 1, 2, or 3, also 1 or 2, or one, where multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of aryl, or aryl substituted on another moiety, are attached at any available atom to provide a stable compound.

[00170] The term “carbocyclyl” as used herein, by itself or as part of another substituent, refers to a mono- or polycyclic saturated or unsaturated, non-aromatic carbocyclic radical having from 3 to 24 carbon atoms (i.e. C_{3-24} carbocyclyl), also 3 to 12 carbon atoms (i.e., C_{3-12} carbocyclyl), 3 to 10 carbon atoms (i.e. C_{3-10} carbocyclyl), 3 to 8 carbon atoms (i.e. C_{3-8} carbocyclyl) or 3 to 6 carbon atoms (i.e. C_{3-6} carbocyclyl). Included within carbocyclyl are fully saturated mono- or polycyclic ring systems referred to as “cycloalkyl”, including bridged or spirocyclic polycyclics, for example cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl,

bicyclo[2.2.1]heptyl, adamantly, and spiro[4.4]nonanyl, and the like. Also included within carbocyclyl are partially unsaturated mono- or polycyclic non-aromatic ring systems having at least one double bond referred to as "cycloalkenyl", for example 1-cyclohexenyl, 3-cyclohexenyl, and the like. In some instances of the compounds described herein, two substituents on the same carbon atom can form a C₃₋₆cycloalkyl spirocyclic ring (e.g. CRR portion of a ring, where the two R along with the carbon forms a spirocyclic ring). A polycyclic carbocyclyl group includes a cycloalkyl or cycloalkenyl group fused to 1 or 2 other aryl, heterocycloalkyl or heteroaryl rings, wherein such polycyclic carbocyclyl ring system is attached to the group it is a substituent of via the cycloalkyl or cycloalkenyl ring portion. Preferred carbocyclyl groups include monocyclic C₃₋₈cycloalkyl, preferably C₃₋₆cycloalkyl. Where it is indicated that carbocyclyl is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4 or 5, also 1, 2, 3, or 4, also 1, 2, or 3, also 1 or 2, or one, where multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of carbocyclyl, or carbocyclyl substituted on another moiety, are attached at any available atom to provide a stable compound.

[00171] The term "heterocyclyl" as used herein, by itself or as part of another substituent, refers to a mono- or polycyclic saturated or unsaturated, non-aromatic ring having, for example, 3- to 24-members, 3- to 12 members, 3- to 10-members, 3- to 8-members, also 4-, 5-, 6- or 7-members, where 1 to 5 members, 1-4 members, 1-3 members, 1-2 members, or one member of the ring are heteroatoms, e.g., N, O, S, Si, B or P, preferably N, O or S, where the remaining ring atoms are carbon atoms, in stable combinations known to those of skill in the art. The heterocyclyl ring nitrogen, sulfur or phosphorus atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. Included within heterocyclyl are "3 - 7 membered heterocycloalkyl", "3 - 6 membered heterocycloalkyl", "4-7 membered heterocycloalkyl", "5-7 membered heterocycloalkyl" or the like which refer to a monocyclic heterocyclic ring having the indicated number of ring atoms (e.g. 3-7 members has 3-, 4-, 5-, 6-, or 7- ring atoms), where 1, 2, or 3 members, 1 or 2 members, or one member is N, O or S, and the remaining ring atoms are carbon atoms. In some instances of the compounds described herein, two substituents on the same carbon atom can form a 3-6 membered heterocycloalkyl spirocyclic ring (e.g. CRR portion of a ring, where the two R along with the carbon forms a spirocyclic ring containing e.g. O or NH). In some preferred

embodiments, heterocyclyl is monocyclic 4-7 or 5-7 membered heterocycloalkyl. Heterocyclyl group includes a heterocycloalkyl fused to 1 or 2 other aryl, heteroaryl or cycloalkyl rings, wherein such polycyclic heterocyclyl ring system is attached to the group it is a substituent of via the heterocycloalkyl portion. The point of attachment of heterocycloalkyl to the group it is a substituent of can be via a carbon atom or via a heteroatom. Exemplary heterocycloalkyl groups for compounds described herein (e.g. compounds of Formula I) include morpholinyl, thiomorpholinyl, thiomorpholinyl S-oxide, thiomorpholinyl S,S-dioxide, piperazinyl, homopiperazinyl, pyrrolidinyl, pyrrolinyl, imidazolidinyl, tetrahydropyranyl, piperidinyl, tetrahydrofuranyl, tetrahydrothienyl, homopiperidinyl, homomorpholinyl, homothiomorpholinyl, homothiomorpholinyl S,S-di oxide, oxazolidinonyl, dihydropyrazolyl, dihydropyrrolyl, dihydropyrazolyl, dihydropyridyl, dihydropyrimidinyl, dihydrofuryl, dihydropyranyl, tetrahydrothienyl S-oxide, tetrahydrothienyl S,S-dioxide, homothiomorpholinyl S-oxide, and the like. In one example, heterocycloalkyl is N-linked heterocycloalkyl as defined herein above. Where it is indicated that heterocycloalkyl is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4 or 5, also 1, 2, 3, or 4, also 1, 2, or 3, also 1 or 2, or one, where multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of heterocycloalkyl, or heterocycloalkyl substituted on another moiety, are attached at any available atom to provide a stable compound.

[00172] The term "heteroaryl", by itself or as part of another substituent means, unless otherwise stated, a monocyclic polyunsaturated, 5-, 6- or 7-membered aromatic ring containing at least one heteroatom (e.g., 1 to 5, 1 to 4, 1 to 3, 1 to 2 or 1 heteroatoms) selected from N, O, S, Si and B (in one example N, O and S), and the remaining ring atoms are carbon atoms, in stable combinations known to those of skill in the art; or such 5-, 6- or 7-membered aromatic ring fused to 1 or 2 other aryl, heteroaryl, cycloalkyl or heterocycloalkyl rings. Where heteroaryl includes additional fused rings, the heteroaryl is attached to the group it is a substituent of via the 5-, 6-, or 7-membered heteroaryl portion. Heteroaryl ring nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. The point of attachment of heteroaryl to the group it is a substituent of can be via a carbon atom or a heteroatom (e.g. nitrogen). In one example, the heteroaryl group has from 1 to 10 carbon atoms and from 1 to 5 heteroatoms selected from O, S and N. In some preferred embodiments, the heteroaryl is

a "5 or 6 membered heteroaryl", meaning a monocyclic heteroaryl ring having 5- or 6-members, where 1, 2, 3, or 4, also 1, 2 or 3, also 1 or 2, also 1 member(s) is N, O or S. Non-limiting examples of heteroaryl groups include pyridyl, pyrimidinyl, quinolinyl, benzothienyl, indolyl, pyridazinyl, pyrazinyl, isoindolyl, isoquinolinyl, quinazolinyl, quinoxalinyl, phthalazinyl, imidazolyl, isoxazolyl, pyrazolyl, oxazolyl, thiazolyl, indoliziny, indazolyl, benzothiazolyl, benzimidazolyl, benzofuranyl, furanyl, thienyl, pyrrolyl, oxadiazolyl, thiadiazolyl, triazolyl, tetrazolyl, isothiazolyl, naphthyridinyl, tetrahydroisoquinolinyl, isoindolinyl, isobenzothienyl, benzoxazolyl, pyridopyridyl, purinyl, benzodioxolyl, triazinyl, pteridinyl, benzothiazolyl, imidazopyridyl, imidazothiazolyl, dihydrobenzoxazinyl, benzisoxazinyl, benzoxazinyl, dihydrobenzisothiazinyl, benzopyranyl, benzothiopyranyl, chromonyl, tetrahydroquinolinyl, dihydroquinolinyl, and the like. In one example, heteroaryl groups include pyrrolyl, furanyl, thienyl, imidazolyl, pyrazolyl, thiadiazolyl, triazolyl, isoxazolyl, oxazolyl, isothiazolyl, thiazolyl, oxadiazolyl, pyridazinyl, pyrazinyl, pyrimidinyl, and pyridyl. Where it is indicated that heteroaryl is optionally substituted with one or more substituents, one or more is typically 1, 2, 3, 4 or 5, also 1, 2, 3, or 4, also 1, 2, or 3, also 1 or 2, or one, where multiple substituents are independently selected unless indicated otherwise. It is understood that any substitutions of heteroaryl, or heteroaryl substituted on another moiety, are attached at any available atom to provide a stable compound.

[00173] As described herein, compounds of the invention may contain "optionally substituted" or "substituted" moieties. Unless indicated otherwise, the "optionally substituted" or "substituted" moiety may be substituted with one or more substituents, such as 1, 2, 3, 4 or 5 substituents, 1, 2, 3 or 4 substituents, 1, 2 or 3 substituents, 1 or 2 substituents, or 1 substituent. Any substitutable position within a moiety can be substituted with a suitable substituent, for example, a substitutable carbon or nitrogen atom within a moiety. When the moiety has more than one substitution, each substituent may be the same or different, i.e. substituents are independently selected from a group of options. More than one substituent may be at the same position within the moiety, for example, where a carbon atom can be substituted by more than one substituent. It is understood that all such substitutions result in a stable or chemically feasible compound.

[00174] The terms "suitable oxygen protecting group" or "suitable nitrogen protecting group" refer to protecting groups used during organic synthesis and are known to one

skilled in the art, e.g. as described in Greene's Protective Groups in Organic Synthesis, 4th Edition, (John Wiley & Sons, Inc.), the disclosure of which is incorporated herein by reference as it relates to nitrogen and oxygen protecting groups. Without limitation, a suitable oxygen protecting group includes silyl protecting groups such as TBS (-Si(CH₃)₂C(CH₃)₃), TMS (-Si(CH₃)₃), TES (-Si(CH₂CH₃)₃), TIPS (-Si(CH(CH₃)₂)₃), TBDPS (-Si(C(CH₃)₃)(phenyl)₂), ester forming protecting groups such as pivalyl (-C(=O)C(CH₃)₃) and acetyl (-C(=O)CH₃), or ether forming protecting groups, such as methyl (-CH₃), methoxymethyl (-CH₂-O-CH₃), t-butyl (-C(CH₃)₃), allyl (-CH₂-CH=CH₂), benzyl (-CH₂-phenyl) and p-methoxybenzyl (-CH₂-(4-methoxy-phenyl)). Without limitation, suitable nitrogen protecting groups include benzyl (-CH₂-phenyl), benzoyl (-C(=O)-phenyl), isobutyryl (-C(=O)-CH(CH₃)₂), acetyl (-C(=O)-CH₃), t-butyl formate (-C(=O)OC(CH₃)₂), and N'N'-dimethylformamidinium (=CH-N(CH₃)₂), i.e. the protected nitrogen is -N=CH-N(CH₃)₂.

[00175] "Administration" as it is used herein with regard to a human, mammal, mammalian subject, animal, veterinary subject, placebo subject, research subject, experimental subject, cell, tissue, organ, or biological fluid, refers without limitation to contact of an exogenous ligand, reagent, placebo, small molecule, pharmaceutical agent, therapeutic agent, diagnostic agent, or composition to the subject, cell, tissue, organ, or biological fluid, and the like. "Administration" can refer, *e.g.*, to therapeutic, pharmacokinetic, diagnostic, research, placebo, and experimental methods. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. "Administration" also encompasses in vitro and ex vivo treatments, *e.g.*, of a cell, by a reagent, diagnostic, binding composition, or by another cell. By "administered together" or "co-administered" it is not meant to be implied that two or more agents be administered as a single composition. Although administration as a single composition is contemplated by the present invention, such agents may be delivered to a single subject as separate administrations, which may be at the same or different time, and which may be by the same route or different routes of administration. By "administered simultaneously" it is meant to be implied that two or more agents be administered at essentially the same time, although not necessarily administered as a single composition or by the same route of administration.

[00176] An “agonist,” as it relates to a ligand and receptor, comprises a molecule, combination of molecules, a complex, or a combination of reagents, that stimulates the receptor. For example, an agonist of granulocyte-macrophage colony stimulating factor (GM-CSF) receptor can encompass GM-CSF, a mutant or derivative of GM-CSF, a peptide mimetic of GM-CSF, a small molecule that mimics the biological function of GM-CSF, or an antibody that stimulates GM-CSF receptor.

[00177] An “antagonist,” as it relates to a ligand and receptor, comprises a molecule, combination of molecules, or a complex, that inhibits, counteracts, downregulates, and/or desensitizes the receptor. “Antagonist” encompasses any reagent that inhibits a constitutive activity of the receptor. A constitutive activity is one that is manifest in the absence of a ligand/receptor interaction. “Antagonist” also encompasses any reagent that inhibits or prevents a stimulated (or regulated) activity of a receptor. By way of example, an antagonist of GM-CSF receptor includes, without implying any limitation, an antibody that binds to the ligand (GM-CSF) and prevents it from binding to the receptor, or an antibody that binds to the receptor and prevents the ligand from binding to the receptor, or where the antibody locks the receptor in an inactive conformation.

[00178] The term “antibody” as used herein refers to a peptide or polypeptide derived from, modeled after or substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, capable of specifically binding an antigen or epitope. See, e.g. Fundamental Immunology, 3rd Edition, W.E. Paul, ed., Raven Press, N.Y. (1993); Wilson (1994; J. Immunol. Methods 175:267-273; Yarmush (1992) J. Biochem. Biophys. Methods 25:85-97. The term antibody includes antigen-binding portions, i.e., “antigen binding sites,” (e.g., fragments, subsequences, complementarity determining regions (CDRs)) that retain capacity to bind antigen, including (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Single chain antibodies are also included by reference in the term “antibody.”

[00179] By “an agent that enhances permeability” or “an agent that enhances uptake” as used herein as it relates to cell permeability or uptake of compound by cells, is an agent that enhances the permeability of a cell to a compound or enhances the uptake of a compound by the cell, either *in vitro*, or *in vivo*. The LNA-CDN compounds as described herein can be compared to a reference compound in an *in vitro* cell based assay, wherein the assay may be performed with or without an agent, such as digitonin, or by electroporation, that allows for the compound to be taken up by the cell. The LNA-CDN compounds as described herein are surprisingly active in such cell based assays without the need for such an agent that enhances permeability of the cell or enhances uptake of the compound by the cell, for example in the THP-1 cell assay as described herein. Compositions comprising the LNA-CDN compounds as described herein can be formulated without an agent that enhances permeability of the cell or enhances uptake of the compound by the cell, for example without a delivery vehicle that enhances permeability of the cell or enhances cellular uptake. Such additives or delivery vehicles include, without limitation, lipid or lipid-like adjuvants, liposomes, interbilayer crosslinked multilamellar vesicles, nanocarriers, nanoparticles and the like, such as nanoparticles comprising Poly(lactic acid) (PLA), Poly(glycolic acid) (PGA), and/or their copolymers such as biodegradable poly(D,L-lactic-co-glycolic acid) [PLGA]-based or poly anhydride-based nanoparticles or microparticles.

[00180] By “substantially purified” with regard to LNA-CDNs of the invention is meant that a specified species accounts for at least 50%, more often accounts for at least 60%, typically accounts for at least 70%, more typically accounts for at least 75%, most typically accounts for at least 80%, usually accounts for at least 85%, more usually accounts for at least 90%, most usually accounts for at least 95%, and conventionally accounts for at least 98% by weight, or greater, of the CDN activity present in a composition. The weights of water, buffers, salts, detergents, reductants, protease inhibitors, stabilizers (including an added protein such as albumin), and excipients are generally not used in the determination of purity.

[00181] “Specifically” or “selectively” binds, when referring to a ligand/receptor, nucleic acid/complementary nucleic acid, antibody/antigen, or other binding pair (e.g., a cytokine to a cytokine receptor) (each generally referred to herein as a “target biomolecule” or a “target”) indicates a binding reaction which is related to the presence of

the target in a heterogeneous population of proteins and other biologics. Specific binding can mean, e.g., that the binding compound, nucleic acid ligand, antibody, or binding composition derived from the antigen-binding site of an antibody, of the contemplated method binds to its target with an affinity that is often at least 25% greater, more often at least 50% greater, most often at least 100% (2-fold) greater, normally at least ten times greater, more normally at least 20-times greater, and most normally at least 100-times greater than the affinity with a non-target molecule.

[00182] “Ligand” refers to a small molecule, nucleic acid, peptide, polypeptide, saccharide, polysaccharide, glycan, glycoprotein, glycolipid, or combinations thereof that binds to a target biomolecule. While such ligands may be agonists or antagonists of a receptor, a ligand also encompasses a binding agent that is not an agonist or antagonist, and has no agonist or antagonist properties. Specific binding of a ligand for its cognate target is often expressed in terms of an “Affinity.” In preferred embodiments, the ligands of the present invention bind with affinities of between about 10^4 M^{-1} and about 10^8 M^{-1} . Affinity is calculated as $K_d = k_{\text{off}}/k_{\text{on}}$ (k_{off} is the dissociation rate constant, K_{on} is the association rate constant and K_d is the equilibrium constant).

[00183] Affinity can be determined at equilibrium by measuring the fraction bound (r) of labeled ligand at various concentrations (c). The data are graphed using the Scatchard equation: $r/c = K(n-r)$; where r = moles of bound ligand/mole of receptor at equilibrium; c = free ligand concentration at equilibrium; K = equilibrium association constant; and n = number of ligand binding sites per receptor molecule. By graphical analysis, r/c is plotted on the Y-axis versus r on the X-axis, thus producing a Scatchard plot. Affinity measurement by Scatchard analysis is well known in the art. *See, e.g., van Erp et al., J. Immunoassay* 12: 425-43, 1991; Nelson and Griswold, *Comput. Methods Programs Biomed.* 27: 65-8, 1988. In an alternative, affinity can be measured by isothermal titration calorimetry (ITC). In a typical ITC experiment, a solution of ligand is titrated into a solution of its cognate target. The heat released upon their interaction (ΔH) is monitored over time. As successive amounts of the ligand are titrated into the ITC cell, the quantity of heat absorbed or released is in direct proportion to the amount of binding. As the system reaches saturation, the heat signal diminishes until only heats of dilution are observed. A binding curve is then obtained from a plot of the heats from each injection

against the ratio of ligand and binding partner in the cell. The binding curve is analyzed with the appropriate binding model to determine K_B , n and ΔH . Note that $K_B = 1/K_d$.

[00184] The term "prodrug" as used herein refers to a modification of contemplated compounds, wherein the modified compound exhibits less pharmacological activity (as compared to the modified compound) and wherein the modified compound is converted within the body (e.g., in a target cell or target organ) back into the unmodified form through enzymatic or non-enzymatic reactions. In certain embodiments, the hydroxyl on one ribose comprises a prodrug leaving group. Prodrugs can modify the physicochemical, biopharmaceutic, and pharmacokinetic properties of drugs. Traditional prodrugs are classified as drugs that are activated by undergoing transformation in vivo to form the active drug. Reasons for prodrug development are typically poor aqueous solubility, chemical instability, low oral bioavailability, lack of blood brain barrier penetration, and high first pass metabolism associated with the parent drug. Suitable prodrug moieties are described in, for example, "Prodrugs and Targeted Delivery," J. Rautico, Ed., John Wiley & Sons, 2011. Examples include, but are not limited to, leaving groups removed by cellular esterases, a C6 to C18 fatty acid ester, a myristoyl ester, a pentanoyl ester, a hexanoyl ester, and a heptanoyl ester. For example, the LNA-CDN compounds as described herein wherein one of the nucleotides does not include a locked portion, but has either a 2' hydroxyl or 3' hydroxyl substituent on the ribose ring can include derivatives at the 2' hydroxyl or 3' hydroxyl, respectively, to form such esters.

[00185] The term "subject" or "individual" as used herein refers to a human or non-human organism. Thus, the methods and compositions described herein are applicable to both human and veterinary disease. In certain embodiments, subjects are "patients," i.e., living humans that are receiving medical care for a disease or condition. This includes persons with no defined illness who are being investigated for signs of pathology. Preferred are subjects who have an existing diagnosis of a particular cancer which is being targeted by the compositions and methods of the present invention. Preferred cancers for treatment with the compositions described herein include, but are not limited to prostate cancer, renal carcinoma, melanoma, pancreatic cancer, cervical cancer, ovarian cancer, colon cancer, head & neck cancer, lung cancer and breast cancer.

[00186] "Therapeutically effective amount" is defined as an amount of a reagent or pharmaceutical composition that is sufficient to show a patient benefit, i.e., to cause a

decrease, prevention, or amelioration of the symptoms of the condition being treated. When the agent or pharmaceutical composition comprises a diagnostic agent, a “diagnostically effective amount” is defined as an amount that is sufficient to produce a signal, image, or other diagnostic parameter. Effective amounts of the pharmaceutical formulation will vary according to factors such as the degree of susceptibility of the individual, the age, gender, and weight of the individual, and idiosyncratic responses of the individual. “Effective amount” encompasses, without limitation, an amount that can ameliorate, reverse, mitigate, prevent, or diagnose a symptom or sign of a medical condition or disorder or a causative process thereof. Unless dictated otherwise, explicitly or by context, an “effective amount” is not limited to a minimal amount sufficient to ameliorate a condition.

[00187] “Treatment” or “treating” (with respect to a condition or a disease) is an approach for obtaining beneficial or desired results including and preferably clinical results. For purposes of this invention, beneficial or desired results with respect to a disease include, but are not limited to, one or more of the following: improving a condition associated with a disease, curing a disease, lessening severity of a disease, delaying progression of a disease, alleviating one or more symptoms associated with a disease, increasing the quality of life of one suffering from a disease, and/or prolonging survival. Likewise, for purposes of this invention, beneficial or desired results with respect to a condition include, but are not limited to, one or more of the following: improving a condition, curing a condition, lessening severity of a condition, delaying progression of a condition, alleviating one or more symptoms associated with a condition, increasing the quality of life of one suffering from a condition, and/or prolonging survival. For instance, in embodiments where the compositions described herein are used for treatment of cancer, the beneficial or desired results include, but are not limited to, one or more of the following: reducing the proliferation of (or destroying) neoplastic or cancerous cells, reducing metastasis of neoplastic cells found in cancers, shrinking the size of a tumor, decreasing symptoms resulting from the cancer, increasing the quality of life of those suffering from the cancer, decreasing the dose of other medications required to treat the disease, delaying the progression of the cancer, and/or prolonging survival of patients having cancer. Depending on the context, “treatment” of a subject can imply that the subject is in need of treatment, e.g., in the situation where the subject comprises a disorder expected to be ameliorated by administration of a reagent.

[00188] “Vaccine” encompasses preventative vaccines. Vaccine also encompasses therapeutic vaccines, e.g., a vaccine administered to a mammal that comprises a condition or disorder associated with the antigen or epitope provided by the vaccine.

Locked Nucleic Acid Cyclic Dinucleotides

[00189] Prokaryotic as well as eukaryotic cells use various small molecules for cell signaling and intra- and intercellular communication. Cyclic purine nucleotides like cGMP, cAMP, etc. are known to have regulatory and initiating activity in pro- and eukaryotic cells. Unlike eukaryotic cells, prokaryotic cells also use cyclic purine dinucleotides as regulatory molecules. In prokaryotes, the condensation of two GTP molecules is catalyzed by the enzyme diguanylate cyclase (DGC) to give c-diGMP, which represents an important regulator in bacteria.

[00190] Recent work suggests that cyclic diGMP or analogs thereof can also stimulate or enhance immune or inflammatory response in a patient or can enhance the immune response to a vaccine by serving as an adjuvant in mammals. Cytosolic detection of pathogen-derived DNA requires signaling through TANK binding kinase 1 (TBK1) and its downstream transcription factor, IFN-regulatory factor 3 (IRF3). A transmembrane protein called STING (stimulator of IFN genes; also known as MITA, ERIS, MPYS and TMEM173) functions as the signaling receptor for these cyclic purine dinucleotides, causing stimulation of the TBK1-IRF3 signalling axis and a STING-dependent type I interferon response. Burdette et al., Nature 478: 515-18, 2011 demonstrated that STING binds directly to cyclic diguanylate monophosphate, but not to other unrelated nucleotides or nucleic acids.

[00191] The Locked Nucleic Acid Cyclic dinucleotides as described herein can be readily derived from nucleotides known in the art, using the synthetic methods as described herein and as known in the art. Such Locked Nucleic Acids for use as nucleotide precursors to derive the LNA-CDNs of the present invention are described in some detail in the literature and patents, including, but not limited to, PCT Publication Nos. WO 99/60855; WO 2007/145593; WO 2008/154401; WO 2009/006478; WO 2009/143369; WO 2010/091308; WO 2011/085102; WO 2013/036868; WO 2014/145356; WO 2015/125783; WO 2015/142735; WO 2015/185565; and WO 2016/017422; US Patent Nos. 6,403,556; 6,833,361; 7,217,805; 7,335,765; 7,427,672;

7,569,686; 8,022,193; 8,278,283; 8,278,425; 8,278,426; 8,461,124; 8,541,562; and 9,005,906; US Patent Publication Nos. 2003/0028013; 2012/071646; 2015/0337002; 2015/0152132; and Seth et al., J. Org. Chem., 2012, 77: 5074-5085; Migawa et al., Org. Letters, 2013, 15(17): 4316-4319; Xu et al., J. Org. Chem. 2009, 74, 6534-6554; and Kovács et al., 1995, Nucleosides & Nucleotides, 14(6): 1259-1267. Synthesis of cyclic dinucleotides, and additional nucleotide precursors useful in preparing the LNA-CDNs of the present invention are also described, for example, in Gao et al., Cell (2013) 153: doi: 10.1016/j.cell.2013.04.046; U.S. Patents 7,709,458 and 7,592,326; PCT Publication Nos. WO2007/054279, WO2014/093936, WO2014/179335, WO2014/189805, WO2015/185565, WO2016/096174, WO2016/145102, WO2017/027645, WO2017/027646, and WO2017/075477; and Yan et al., Bioorg. Med. Chem Lett. 18:5631-4, 2008. The disclosure of the references in this paragraph are hereby incorporated by reference as it relates to compounds for use in synthetic methods as described herein for the synthesis of compounds of the invention as described herein. Such nucleotides may be modified using standard organic chemistry techniques in order to produce the LNA-CDNs of the present invention.

[00192] In some embodiments, the LNA-CDN compounds of the present invention as described herein are potent STING agonists, and are compared to known CDN STING agonists as a reference compound, such as the natural ligand 2'3'-(G)(G), and 2'3'-RR-(A)(A), a STING agonist which has been shown to be efficacious in mouse tumor models (Corrales *et al.*, Cell Reports 2015, 11:1018-1031). The properties of the LNA-CDN compounds are demonstrated in Examples 7-12 below, where the compounds demonstrate an improvement over the reference compound as having one or more of i) a higher T_m shift in a DSF assay as described in Example 10 for one or more of hSTING (WT), hSTING (HAQ) or hSTING (REF); ii) a higher relative expression of IFN- β transcript in an hPBMC assay as described in Example 11 for one or more of hSTING (WT), hSTING (HAQ) or hSTING (REF); or iii) a lower EC50 in a THP1 cell assay as described in Example 12, where preferably the THP1 assay is performed in the absence of digitonin. In some embodiments, the LNA-CDN demonstrates an improvement over the reference compound as having two or more of i) a higher T_m shift in a DSF assay as described in Example 10 for one or more of hSTING (WT), hSTING (HAQ) or hSTING (REF); ii) a higher relative expression of IFN- β transcript in an hPBMC assay as described in Example 11 for one or more of hSTING (WT), hSTING (HAQ) or hSTING

(REF); or iii) a lower EC₅₀ in a THP1 cell assay as described in Example 12, where preferably the THP1 assay is performed in the absence of digitonin. In some embodiments, the LNA-CDN demonstrates an improvement over the reference compound as having each of i) a higher T_m shift in a DSF assay as described in Example 10 for one or more of hSTING (WT), hSTING (HAQ) or hSTING (REF); ii) a higher relative expression of IFN- β transcript in an hPBMC assay as described in Example 11 for one or more of hSTING (WT), hSTING (HAQ) or hSTING (REF); or iii) a lower EC₅₀ in a THP1 cell assay as described in Example 12, where preferably the THP1 assay is performed in the absence of digitonin. In some embodiments of the LNA-CDN compounds as described herein, the LNA-CDN compound has at least a 2-fold, at least a 5-fold, at least a 10-fold, at least a 50-fold, at least a 100-fold, at least a 500-fold, or at least a 1000-fold higher relative expression of IFN- β transcript in an hPBMC assay as described in Example 11 for hSTING REF allele as compared to 2'3'-RR-(A)(A). In some embodiments, the LNA-CDN compound has an EC₅₀ in the THP1 assay described in Example 12 without addition of digitonin that is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, or at least 8-fold lower than the EC₅₀ of a reference compound. In some embodiments, the LNA-CDN compound has an EC₅₀ in the THP1 cell assay without addition of an agent that enhances the permeability of the compound to the cell or an agent that enhances the uptake of the compound by the cell that is less than 40 μ M, less than 30 μ M, less than 20 μ M, less than 15 μ M, less than 10 μ M or less than 5 μ M. In some embodiments, the LNA-CDN compound has an EC₅₀ in the THP1 assay described in Example 12 without addition of digitonin or other cell permeabilizing agent that is less than 40 μ M, less than 30 μ M, less than 20 μ M, less than 15 μ M, less than 10 μ M or less than 5 μ M.

[00193] The LNA-CDN compounds of the present invention as described herein can be phosphorothioate analogues, referred to herein as “thiophosphates”. Phosphorothioates are a variant of normal nucleotides in which one of the nonbridging oxygens is replaced by a sulfur. The sulfurization of the internucleotide bond dramatically reduces the action of endo- and exonucleases, including 5' to 3' and 3' to 5' DNA POL 1 exonuclease, nucleases S1 and P1, RNases, serum nucleases and snake venom phosphodiesterase. In addition, the potential for crossing the lipid bilayer increases.

[00194] A phosphorothioate linkage is inherently chiral. The skilled artisan will recognize that the phosphates in this structure may each exist in R or S forms. Thus, Rp,Rp, Sp,Sp, Sp,Rp, and Rp,Sp forms are possible. The dithio LNA-CDN compounds of the present invention are preferably of the Rp,Rp form.

[00195] As noted above, the LNA-CDN compounds also include prodrug forms of the LNA-CDNs. Prodrugs can modify the physicochemical, biopharmaceutic, and pharmacokinetic properties of drugs. Traditional prodrugs are classified as drugs that are activated by undergoing transformation in vivo to form the active drug. Reasons for prodrug development are typically poor aqueous solubility, chemical instability, low oral bioavailability, lack of blood brain barrier penetration, and high first pass metabolism associated with the parent drug. Suitable prodrug moieties are described in, for example, "Prodrugs and Targeted Delivery," J. Rautico, Ed., John Wiley & Sons, 2011.

[00196] The term "substantially pure" as used herein with regard to dithio-diphosphate cyclic dinucleotides refers to an Rp,Rp form which is at least 75% pure relative to other possible stereochemistries at the chiral phosphorus centers indicated in the dithio LNA-CDN compounds as described herein. By way of example, a "substantially pure 2'3'-RR-LNA-CDN" would be at least 75% pure with regard to the Rp,Sp, Sp,Rp and Sp,Sp, i.e. with respect to 2'3'-RS-LNA-CDN, 2'3'-SR-LNA-CDN, and 2'3'-SS-LNA-CDN. In preferred embodiments, a substantially pure cyclic dinucleotide is at least 85% pure, at least 90% pure, at least 95% pure, at least 97% pure, and at least 99% pure. Similarly, any of the other isomers can be substantially pure with respect to the other three isomers, e.g. substantially pure Rp,Sp form relative to the Rp,Rp, Sp,Sp and Sp,Rp forms. While a substantially pure cyclic dinucleotide preparation of the invention is "stereochemically pure," this is not meant to indicate that all CDNs within the preparation having a particular stereochemistry at these chiral centers are otherwise identical. For example, a substantially pure cyclic dinucleotide preparation may contain a combination of 2'3'-RR-(G)(2'O,4'C-LNA-A) thiophosphate and 2'3'-RR-(A)(2'O,4'C-LNA-A) thiophosphate and still be a substantially pure cyclic dinucleotide preparation. Such a preparation may also include other components as described hereinafter that are advantageous for patient treatment, provided that all CDNs within the preparation having a particular stereochemistry at these chiral centers.

[00197] The LNA-CDN compounds and compositions thereof described herein can be administered to a host, either alone or in combination with a pharmaceutically acceptable excipient, in an amount sufficient to induce, modify, or stimulate an appropriate immune response. The immune response can comprise, without limitation, 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 LNA-CDN and compositions thereof described herein are administered in conjunction with one or more additional compositions including vaccines intended to stimulate an immune response to one or more predetermined antigens; adjuvants; CTLA-4 and PD-1 pathway antagonists, lipids, liposomes, chemotherapeutic agents, immunomodulatory cell lines, etc..

[00198] The LNA-CDN compounds and compositions thereof described herein may be administered before, after, and/or simultaneously with an additional therapeutic or prophylactic composition or modality. These include, without limitation, B7 costimulatory molecule, interleukin-2, interferon- γ , GM-CSF, CTLA-4 antagonists, OX-40/OX-40 ligand, CD40/CD40 ligand, sargramostim, levamisole, 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. Carriers for inducing a T cell immune response which preferentially stimulate a cytolytic T cell response versus an antibody response are preferred, although those that stimulate both types of response can be used as well. In cases where the agent is a polypeptide, the polypeptide itself or a polynucleotide encoding the polypeptide can be administered. The carrier can be a cell, such as an antigen presenting cell (APC) or a dendritic cell. Antigen presenting cells include such cell types as macrophages, dendritic cells and B cells. Other professional antigen-presenting cells include monocytes, marginal zone Kupffer cells, microglia, Langerhans' cells, interdigitating dendritic cells, follicular dendritic cells, and T cells. Facultative antigen-presenting cells can also be used. Examples of facultative antigen-presenting cells include astrocytes, follicular cells, endothelium and fibroblasts. The carrier can be a bacterial cell that is transformed to express the polypeptide or to deliver a polynucleotide which is subsequently expressed in cells of the vaccinated individual. 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, monophosphoryl lipid 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. Other representative examples of adjuvants include the synthetic adjuvant QS-21 comprising a homogeneous saponin purified from the bark of *Quillaja saponaria* and *Corynebacterium parvum* (McCune et al., Cancer, 1979; 43:1619). It will be understood that the adjuvant is subject to optimization. In other words, the skilled artisan can engage in routine experimentation to determine the best adjuvant to use.

[00199] Methods for co-administration with an additional therapeutic agent are well known in the art (Hardman, et al. (eds.) (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill, New York, NY; Poole and Peterson (eds.) (2001) Pharmacotherapeutics for Advanced Practice: A Practical Approach, Lippincott, Williams & Wilkins, Phila., PA; Chabner and Longo (eds.) (2001) Cancer Chemotherapy and Biotherapy, Lippincott, Williams & Wilkins, Phila., PA). Generally, co-administration or administration together indicates treating a subject with two or more agents, where the agents can be administered simultaneously or at different times. For example, such agents may be delivered to a single subject as separate administrations, which may be at essentially the same time or different times, and which may be by the same route or different routes of administration. Such agents may be delivered to a single subject in the same administration (e.g. same formulation) such that they are administered at the same time by the same route of administration.

[00200] Because of the adjuvant properties of the compounds of the present invention, their use may also be combined with other therapeutic modalities including other vaccines, adjuvants, antigen, antibodies, and immune modulators. Examples are provided below.

Adjuvants

[00201] In addition to the LNA-CDN compounds and compositions thereof described herein described above, the compositions or methods of the present invention may further comprise one or more additional substances which, because of their nature, can act to stimulate or otherwise utilize the immune system to respond to the cancer antigens present on the targeted tumor cell(s). Such adjuvants include, but are not limited to, lipids, liposomes, inactivated bacteria which induce innate immunity (e.g., inactivated or attenuated *Listeria monocytogenes*), compositions which mediate innate immune activation via Toll-like Receptors (TLRs), (NOD)-like receptors (NLRs), Retinoic acid inducible gene-based (RIG)-I-like receptors (RLRs), C-type lectin receptors (CLRs), and/or pathogen-associated molecular patterns (“PAMPs”). Examples of PAMPs include lipoproteins, lipopolypeptides, peptidoglycans, zymosan, lipopolysaccharide, neisserial porins, flagellin, proflagellin, galactoceramide, muramyl dipeptide. Peptidoglycans, lipoproteins, and lipoteichoic acids are cell wall components of Gram-positive. Lipopolysaccharides are expressed by most bacteria, with MPL being one example. Flagellin refers to the structural component of bacterial flagella that is secreted by pathogenic and commensal bacterial. α -Galactosylceramide (α -GalCer) is an activator of natural killer T (NKT) cells. Muramyl dipeptide is a bioactive peptidoglycan motif common to all bacteria. This list is not meant to be limiting. Preferred adjuvant compositions are described below.

Immune Checkpoint Inhibitors

[00202] The LNA-CDN compounds as described herein can be used in combination with an immune checkpoint inhibitor, such as an immune checkpoint inhibitor selected from the group consisting of a CTLA-4 pathway antagonist, a PD-1 pathway antagonist, a Tim-3 pathway antagonist, a Vista pathway antagonist, a BTLA pathway antagonist, a LAG-3 pathway antagonist, or a TIGIT pathway antagonist. In some embodiments, the immune checkpoint inhibitor is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, an anti-Tim-3 antibody, an anti-Vista antibody, an anti-BTLA antibody, an anti-LAG-3 antibody, or an anti-TIGIT antibody.

[00203] The LNA-CDN compounds as described herein can be used in combination with CTLA-4 pathway antagonists. In some embodiments, the combination is used to treat a solid tumor or a hematologic malignancy. CTLA-4 is thought to be an important negative regulator of the adaptive immune response. Activated T cells upregulate CTLA-

4, which binds CD80 and CD86 on antigen-presenting cells with higher affinity than CD28, thus inhibiting T-cell stimulation, IL-2 gene expression and T-cell proliferation. Anti-tumor effects of CTLA4 blockade have been observed in murine models of colon carcinoma, metastatic prostate cancer, and metastatic melanoma. In some embodiments, the CTLA-4 pathway antagonist is an anti-CTLA-4 antibody molecule selected from the group consisting of tremelimumab and ipilimumab. In some embodiments, the anti-CTLA-4 antibody is an anti-CTLA-4 antibody as disclosed in *e.g.*, U.S. Pat. No. 5,811,097.

[00204] Ipilimumab (YervoyTM, a CTLA-4 antibody, also known as MDX-010, CAS No. 477202-00-9) and tremelimumab (IgG2 monoclonal antibody available from Pfizer, formerly known as ticilimumab, CP-675,206) are humanized monoclonal antibodies that bind to human CTLA4 and prevent its interaction with CD80 and CD86. Phase I and II studies using ipilimumab and tremelimumab have demonstrated clinical activity in cancer patients. Other negative immune regulators which may be targeted by a similar strategy include programmed cell death 1 (PD-1), B and T lymphocyte attenuator, transforming growth factor beta β , interleukin-10, and vascular endothelial growth factor.

[00205] In some embodiments, the LNA-CDN compounds as described herein can be used in combination with an anti-CTLA-4 antibody and an anti-PD-1 antibody. In one embodiment, the combination includes an anti-PD-1 antibody molecule, *e.g.*, as described herein, and an anti-CTLA-4 antibody, *e.g.*, ipilimumab. Exemplary doses that can be used include a dose of anti-PD-1 antibody molecule of about 1 to 10 mg/kg, *e.g.*, 3 mg/kg, and a dose of an anti-CTLA-4 antibody, *e.g.*, ipilimumab, of about 3 mg/kg.

[00206] The LNA-CDN compounds as described herein can be used in combination with PD-1 pathway antagonists. In some embodiments, the combination is used to treat a solid tumor or a hematologic malignancy. PD-1 is another negative regulator of adaptive immune response that is expressed on activated T-cells. PD-1 binds to B7-H1 and B7-DC, and the engagement of PD-1 suppresses T-cell activation. Anti-tumor effects have been demonstrated with PD-1 pathway blockade. Anti-PD-1 antibody molecules (*e.g.* Nivolumab (OpdivoTM), pembrolizumab (KeytrudaTM), and pidilizumab), and AMP-224 have been reported in the literature to be examples of PD-1 pathway blockers which may find use in the present invention. In some embodiments, the PD-1 pathway antagonist is

an anti-PD-1 antibody molecule selected from the group consisting of nivolumab, pembrolizumab or pidilizumab.

[00207] In some embodiments, the anti-PD-1 antibody is nivolumab. Alternative names for nivolumab include MDX- 1106, MDX-1106-04, ONO-4538, or BMS-936558. In some embodiments, the anti-PD- 1 antibody is nivolumab (CAS Registry Number: 946414-94-4). Nivolumab is a fully human IgG4 monoclonal antibody which specifically blocks PD1. Nivolumab (clone 5C4) and other human monoclonal antibodies that specifically bind to PD1 are disclosed in US 8,008,449 and WO2006/121168. In one embodiment, the inhibitor of PD-1 is nivolumab, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, *e.g.*, a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00208] The heavy chain amino acid sequence of nivolumab is as follows:

QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWDGSKRYY
ADSVKGRFTISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGLTVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYTCNV DHKPSNTKVDKRVE SKYGPCCPPCPAPEFLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQVYTLPPSQEEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLGLK (SEQ ID NO: 1)

[00209] The light chain amino acid sequence of nivolumab is as follows:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPA
RFGSGSGTDFTLTITSSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 2)

[00210] In some embodiments, the anti-PD-1 antibody is pembrolizumab.

Pembrolizumab (also referred to as lambrolizumab, MK-3475, MK03475, SCH-900475 or KEYTRUDA®; Merck) is a humanized IgG4 monoclonal antibody that binds to PD-1. Pembrolizumab and other humanized anti-PD-1 antibodies are disclosed in Hamid, O. *et al.* (2013) *New England Journal of Medicine* 369 (2): 134–44, US 8,354,509 and WO2009/114335. In one embodiment, the inhibitor of PD-1 is pembrolizumab, and

having a sequence disclosed herein (or a sequence substantially identical or similar thereto, *e.g.*, a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00211] The heavy chain amino acid sequences of pembrolizumab is as follows:

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QVQLVQSGVE VKKPGASVKV SCKASGYTFT NYMYWVRQA PGQGLEWMGG 50
INPSNGGTNF NEKFKNRVTL TTDSSTTTAY MELKSLQFDD TAVYYCARRD 100
YRFDMGFDYW GQGTTVTVSS ASTKGPSVFP LAPCSRSTSE STAALGCLVK 150
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT 200
YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT 250
LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY 300
RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT 350
LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLD 400
DGSFFLYSRL TVDKSRWQEG NVFSCSVME ALHNHYTQKS LSLSLGK 447

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(SEQ ID NO: 3)

[00212] The light chain amino acid sequences of pembrolizumab is as follows:

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EIVLTQSPAT LSLSPGERAT LSCRASKGVS TSGYSYLHWY QQKPGQAPRL 50
LIYLA SYLES GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRDLPL 100
TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYFPREAKV 150
QWKVDNALQS GNSQESVTEQ DSKDSTYSL S TLTL SKADY EKHKVYACEV 200
THQGLSSPVT KSFNRGEC (SEQ ID NO: 4) 218

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[00213] In some embodiments, the anti-PD-1 antibody is pidilizumab. Pidilizumab (CT-011; Cure Tech) is a humanized IgG1k monoclonal antibody that binds to PD-1. Pidilizumab and other humanized anti-PD-1 monoclonal antibodies are disclosed in WO2009/101611.

[00214] In some embodiments, the anti-PD-1 antibody is AMP 514 (Amplimmune), or an anti-PD-1 antibody as disclosed in US 8,609,089, US 2010028330, and/or US 20120114649.

[00215] In some embodiments the PD-1 pathway antagonist is an anti-PD-1 antibody molecule disclosed in US 2015/0210769, published on July 30, 2015, entitled “Antibody Molecules to PD-1 and Uses Thereof”.

[00216] In one embodiment, the anti-PD-1 antibody molecule includes at least one or two heavy chain variable domain (optionally including a constant region), at least one or two light chain variable domain (optionally including a constant region), or both, comprising the amino acid sequence of BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1 of US 2015/0210769, or encoded by the nucleotide sequence in Table 1 therein; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences. The anti-PD-1 antibody molecule, optionally, comprises a leader sequence from a heavy chain, a light chain, or both, as shown in Table 4 of US 2015/0210769; or a sequence substantially identical thereto.

[00217] In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region and/or a light chain variable region of an antibody described in US 2015/0210769, *e.g.*, an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1 therein, or encoded by the nucleotide sequence in Table 1 therein; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00218] In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0210769, or encoded by a nucleotide sequence shown in Table 1 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1 therein.

[00219] In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0210769, or

encoded by a nucleotide sequence shown in Table 1 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1 therein, or encoded by a nucleotide sequence shown in Table 1 therein. In certain embodiments, the anti-PD-1 antibody molecule includes a substitution in a light chain CDR, *e.g.*, one or more substitutions in a CDR1, CDR2 and/or CDR3 of the light chain. In one embodiment, the anti-PD-1 antibody molecule includes a substitution in the light chain CDR3 at position 102 of the light variable region, *e.g.*, a substitution of a cysteine to tyrosine, or a cysteine to serine residue, at position 102 of the light variable region according to Table 1 (*e.g.*, SEQ ID NO: 16 or 24 for murine or chimeric, unmodified; or any of SEQ ID NOs: 34, 42, 46, 54, 58, 62, 66, 70, 74, or 78 for a modified sequence).

[00220] In another embodiment, the anti-PD-1 antibody molecule includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and light chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0210769, or encoded by a nucleotide sequence shown in Table 1 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1 therein, or encoded by a nucleotide sequence shown in Table 1 therein.

[00221] In one embodiment, the anti-PD-1 antibody molecule includes: (a) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence of SEQ ID NO: 4, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33, each as disclosed in Table 1 of US 2015/0210769; (b) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32, each as disclosed in Table 1 of US 2015/0210769; (c) a VH comprising a VHCDR1 amino acid

sequence of SEQ ID NO: 224, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33, each as disclosed in Table 1 of US 2015/0210769; or (d) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32, each as disclosed in Table 1 of US 2015/0210769.

[00222] In another embodiment, the anti-PD-1 antibody molecule comprises (i) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4, or SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 5; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and (ii) a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 32 or SEQ ID NO: 33, each as disclosed in Table 1 of US 2015/0210769.

[00223] In some embodiments the PD-1 pathway antagonist is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence)). In some embodiments, the PD-1 inhibitor is AMP-224 (B7-DCIg; Amplimmune; *e.g.*, disclosed in WO2010/027827 and WO2011/066342) is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD-1 and B7-H1.

[00224] In some embodiments the PD-1 pathway antagonist is a PD-L1 or PD-L2 inhibitor. In some embodiments the PD-L1 or PD-L2 inhibitor is an anti-PD-L1 antibody or an anti-PD-L2 antibody.

[00225] In some embodiments, the PD-L1 inhibitor is an anti-PD-L1 antibody. Exemplary non-limiting combinations and uses of the anti-PD-L1 antibody molecules are disclosed in US 2016/0108123, published April 21, 2016, entitled “Antibody Molecules to PD-L1 and Uses Thereof”.

[00226] In one embodiment, the anti-PD-L1 antibody molecule includes at least one or two heavy chain variable domain (optionally including a constant region), at least one or two light chain variable domain (optionally including a constant region), or both, comprising the amino acid sequence of any of BAP058-hum01, BAP058-hum02, BAP058-hum03, BAP058-hum04, BAP058-hum05, BAP058-hum06, BAP058-hum07, BAP058-hum08, BAP058-hum09, BAP058-hum10, BAP058-hum11, BAP058-hum12, BAP058-hum13, BAP058-hum14, BAP058-hum15, BAP058-hum16, BAP058-hum17, BAP058-Clone-K, BAP058-Clone-L, BAP058-Clone-M, BAP058-Clone-N, or BAP058-Clone-O; or as described in Table 1 of US 2016/0108123, or encoded by the nucleotide sequence in Table 1 therein; or a sequence substantially identical (e.g., at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00227] In yet another embodiment, the anti-PD-L1 antibody molecule includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region and/or a light chain variable region of an antibody described herein, e.g., an antibody chosen from any of BAP058-hum01, BAP058-hum02, BAP058-hum03, BAP058-hum04, BAP058-hum05, BAP058-hum06, BAP058-hum07, BAP058-hum08, BAP058-hum09, BAP058-hum10, BAP058-hum11, BAP058-hum12, BAP058-hum13, BAP058-hum14, BAP058-hum15, BAP058-hum16, BAP058-hum17, BAP058-Clone-K, BAP058-Clone-L, BAP058-Clone-M, BAP058-Clone-N, or BAP058-Clone-O; or as described in Table 1 of US 2016/0108123, or encoded by the nucleotide sequence in Table 1 therein; or a sequence substantially identical (e.g., at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00228] In yet another embodiment, the anti-PD-L1 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in Table 1 of US 2016/0108123, or encoded by a nucleotide sequence shown in Table 1 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1.

[00229] In yet another embodiment, the anti-PD-L1 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable

region comprising an amino acid sequence shown in Table 1 of US 2016/0108123, or encoded by a nucleotide sequence shown in Table 1 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1. In certain embodiments, the anti-PD-L1 antibody molecule includes a substitution in a light chain CDR, e.g., one or more substitutions in a CDR1, CDR2 and/or CDR3 of the light chain.

[00230] In another embodiment, the anti-PD-L1 antibody molecule includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and light chain variable region comprising an amino acid sequence shown in Table 1 of US 2016/0108123, or encoded by a nucleotide sequence shown in Table 1 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1.

[00231] In one embodiment, the anti-PD-L1 antibody molecule includes: (i) a heavy chain variable region (VH) including a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 195; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3, each disclosed in Table 1 of US 2016/0108123; and (ii) a light chain variable region (VL) including a VLCDR1 amino acid sequence of SEQ ID NO: 9, a VLCDR2 amino acid sequence of SEQ ID NO: 10, and a VLCDR3 amino acid sequence of SEQ ID NO: 11, each disclosed in Table 1 of US 2016/0108123.

[00232] In one embodiment, the anti-PD-L1 antibody molecule includes: (i) a heavy chain variable region (VH) including a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 195; a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3, each disclosed in Table 1 of US 2016/0108123; and (ii) a light chain variable region (VL) including a VLCDR1 amino acid sequence of SEQ ID NO: 12, a VLCDR2 amino acid sequence of SEQ ID NO: 13, and a VLCDR3 amino acid sequence of SEQ ID NO: 14, each disclosed in Table 1 of US 2016/0108123.

[00233] In one embodiment, the anti-PD-L1 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 1. In another embodiment, the anti-PD-L1 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 4. In yet another embodiment, the anti-PD-L1 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 195, each disclosed in Table 1 of US 2016/0108123.

[00234] In some embodiments, the anti-PD-L1 inhibitor is chosen from YW243.55.S70, MPDL3280A, MEDI-4736, MSB-0010718C, or MDX-1105. In some embodiments, the PD-L1 inhibitor is an anti-PD-L1 antibody MSB0010718C. MSB0010718C (also referred to as A09-246-2; Merck Serono) is a monoclonal antibody that binds to PD-L1. MSB0010718C and other humanized anti-PD-L1 antibodies are disclosed in WO2013/079174, and having a sequence disclosed therein (or a sequence substantially identical or similar thereto, *e.g.*, a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00235] The heavy chain amino acid sequence (SEQ ID NO: 24 as disclosed in WO2013/079174) of MSB0010718C includes at least the following:
 EVQLLES GGG LVQP GGS LRLSCAASGFT FSSYIMMWVRQAPGKGLEWVSSIYPSSGGITFY
 ADKGRFTISRDN SKNTLYLQMNSLR AEDTAVYYCARIKLGT VTTVDYWGQGT LTVTVSS
 (SEQ ID NO: 5)

[00236] The light chain amino acid sequence (SEQ ID NO: 25 as disclosed in WO2013/079174) of MSB0010718C includes at least the following:
 QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIDVSNRPSGV
 SNRFSGSKSGNTASLTISGLQAED EADYYCSSYTSSSTRVFGTGTKVTVL (SEQ ID NO:
 6)

[00237] In one embodiment, the PD-L1 inhibitor is YW243.55.S70. The YW243.55.S70 antibody is an anti-PD-L1 antibody as described in WO 2010/077634 (heavy and light chain variable region sequences shown in SEQ ID Nos. 20 and 21, respectively), and having a sequence disclosed therein (or a sequence substantially identical or similar thereto, *e.g.*, a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00238] In one embodiment, the PD-L1 inhibitor is MDX-1105. MDX-1105, also known as BMS-936559, is an anti-PD-L1 antibody described in WO2007/005874, and

having a sequence disclosed therein (or a sequence substantially identical or similar thereto, *e.g.*, a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00239] In one embodiment, the PD-L1 inhibitor is MDPL3280A (Genentech / Roche). MDPL3280A is a human Fc optimized IgG1 monoclonal antibody that binds to PD-L1. MDPL3280A and other human monoclonal antibodies to PD-L1 are disclosed in U.S. Patent No.: 7,943,743 and U.S Publication No.: 20120039906.

[00240] In other embodiments, the PD-L2 inhibitor is AMP-224. AMP-224 is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD1 and B7-H1 (B7-DCIg; Amplimmune; *e.g.*, disclosed in WO2010/027827 and WO2011/066342

[00241] The LNA-CDN compounds as described herein can be used in combination with TIM-3 pathway antagonists. In some embodiments, the combination is used to treat a solid tumor or a hematologic malignancy. In some embodiments, the TIM-3 pathway antagonist is an anti-TIM-3 antibody. In some embodiments, anti-TIM-3 antibody molecules are disclosed in US 2015/0218274, published on August 6, 2015, entitled “Antibody Molecules to TIM-3 and Uses Thereof”.

[00242] In one embodiment, the anti- TIM-3 antibody molecule includes at least one or two heavy chain variable domain (optionally including a constant region), at least one or two light chain variable domain (optionally including a constant region), or both, comprising the amino acid sequence of ABTIM3, ABTIM3-hum01, ABTIM3-hum02, ABTIM3-hum03, ABTIM3-hum04, ABTIM3-hum05, ABTIM3-hum06, ABTIM3-hum07, ABTIM3-hum08, ABTIM3-hum09, ABTIM3-hum10, ABTIM3-hum11, ABTIM3-hum12, ABTIM3-hum13, ABTIM3-hum14, ABTIM3-hum15, ABTIM3-hum16, ABTIM3-hum17, ABTIM3-hum18, ABTIM3-hum19, ABTIM3-hum20, ABTIM3-hum21, ABTIM3-hum22, ABTIM3-hum23; or as described in Tables 1-4 of US 2015/0218274; or encoded by the nucleotide sequence in Tables 1-4 therein; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences. The anti-TIM-3 antibody molecule, optionally, comprises a leader sequence from a heavy chain, a light chain, or both, as shown in US 2015/0218274; or a sequence substantially identical thereto.

[00243] In yet another embodiment, the anti- TIM-3 antibody molecule includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region and/or a light chain variable region of an antibody described in US 2015/0218274, *e.g.*, an antibody chosen from any of ABTIM3, ABTIM3-hum01, ABTIM3-hum02, ABTIM3-hum03, ABTIM3-hum04, ABTIM3-hum05, ABTIM3-hum06, ABTIM3-hum07, ABTIM3-hum08, ABTIM3-hum09, ABTIM3-hum10, ABTIM3-hum11, ABTIM3-hum12, ABTIM3-hum13, ABTIM3-hum14, ABTIM3-hum15, ABTIM3-hum16, ABTIM3-hum17, ABTIM3-hum18, ABTIM3-hum19, ABTIM3-hum20, ABTIM3-hum21, ABTIM3-hum22, ABTIM3-hum23; or as described in Tables 1-4 of US 2015/0218274; or encoded by the nucleotide sequence in Tables 1-4 therein; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00244] In yet another embodiment, the anti- TIM-3 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in Tables 1-4 of US 2015/0218274, or encoded by a nucleotide sequence shown in Tables 1-4 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Tables 1-4 therein, or encoded by a nucleotide sequence shown in Table 1-4 therein.

[00245] In yet another embodiment, the anti- TIM-3 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable region comprising an amino acid sequence shown in Tables 1-4 of US 2015/0218274, or encoded by a nucleotide sequence shown in Tables 1-4 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Tables 1-4 therein, or encoded by a nucleotide sequence shown in Tables 1-4 therein. In certain embodiments, the anti-TIM-3 antibody molecule includes a substitution in a light chain CDR, *e.g.*, one or more substitutions in a CDR1, CDR2 and/or CDR3 of the light chain.

[00246] In another embodiment, the anti-TIM-3 antibody molecule includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and

light chain variable region comprising an amino acid sequence shown in Tables 1-4 of US 2015/0218274, or encoded by a nucleotide sequence shown in Tables 1-4 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Tables 1-4 therein, or encoded by a nucleotide sequence shown in Tables 1-4 therein.

[00247] In one embodiment, the anti- TIM-3 antibody molecule includes: (a) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 9; a VHCDR2 amino acid sequence of SEQ ID NO: 10; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 12, a VLCDR2 amino acid sequence of SEQ ID NO: 13, and a VLCDR3 amino acid sequence of SEQ ID NO: 14, each as disclosed in Tables 1-4 of US 2015/0218274; (b) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 3; a VHCDR2 amino acid sequence of SEQ ID NO: 4; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6, a VLCDR2 amino acid sequence of SEQ ID NO: 7, and a VLCDR3 amino acid sequence of SEQ ID NO: 8, each as disclosed in Tables 1-4 of US 2015/0218274; (c) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 9; a VHCDR2 amino acid sequence of SEQ ID NO: 25; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 12, a VLCDR2 amino acid sequence of SEQ ID NO: 13, and a VLCDR3 amino acid sequence of SEQ ID NO: 14, each as disclosed in Tables 1-4 of US 2015/0218274; (d) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 3; a VHCDR2 amino acid sequence of SEQ ID NO: 24; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6, a VLCDR2 amino acid sequence of SEQ ID NO: 7, and a VLCDR3 amino acid sequence of SEQ ID NO: 8, each as disclosed in Tables 1-4 of US 2015/0218274; (e) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 9; a VHCDR2 amino acid sequence of SEQ ID NO: 31; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 12, a VLCDR2 amino acid sequence of SEQ ID NO: 13, and a VLCDR3 amino acid sequence of SEQ ID NO: 14, each as disclosed in Tables 1-4 of US 2015/0218274; or (f) a VH comprising a VHCDR1 amino

acid sequence chosen from SEQ ID NO: 3; a VHCDR2 amino acid sequence of SEQ ID NO: 30; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6, a VLCDR2 amino acid sequence of SEQ ID NO: 7, and a VLCDR3 amino acid sequence of SEQ ID NO: 8, each as disclosed in Tables 1-4 of US 2015/0218274.

[00248] In some embodiments, the TIM-3 pathway antagonist is an anti-TIM-3 antibody as disclosed in U.S. Patent No.: 8,552,156, WO 2011/155607, EP 2581113 or U.S Publication No.: 2014/044728.

[00249] The LNA-CDN compounds as described herein can be used in combination with LAG-3 pathway antagonists. In some embodiments, the combination is used to treat a solid tumor or a hematologic malignancy. In some embodiments, the LAG-3 pathway antagonist is an anti-LAG-3 antibody. In some embodiments the anti-LAG-3 antibody molecules are disclosed in US 2015/0259420, filed March 13, 2015, entitled “Antibody Molecules to LAG-3 and Uses Thereof”.

[00250] In one embodiment, the anti- LAG-3antibody molecule includes at least one or two heavy chain variable domain (optionally including a constant region), at least one or two light chain variable domain (optionally including a constant region), or both, comprising the amino acid sequence of any of BAP050-hum01, BAP050-hum02, BAP050-hum03, BAP050-hum04, BAP050-hum05, BAP050-hum06, BAP050-hum07, BAP050-hum08, BAP050-hum09, BAP050-hum10, BAP050-hum11, BAP050-hum12, BAP050-hum13, BAP050-hum14, BAP050-hum15, BAP050-hum16, BAP050-hum17, BAP050-hum18, BAP050-hum19, BAP050-hum20, huBAP050(Ser) (*e.g.*, BAP050-hum01-Ser, BAP050-hum02-Ser, BAP050-hum03-Ser, BAP050-hum04-Ser, BAP050-hum05-Ser, BAP050-hum06-Ser, BAP050-hum07-Ser, BAP050-hum08-Ser, BAP050-hum09-Ser, BAP050-hum10-Ser, BAP050-hum11-Ser, BAP050-hum12-Ser, BAP050-hum13-Ser, BAP050-hum14-Ser, BAP050-hum15-Ser, BAP050-hum18-Ser, BAP050-hum19-Ser, or BAP050-hum20-Ser), BAP050-Clone-F, BAP050-Clone-G, BAP050-Clone-H, BAP050-Clone-I, or BAP050-Clone-J; or as described in Table 1 of US 2015/0259420, or encoded by the nucleotide sequence in Table 1 therein; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00251] In yet another embodiment, the anti- LAG-3 antibody molecule includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region and/or a light chain variable region of an antibody described herein, *e.g.*, an antibody chosen from any of BAP050-hum01, BAP050-hum02, BAP050-hum03, BAP050-hum04, BAP050-hum05, BAP050-hum06, BAP050-hum07, BAP050-hum08, BAP050-hum09, BAP050-hum10, BAP050-hum11, BAP050-hum12, BAP050-hum13, BAP050-hum14, BAP050-hum15, BAP050-hum16, BAP050-hum17, BAP050-hum18, BAP050-hum19, BAP050-hum20, huBAP050(Ser) (*e.g.*, BAP050-hum01-Ser, BAP050-hum02-Ser, BAP050-hum03-Ser, BAP050-hum04-Ser, BAP050-hum05-Ser, BAP050-hum06-Ser, BAP050-hum07-Ser, BAP050-hum08-Ser, BAP050-hum09-Ser, BAP050-hum10-Ser, BAP050-hum11-Ser, BAP050-hum12-Ser, BAP050-hum13-Ser, BAP050-hum14-Ser, BAP050-hum15-Ser, BAP050-hum18-Ser, BAP050-hum19-Ser, or BAP050-hum20-Ser), BAP050-Clone-F, BAP050-Clone-G, BAP050-Clone-H, BAP050-Clone-I, or BAP050-Clone-J; or as described in Table 1 of US 2015/0259420, or encoded by the nucleotide sequence in Table 1 therein; or a sequence substantially identical (*e.g.*, at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00252] In yet another embodiment, the anti- LAG-3 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0259420, or encoded by a nucleotide sequence shown in Table 1 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1 therein, or encoded by a nucleotide sequence shown in Table 1 therein.

[00253] In yet another embodiment, the anti-LAG-3 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0259420, or encoded by a nucleotide sequence shown in Table 1 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1 therein, or encoded by a nucleotide sequence shown in Table

1 therein. In certain embodiments, the anti-PD-L1 antibody molecule includes a substitution in a light chain CDR, *e.g.*, one or more substitutions in a CDR1, CDR2 and/or CDR3 of the light chain.

[00254] In another embodiment, the anti- LAG-3 antibody molecule includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and light chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0259420, or encoded by a nucleotide sequence shown in Table 1 therein. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, *e.g.*, amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1 therein, or encoded by a nucleotide sequence shown in Table 1 therein.

[00255] In one embodiment, the anti- LAG-3 antibody molecule includes: (i) a heavy chain variable region (VH) including a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 286; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3, each as disclosed in Table 1 of US 2015/0259420; and (ii) a light chain variable region (VL) including a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 12, each as disclosed in Table 1 of US 2015/0259420.

[00256] In another embodiment, the anti-LAG-3 antibody molecule includes: (i) a heavy chain variable region (VH) including a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 286; a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3, each as disclosed in Table 1 of US 2015/0259420; and (ii) a light chain variable region (VL) including a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 15, each as disclosed in Table 1 of US 2015/0259420.

[00257] In one embodiment, the anti-LAG-3 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 1 as disclosed in Table 1 of US 2015/0259420. In another embodiment, the anti-LAG-3 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 4 as disclosed in Table 1 of US

2015/0259420. In yet another embodiment, the anti-LAG-3 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 286, as disclosed in Table 1 of US 2015/0259420.

[00258] In some embodiments, the anti-LAG-3 antibody is BMS-986016. BMS-986016 (also referred to as BMS986016; Bristol-Myers Squibb) is a monoclonal antibody that binds to LAG-3. BMS-986016 and other humanized anti-LAG-3 antibodies are disclosed in US 2011/0150892, WO2010/019570, and WO2014/008218.

T-cell Receptor Agonists

[00259] The LNA-CDN compounds as described herein can be used in combination with a T-cell receptor agonist, such as a CD28 agonist, an OX40 agonist, a GITR agonist, a CD137 agonist, a CD27 agonist or an HVEM agonist.

[00260] The LNA-CDN compounds as described herein can be used in combination with a CD27 agonist. Exemplary CD27 agonists include an anti-CD27 agonistic antibody, e.g. as described in PCT Publication No. WO 2012/004367.

[00261] The LNA-CDN compounds as described herein can be used in combination with a GITR agonist. In some embodiments, the combination is used to treat a solid tumor or a hematologic malignancy. Exemplary GITR agonists include, *e.g.*, GITR fusion proteins and anti-GITR antibodies (*e.g.*, bivalent anti-GITR antibodies), such as, a GITR fusion protein described in U.S. Patent No.: 6,111,090, European Patent No.: 0920505B1, U.S. Patent No.: 8,586,023, PCT Publication Nos.: WO 2010/003118 and 2011/090754, or an anti-GITR antibody described, *e.g.*, in U.S. Patent No.: 7,025,962, European Patent No.: 1947183B1, U.S. Patent No.: 7,812,135, U.S. Patent No.: 8,388,967, U.S. Patent No.: 8,591,886, European Patent No.: EP 1866339, PCT Publication No.: WO 2011/028683, U.S. Patent No.: 8,709,424, PCT Publication No.: WO 2013/039954, International Publication No.: WO2013/039954, U.S. Publication No.: US2014/0072566, International Publication NO.: WO2015/026684, PCT Publication No.: WO2005/007190, PCT Publication No.: WO 2007/133822, PCT Publication No.: WO2005/055808, PCT Publication No.: WO 99/40196, PCT Publication No.: WO 2001/03720, PCT Publication No.: WO99/20758, U.S. Patent No.: 6,689,607, PCT Publication No.: WO2006/083289, PCT Publication No.: WO 2005/115451, U.S. Patent

No.: 7,618,632, PCT Publication No.: WO 2011/051726, International Publication No.: WO2004060319, and International Publication No.: WO2014012479.

[00262] In one embodiment, the LNA-CDN compounds as described herein is used in combination with a GITR agonist used in combination with a PD-1 inhibitor, *e.g.*, as described in WO2015/026684.

[00263] In another embodiment, the LNA-CDN compounds as described herein is used in combination with a GITR agonist used in combination with a TLR agonist, *e.g.*, as described in WO2004060319, and International Publication No.: WO2014012479.

TLR Agonists

[00264] The LNA-CDN compounds as described herein can be used in combination with a Toll like receptor agonist. The term “Toll like receptor” (or “TLR”) as used herein refers to a member of the Toll-like receptor family of proteins or a fragment thereof that senses a microbial product and/or initiates an adaptive immune response. In one embodiment, a TLR activates a dendritic cell (DC). Toll like receptors (TLRs) are a family of pattern recognition receptors that were initially identified as sensors of the innate immune system that recognize microbial pathogens. TLRs comprise a family of conserved membrane spanning molecules containing an ectodomain of leucine-rich repeats, a transmembrane domain and an intracellular TIR (Toll/IL-1R) domain. TLRs recognize distinct structures in microbes, often referred to as “PAMPs” (pathogen associated molecular patterns). Ligand binding to TLRs invokes a cascade of intracellular signaling pathways that induce the production of factors involved in inflammation and immunity.

[00265] In humans, ten TLR have been identified. TLRs that are expressed on the surface of cells include TLR-1,-2,-4,-5, and -6, while TLR-3, -7/8, and -9 are expressed with the ER compartment. Human dendritic cell subsets can be identified on the basis of distinct TLR expression patterns. By way of example, the myeloid or “conventional” subset of DC (mDC) expresses TLRs 1–8 when stimulated, and a cascade of activation markers (*e.g.* CD80, CD86, MHC class I and II, CCR7), pro-inflammatory cytokines, and chemokines are produced. A result of this stimulation and resulting expression is antigen-specific CD4+ and CD8+ T cell priming. These DCs acquire an enhanced capacity to take up antigens and present them in an appropriate form to T cells. In contrast, the

plasmacytoid subset of DC (pDC) expresses only TLR7 and TLR9 upon activation, with a resulting activation of NK cells as well as T-cells. As dying tumor cells may adversely affect DC function, it has been suggested that activating DC with TLR agonists may be beneficial for priming anti-tumor immunity in an immunotherapy approach to the treatment of cancer. It has also been suggested that successful treatment of breast cancer using radiation and chemotherapy requires TLR4 activation.

[00266] TLR agonists known in the art and finding use in the present invention include, but are not limited to, the following:

Pam3Cys, a TLR-1/2 agonist;

CFA, a TLR-2 agonist;

MALP2, a TLR-2 agonist;

Pam2Cys, a TLR-2 agonist;

FSL-1, a TLR-2 agonist;

Hib-OMPC, a TLR-2 agonist;

polyribosinic:polyribocytidic acid (Poly I:C), a TLR-3 agonist;

polyadenosine-polyuridylic acid (poly AU), a TLR-3 agonist;

Polyinosinic-Polycytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose (Hiltonol®), a TLR-3 agonist;

monophosphoryl lipid A (MPL), a TLR-4 agonist;

LPS, a TLR-4 agonist;

bacterial flagellin, a TLR-5 agonist;

sialyl-Tn (STn), a carbohydrate associated with the MUC1 mucin on a number of human cancer cells and a TLR-4 agonist;

imiquimod, a TLR-7 agonist;

resiquimod, a TLR-7/8 agonist;

loxoribine, a TLR-7/8 agonist; and

unmethylated CpG dinucleotide (CpG-ODN), a TLR-9 agonist.

[00267] Because of their adjuvant qualities, TLR agonists are preferably used in combinations with other vaccines, adjuvants and/or immune modulators, and may be combined in various combinations. Thus, in certain embodiments, the LNA-CDN compounds that bind to STING and induce STING-dependent TBK1 activation and an inactivated tumor cell which expresses and secretes one or more cytokines which stimulate dendritic cell induction, recruitment and/or maturation, as described herein can be administered together with one or more TLR agonists for therapeutic purposes.

Antibody Therapeutics

[00268] The LNA-CDN compounds as described herein can be used in combination with therapeutic antibodies. In some embodiments, the mechanism of action of the therapeutic antibody is Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)/phagocytosis (ADCP). ADCC is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies. It is one of the mechanisms through which antibodies, as part of the humoral immune response, can act to limit and contain infection. Classical ADCC is mediated by natural killer (NK) cells; macrophages, neutrophils and eosinophils can also mediate ADCC. ADCC is an important mechanism of action of therapeutic monoclonal antibodies, including trastuzumab and rituximab, against tumors. Compounds of the present invention may act to potentiate ADCC.

[00269] The following are an exemplary list of antibodies which may be used together with the LNA-CDN compounds of the present invention.

[00270] Muromonab-CD3: Used to prevent acute rejection of organ, e.g., kidney, transplants. The humanized versions show promise in inhibiting the autoimmune destruction of beta cells in Type 1 diabetes mellitus.

[00271] Infliximab (Remicade®) and adalimumab (Humira®): Bind to tumor necrosis factor- α (TNF- α). Used in some inflammatory diseases such as rheumatoid arthritis, psoriasis, Crohn's disease.

[00272] Omalizumab (Xolair®). Binds to IgE thus preventing IgE from binding to mast cells. Used against allergic asthma.

[00273] Daclizumab (Zenapax®). Binds to part of the IL-2 receptor exposed at the surface of activated T cells. Used to prevent acute rejection of transplanted kidneys.

[00274] Rituximab (trade name = Rituxan®). Binds to the CD20 molecule found on most B-cells and is used to treat B-cell lymphomas.

[00275] Ibritumomab (trade name = Zevalin®). This is a monoclonal antibody against the CD20 molecule on B cells (and lymphomas) conjugated to isotopes. Given to the lymphoma patient supplemented with Rituxan.

[00276] Tositumomab (Bexxar®). This is a conjugate of a monoclonal antibody against CD20 and the radioactive isotope iodine-131 (^{131}I).

[00277] Cetuximab (Erbix®). Blocks HER1, a receptor for epidermal growth factor (EGF) that is found on some tumor cells (some breast cancers, lymphomas).

[00278] Trastuzumab (Herceptin®). Blocks HER2, a growth factor receptor over-expressed in some 20% of breast cancers.

[00279] Brentuximab Vedotin (Adcetris®). A conjugate of a monoclonal antibody that binds CD30, a cell-surface molecule expressed by the cells of some lymphomas but not found on the normal stem cells needed to repopulate the bone marrow.

[00280] Alemtuzumab (Campath-1H®). Binds to CD52, a molecule found on lymphocytes and depletes both T cells and B cells. Has produced complete remission of chronic lymphocytic leukemia and shows promise in preventing rejection of kidney transplants.

[00281] Lym-1 (Oncolym®). Binds to the HLA-DR-encoded histocompatibility antigen that can be expressed at high levels on lymphoma cells.

[00282] Ipilimumab (Yervoy®) that acts to enhance the body's own immune response to tumors.

[00283] Vitaxin. Binds to a vascular integrin (alpha-v/beta-3) found on the blood vessels of tumors but not on the blood vessels supplying normal tissues. In Phase II clinical trials, Vitaxin has shown some promise in shrinking solid tumors without harmful side effects.

[00284] Bevacizumab (Avastin®). Binds to vascular endothelial growth factor (VEGF) preventing it from binding to its receptor. Used for the treatment of colorectal cancers.

[00285] Abciximab (ReoPro®). Inhibits the clumping of platelets by binding the receptors on their surface that normally are linked by fibrinogen. Helpful in preventing reclogging of the coronary arteries in patients who have undergone angioplasty.

[00286] Additional therapeutic antibodies that may be used in combination with the LNA-CDN compounds as described herein include a prolactin receptor (PRLR) inhibitor, *e.g.* as disclosed in US Patent 7,867,493, a HER3 inhibitor, *e.g.* as disclosed in PCT Publication No. WO 2012/022814, an EGFR2 and/or EGFR4 inhibitor, *e.g.* as disclosed in PCT Publication No. WO 2014/160160, an M-CSF inhibitor, *e.g.* as disclosed in PCT Publication No. WO 2004/045532, an anti-APRIL antibody, *e.g.* as disclosed in US Patent 8,895,705, or an anti-SIRP α antibody *e.g.*, as disclosed in US Patent 8,728,476, WO2015138600 and US20140242095, an anti-CD47 antibody, *e.g.* as disclosed in US Patent 8,562,997.

[00287] In one embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination a prolactin receptor (PRLR) inhibitor, a human monoclonal antibody molecule (Compound A26) as disclosed in US Patent 7,867,493), to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PRLR inhibitor is a human monoclonal antibody (Compound A26) disclosed in US 7,867,493. In one embodiment, the LNA-CDN compound is used in combination with human monoclonal antibody molecule (Compound A26) described in US Patent 7,867,493 to treat a disorder such as, a cancer, a prostate cancer, or a breast cancer.

[00288] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a HER3 inhibitor, Compound A31, or a compound disclosed in PCT Publication No. WO 2012/022814, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the HER3 inhibitor is Compound A31 or a compound disclosed in PCT Publication WO 2012/022814. In one embodiment, the LNA-CDN compound is used in combination with Compound A31, or a compound disclosed in PCT Publication WO 2012/022814, to treat a disorder such as a gastric cancer, an esophageal cancer, a head and neck cancer, a squamous cell carcinoma, a stomach cancer, a breast cancer (*e.g.*, metastatic breast cancer), or a digestive/gastrointestinal cancer. In some embodiments, Compound A31 is a human monoclonal antibody molecule. In one embodiment, the HER3 inhibitor or Compound A31 is administered at a dose of about 3, 10, 20, or 40 mg/kg, *e.g.*, once weekly (QW). In one embodiment, the compound is administered at a dose of about 3-10, 10-20, or 20-40 mg/kg.

[00289] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination an FGFR2 and/or FGFR4 inhibitor, Compound A32, or a compound disclosed in a publication PCT Publication No. WO 2014/160160 (*e.g.*, an antibody molecule drug conjugate against an FGFR2 and/or FGFR4, *e.g.*, mAb 12425 as described therein), to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the FGFR2 and/or FGFR4 inhibitor is Compound A32 or a compound disclosed in a publication PCT Publication No. WO 2014/160160. In one embodiment, the LNA-CDN compound is used in combination with Compound A32, or in further combination with a compound as described in Table 2, to treat a disorder such as a cancer, a gastric cancer, a breast cancer, a rhabdomyosarcoma, a liver cancer, an adrenal cancer, a lung cancer, an esophageal cancer, a colon cancer, or an endometrial cancer. In some embodiments, Compound A32 is an antibody molecule drug conjugate against an FGFR2 and/or FGFR4, *e.g.*, mAb 12425.

[00290] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound described herein, is used in combination an M-CSF inhibitor, Compound A33, or a compound disclosed in PCT Publication No. WO 2004/045532 (*e.g.*, an antibody molecule or Fab fragment against M-CSF), to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the M-CSF inhibitor is Compound A33 or

a compound disclosed in PCT Publication No. WO 2004/045532. In one embodiment, the LNA-CDN compound is used in combination with Compound A33, or a compound as described in PCT Publication No. WO 2004/045532, to treat a disorder such as a cancer, a prostate cancer, a breast cancer, or pigmented villonodular synovitis (PVNS). In embodiments, Compound A33 is a monoclonal antibody molecule against M-CSF or a fragment (*e.g.*, Fab fragment) thereof. In embodiments, the M-CSF inhibitor or Compound A33 is administered at an average dose of about 10mg/kg.

Delivery Agents

[00291] Liposomes are vesicles formed from one (“unilamellar”) or more (“multilamellar”) layers of phospholipid. Because of the amphipathic character of the phospholipid building blocks, liposomes typically comprise a hydrophilic layer presenting a hydrophilic external face and enclosing a hydrophilic core. The versatility of liposomes in the incorporation of hydrophilic/hydrophobic components, their non-toxic nature, biodegradability, biocompatibility, adjuvanticity, induction of cellular immunity, property of sustained release and prompt uptake by macrophages, makes them attractive candidates for the delivery of antigens.

[00292] WO2010/104833 describes suitable liposomal preparations. Such liposomal formulations, referred to herein as VesiVax® (Molecular Express, Inc.), with or without the “immunogenic polypeptide(s) or carbohydrate(s)” referred to above, can contain one or more additional components such as peptidoglycan, lipopeptide, lipopolysaccharide, monophosphoryl lipid A, lipoteichoic acid, resiquimod, imiquimod, flagellin, oligonucleotides containing unmethylated CpG motifs, beta-galactosylceramide, muramyl dipeptide, all-trans retinoic acid, double-stranded viral RNA, heat shock proteins, dioctadecyldimethylammonium bromide, cationic surfactants, toll-like receptor agonists, dimyristoyltrimethylammoniumpropane, and nod-like receptor agonists. Advantageously, these liposomal formulations can be used to deliver one or more LNA-CDN compounds and compositions thereof described herein in accordance with the present invention.

[00293] Moreover, while the liposomal formulations discussed above employ a “steroid derivative” as an anchor for attaching an immunogenic polypeptide or carbohydrate to a liposome, the steroid may simply be provided as an unconjugated steroid such as cholesterol.

[00294] Suitable methods for preparing liposomes from lipid mixtures are well known in the art. *See, e.g.*, Basu & Basu, *Liposome Methods and Protocols* (Methods in Molecular Biology), Humana Press, 2002; Gregoriadis, *Liposome Technology*, 3rd Edition, Informa HealthCare, 2006. Preferred methods include extrusion, homogenization, and sonication methods described therein. An exemplary method for preparing liposomes for use in the present invention, which comprises drying a lipid mixture, followed by hydration in an aqueous vehicle and sonication to form liposomes, is described in WO2010/104833.

[00295] In certain embodiments, the liposomes are provided within a particular average size range. Liposome size can be selected, for example, by extrusion of an aqueous vehicle comprising liposomes through membranes having a preselected pore size and collecting the material flowing through the membrane. In preferred embodiments, the liposomes are selected to be substantially between 50 and 500 nm in diameter, more preferably substantially between 50 and 200 nm in diameter, and most preferably substantially between 50 and 150 nm in diameter. The term “substantially” as used herein in this context means that at least 75%, more preferably 80%, and most preferably at least 90% of the liposomes are within the designated range.

[00296] Other lipid and lipid-like adjuvants which may find use in the present invention include oil-in-water (o/w) emulsions (*see, e.g.*, Muderhwa et al., *J. Pharmaceut. Sci.* 88: 1332–9, 1999)), VesiVax® TLR (Molecular Express, Inc.), digitonin (*see, e.g.*, U.S. Patent 5,698,432), and glucopyranosyl lipids (*see, e.g.*, United States Patent Application 20100310602).

[00297] Nanoparticles also represent drug delivery systems suitable for most administration routes. Over the years, a variety of natural and synthetic polymers have been explored for the preparation of nanoparticles, of which Poly(lactic acid) (PLA), Poly(glycolic acid) (PGA), and their copolymers (PLGA) have been extensively investigated because of their biocompatibility and biodegradability. Nanoparticles and other nanocarriers act as potential carriers for several classes of drugs such as anticancer agents, antihypertensive agents, immunomodulators, and hormones; and macromolecules such as nucleic acids, proteins, peptides, and antibodies. *See, e.g.*, *Crit. Rev. Ther. Drug Carrier Syst.* 21:387-422, 2004; *Nanomedicine: Nanotechnology, Biology and Medicine* 1:22-30, 2005.

Chemotherapeutic Agents

[00298] In additional embodiments the methods described herein, the LNA-CDN compounds as described herein are used in combination with chemotherapeutic agents (e.g. small molecule pharmaceutical compounds). Thus the methods further involve administering to the subject an effective amount of one or more chemotherapeutic agents as an additional treatment or a combination treatment. In certain embodiments the one or more chemotherapeutic agents is selected from the group consisting of 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-prolyl-L-proline-t-butylamide, cachectin, cemadotin, chlorambucil, cyclophosphamide, 3',4'-didehydro-4'-deoxy-8'-norvin-calceculastine, docetaxol, doxorubicin, 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), enzalutamide, mechlorethamine (nitrogen mustard), melphalan, mivobulin isethionate, rhizoxin, sertenef, streptozocin, mitomycin, methotrexate, taxanes, nilutamide, onapristone, paclitaxel, prednimustine, procarbazine, RPR109881, stramustine phosphate, tamoxifen, tasonermin, taxol, tretinoin, vinblastine, vincristine, vindesine sulfate, and vinflunine.

[00299] In additional embodiments the methods described herein, the LNA-CDN compounds as described herein are used in combination with chemotherapeutic agents and/or additional agents for treating the indications as described in the methods herein. In some embodiments, the LNA-CDN compounds as described herein are used in combination with one or more agents selected from the group consisting of sotrastaurin, nilotinib, 5-(2,4-dihydroxy-5-isopropylphenyl)-N-ethyl-4-(4-(morpholinomethyl)phenyl)isoxazole-3-carboxamide, dactolisib, 8-(6-Methoxy-pyridin-3-yl)-3-methyl-1-(4-piperazin-1-yl-3-trifluoromethyl-phenyl)-1,3-dihydro-imidazo[4,5-c]quinolin-2-one, 3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-(6-((4-(4-ethylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)-1-methylurea, buparlisib, 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-carboxamide, (S)-N1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-

yl)thiazol-2-yl)pyrrolidine-1,2-dicarboxamide, (S)-1-(4-chlorophenyl)-7-isopropoxy-6-methoxy-2-(4-(methyl(((1R,4S)-4-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)methyl)amino)phenyl)-1,2-dihydroisoquinolin-3(4H)-one, deferasirox, letrozole, (4S,5R)-3-(2'-amino-2-morpholino-4'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)-4-(hydroxymethyl)-5-methyloxazolidin-2-one, (S)-5-(5-chloro-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-6-(4-chlorophenyl)-2-(2,4-dimethoxypyrimidin-5-yl)-1-isopropyl-5,6-dihydropyrrolo[3,4-d]imidazol-4(1H)-one, 4-((2-(((1R,2R)-2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-yl)oxy)-N-methylpicolinamide, imatinib mesylate, 2-fluoro-N-methyl-4-(7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2-yl)benzamide, ruxolitinib, panobinostat, osilodrostat, (S)-N-((S)-1-cyclohexyl-2-((S)-2-(4-(4-fluorobenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-2-(methylamino)propanamide, (S)-N-((S)-1-cyclohexyl-2-((S)-2-(4-(4-fluorobenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-2-(methylamino)propanamide, sonidegib phosphate, ceritinib, 7-cyclopentyl-N,N-dimethyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide, N-(4-((1R,3S,5S)-3-amino-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide, 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide, encorafenib, 7-cyclopentyl-N,N-dimethyl-2-((5-((1R,6S)-9-methyl-4-oxo-3,9-diazabicyclo[4.2.1]nonan-3-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide, binimetinib, midostaurin, everolimus, 1-methyl-5-((2-(5-(trifluoromethyl)-1H-imidazol-2-yl)pyridin-4-yl)oxy)-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-2-amine, pasireotide diaspertate, dovitinib, (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide, N⁶-(2-isopropoxy-5-methyl-4-(1-methylpiperidin-4-yl)phenyl)-N⁴-(2-(isopropylsulfonyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine, 3-(4-(4-((5-chloro-4-((5-methyl-1H-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)-5-fluoro-2-methylphenyl)piperidin-1-yl)thietane 1,1-dioxide, 5-chloro-N²-(2-fluoro-5-methyl-4-(1-(tetrahydro-2H-pyran-4-yl)piperidin-4-yl)phenyl)-N⁴-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine, 5-chloro-N²-(4-(1-ethylpiperidin-4-yl)-2-fluoro-5-methylphenyl)-N⁴-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine, valspodar, and vatalanib succinate.

[00300] In one embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a PKC inhibitor,

Sotrastaurin (Compound A1), or a compound disclosed in PCT Publication No. WO 2005/039549, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PKC inhibitor is Sotrastaurin (Compound A1) or a compound disclosed in PCT Publication No. WO 2005/039549. In one embodiment, the LNA-CDN compound is used in combination with Sotrastaurin (Compound A1), or a compound as described in PCT Publication No. WO 2005/039549, to treat a disorder such as a cancer, a melanoma, a non-Hodgkin lymphoma, an inflammatory bowel disease, transplant rejection, an ophthalmic disorder, or psoriasis. In certain embodiments, Sotrastaurin (Compound A1) is administered at a dose of about 20 to 600 mg, *e.g.*, about 200 to about 600 mg, about 50 mg to about 450 mg, about 100 mg to 400 mg, about 150 mg to 350 mg, or about 200 mg to 300 mg, *e.g.*, about 50 mg, 100 mg, 150mg, 200 mg, 300 mg, 400 mg, 500 mg, or 600 mg. The dosing schedule can vary from *e.g.*, every other day to daily, twice or three times a day.

[00301] In one embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a BCR-ABL inhibitor, TASIGNA (Compound A2, nilotinib), or a compound disclosed in PCT Publication No. WO 2004/005281, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the BCR-ABL inhibitor is TASIGNA, or a compound disclosed in PCT Publication No. WO 2004/005281. In one embodiment, the LNA-CDN compound is used in combination with TASIGNA (Compound A2), or a compound as described in PCT Publication No. WO 2004/005281, to treat a disorder such as a lymphocytic leukemia, Parkinson's Disease, a neurologic cancer, a melanoma, a digestive/gastrointestinal cancer, a colorectal cancer, a myeloid leukemia, a head and neck cancer, or pulmonary hypertension. In one embodiment, the BCR-ABL inhibitor or TASIGNA is administered at a dose of about 300 mg (*e.g.*, twice daily, *e.g.*, for newly diagnosed Ph+ CML-CP), or about 400 mg, *e.g.*, twice daily, *e.g.*, for resistant or intolerant Ph+ CML-CP and CML-AP). BCR-ABL inhibitor or a Compound A2 is administered at a dose of about 300-400 mg.

[00302] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an HSP90 inhibitor, such as 5-(2,4-dihydroxy-5-isopropylphenyl)-N-ethyl-4-(4-(morpholinomethyl)phenyl)isoxazole-3-carboxamide (Compound A3), or a compound

disclosed in PCT Publication No. WO 2010/060937 or WO 2004/072051, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the HSP90 inhibitor is 5-(2,4-dihydroxy-5-isopropylphenyl)-N-ethyl-4-(4-(morpholinomethyl)phenyl)isoxazole-3-carboxamide (Compound A3), or a compound disclosed in PCT Publication No. WO 2010/060937 or WO 2004/072051. In one embodiment, the LNA-CDN compound is used in combination with 5-(2,4-dihydroxy-5-isopropylphenyl)-N-ethyl-4-(4-(morpholinomethyl)phenyl)isoxazole-3-carboxamide (Compound A3), or a compound as described in PCT Publication No. WO 2010/060937 or WO 2004/072051, to treat a disorder such as a cancer, a multiple myeloma, a non-small cell lung cancer, a lymphoma, a gastric cancer, a breast cancer, a digestive/gastrointestinal cancer, a pancreatic cancer, a colorectal cancer, a solid tumor, or a hematopoiesis disorder.

[00303] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an inhibitor of PI3K and/or mTOR, Dactolisib (Compound A4) or 8-(6-Methoxy-pyridin-3-yl)-3-methyl-1-(4-piperazin-1-yl-3-trifluoromethyl-phenyl)-1,3-dihydro-imidazo[4,5-c]quinolin-2-one (Compound A41), or a compound disclosed in PCT Publication No. WO 2006/122806, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PI3K and/or mTOR inhibitor is Dactolisib (Compound A4), 8-(6-Methoxy-pyridin-3-yl)-3-methyl-1-(4-piperazin-1-yl-3-trifluoromethyl-phenyl)-1,3-dihydro-imidazo[4,5-c]quinolin-2-one (Compound A41), or a compound disclosed in PCT Publication No. WO 2006/122806. In one embodiment, the LNA-CDN compound is used in combination with Dactolisib (Compound A4), 8-(6-Methoxy-pyridin-3-yl)-3-methyl-1-(4-piperazin-1-yl-3-trifluoromethyl-phenyl)-1,3-dihydro-imidazo[4,5-c]quinolin-2-one (Compound A41), or a compound described in PCT Publication No. WO 2006/122806, to treat a disorder such as a cancer, a prostate cancer, a leukemia (*e.g.*, lymphocytic leukemia), a breast cancer, a brain cancer, a bladder cancer, a pancreatic cancer, a renal cancer, a solid tumor, or a liver cancer.

[00304] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an FGFR inhibitor, 3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-(6-((4-(4-ethylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)-1-methylurea (Compound A5) or a compound disclosed in US Patent 8,552,002, to treat a disorder, *e.g.*, a disorder described herein. In one

embodiment, the FGFR inhibitor is 3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-(6-((4-(4-ethylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)-1-methylurea (Compound A5) or a compound disclosed in US Patent 8,552,002. In one embodiment, the LNA-CDN compound is used in combination with Compound A5, or a compound as described in US 8,552,002, to treat a disorder such as a digestive/gastrointestinal cancer, a hematological cancer, or a solid tumor. In one embodiment, the FGFR inhibitor or 3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-(6-((4-(4-ethylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)-1-methylurea (Compound A5) is administered at a dose of about 100-125 mg (*e.g.*, per day), *e.g.*, about 100 mg or about 125 mg.

[00305] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a PI3K inhibitor, Buparlisib (Compound A6), or a compound disclosed in PCT Publication No. WO 2007/084786, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PI3K inhibitor is Buparlisib (Compound A6) or a compound disclosed in PCT Publication No. WO 2007/084786. In one embodiment, the LNA-CDN compound is used in combination with Buparlisib (Compound A6), or a compound disclosed in PCT Publication No. WO 2007/084786, to treat a disorder such as, a prostate cancer, a non-small cell lung cancer, an endocrine cancer, a leukemia, an ovarian cancer, a melanoma, a bladder cancer, a breast cancer, a female reproductive system cancer, a digestive/gastrointestinal cancer, a colorectal cancer, a glioblastoma multiforme, a solid tumor, a non-Hodgkin lymphoma, a hematopoiesis disorder, or a head and neck cancer. In one embodiment, the PI3K inhibitor or Buparlisib (Compound A6) is administered at a dose of about 100 mg (*e.g.*, per day).

[00306] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an FGFR inhibitor, 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-carboxamide (Compound A7) or a compound disclosed in PCT Publication No. WO 2009/141386, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the FGFR inhibitor is 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-carboxamide (Compound A7) or a compound disclosed in PCT Publication No. WO 2009/141386. In one embodiment, the FGFR inhibitor is 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-

((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-carboxamide(Compound A7). In one embodiment, the LNA-CDN compound is used in combination with 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-carboxamide(Compound A7), or a compound disclosed in PCT Publication No. WO 2009/141386, to treat a disorder such as a cancer characterized by angiogenesis. In one embodiment, the FGFR inhibitor or 8-(2,6-difluoro-3,5-dimethoxyphenyl)-N-(4-((dimethylamino)methyl)-1H-imidazol-2-yl)quinoxaline-5-carboxamide (Compound A7) is administered at a dose of *e.g.*, from approximately 3 mg to approximately 5 g, more preferably from approximately 10 mg to approximately 1.5 g per person per day, optionally divided into 1 to 3 single doses which may, for example, be of the same size.

[00307] In another embodiment the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a PI3K inhibitor, (S)-N1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)pyrrolidine-1,2-dicarboxamide (Compound A8) or a compound disclosed PCT Publication No. WO 2010/029082, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PI3K inhibitor is (S)-N1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)pyrrolidine-1,2-dicarboxamide (Compound A8) or a compound disclosed PCT Publication No. WO 2010/029082. In one embodiment, the LNA-CDN compound is used in combination with (S)-N1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)pyrrolidine-1,2-dicarboxamide (Compound A8), or a compound disclosed PCT Publication No. WO 2010/029082, to treat a disorder such as a gastric cancer, a breast cancer, a pancreatic cancer, a digestive/ gastrointestinal cancer, a solid tumor, and a head and neck cancer. In one embodiment, the PI3K inhibitor or (S)-N1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)pyrrolidine-1,2-dicarboxamide (Compound A8) is administered at a dose of about 150-300, 200-300, 200-400, or 300-400 mg (*e.g.*, per day), *e.g.*, about 200, 300, or 400 mg.

[00308] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an inhibitor of cytochrome P450 (*e.g.*, a CYP17 inhibitor) or a compound disclosed in PCT Publication No. WO 2010/149755, to treat a disorder, *e.g.*, a disorder described herein. In one

embodiment, the cytochrome P450 inhibitor (*e.g.*, the CYP17 inhibitor) is a compound disclosed in PCT Publication No. WO 2010/149755. In one embodiment, the LNA-CDN compound is used in combination with a compound disclosed in PCT Publication No. WO 2010/149755, to treat prostate cancer.

[00309] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an HDM2 inhibitor, (S)-1-(4-chlorophenyl)-7-isopropoxy-6-methoxy-2-(4-(methyl(((1R,4S)-4-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)methyl)amino)phenyl)-1,2-dihydroisoquinolin-3(4H)-one (Compound A10) or a compound disclosed in PCT Publication No. WO 2011/076786, to treat a disorder, *e.g.*, a disorder described herein). In one embodiment, the HDM2 inhibitor is (S)-1-(4-chlorophenyl)-7-isopropoxy-6-methoxy-2-(4-(methyl(((1R,4S)-4-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)methyl)amino)phenyl)-1,2-dihydroisoquinolin-3(4H)-one (Compound A10) or a compound disclosed in PCT Publication No. WO 2011/076786. In one embodiment, the LNA-CDN compound is used in combination with (S)-1-(4-chlorophenyl)-7-isopropoxy-6-methoxy-2-(4-(methyl(((1R,4S)-4-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)methyl)amino)phenyl)-1,2-dihydroisoquinolin-3(4H)-one (Compound A10), or a compound disclosed in PCT Publication No. WO 2011/076786, to treat a disorder such as a solid tumor. In one embodiment, the HDM2 inhibitor or (S)-1-(4-chlorophenyl)-7-isopropoxy-6-methoxy-2-(4-(methyl(((1R,4S)-4-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)methyl)amino)phenyl)-1,2-dihydroisoquinolin-3(4H)-one (Compound A10) is administered at a dose of about 400 to 700 mg, *e.g.*, administered three times weekly, 2 weeks on and one week off. In some embodiments, the dose is about 400, 500, 600, or 700 mg; about 400-500, 500-600, or 600-700 mg, *e.g.*, administered three times weekly.

[00310] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an iron chelating agent, Deferasirox (also known as EXJADE; Compound A11), or a compound disclosed in PCT Publication No. WO 1997/049395, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the iron chelating agent is Deferasirox or a compound disclosed in PCT Publication No. WO 1997/049395. In one embodiment, the iron chelating agent is Deferasirox (Compound A11). In one embodiment, the LNA-CDN compound is used in combination with Deferasirox (Compound A11), or a compound

disclosed in PCT Publication No. WO 1997/049395, to treat iron overload, hemochromatosis, or myelodysplasia.

[00311] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an aromatase inhibitor, Letrozole (also known as FEMARA; Compound A12), or a compound disclosed in US 4,978,672, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the aromatase inhibitor is Letrozole (Compound A12) or a compound disclosed in US Patent 4,978,672. In one embodiment, the LNA-CDN compound is used in combination with Letrozole (Compound A12), or a compound disclosed in US Patent 4,978,672, to treat a disorder such as a cancer, a leiomyosarcoma, an endometrium cancer, a breast cancer, a female reproductive system cancer, or a hormone deficiency.

[00312] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a PI3K inhibitor, *e.g.*, a pan-PI3K inhibitor, (4S,5R)-3-(2'-amino-2-morpholino-4'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)-4-(hydroxymethyl)-5-methyloxazolidin-2-one (Compound A13) or a compound disclosed in PCT Publication No. WO2013/124826, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PI3K inhibitor is (4S,5R)-3-(2'-amino-2-morpholino-4'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)-4-(hydroxymethyl)-5-methyloxazolidin-2-one (Compound A13) or a compound disclosed in PCT Publication No. WO2013/124826. In one embodiment, the LNA-CDN compound is used in combination with (4S,5R)-3-(2'-amino-2-morpholino-4'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)-4-(hydroxymethyl)-5-methyloxazolidin-2-one (Compound A13), or a compound disclosed in PCT Publication No. WO2013/124826, to treat a disorder such as a cancer or an advanced solid tumor.

[00313] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an inhibitor of p53 and/or a p53/Mdm2 interaction, (S)-5-(5-chloro-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-6-(4-chlorophenyl)-2-(2,4-dimethoxypyrimidin-5-yl)-1-isopropyl-5,6-dihydropyrrolo[3,4-d]imidazol-4(1H)-one (Compound A14), or a compound disclosed in PCT Publication No. WO2013/111105, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the p53 and/or a p53/Mdm2 interaction inhibitor is (S)-5-(5-chloro-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-6-(4-chlorophenyl)-2-(2,4-

dimethoxypyrimidin-5-yl)-1-isopropyl-5,6-dihydropyrrolo[3,4-d]imidazol-4(1H)-one (Compound A14) or a compound disclosed in PCT Publication No. WO2013/111105. In one embodiment, the LNA-CDN compound is used in combination with (S)-5-(5-chloro-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-6-(4-chlorophenyl)-2-(2,4-dimethoxypyrimidin-5-yl)-1-isopropyl-5,6-dihydropyrrolo[3,4-d]imidazol-4(1H)-one (Compound A14), or a compound disclosed in PCT Publication No. WO2013/111105, to treat a disorder such as a cancer or a soft tissue sarcoma.

[00314] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a CSF-1R tyrosine kinase inhibitor, 4-((2-(((1R,2R)-2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-yl)oxy)-N-methylpicolinamide (Compound A15), or a compound disclosed in PCT Publication No. WO 2005/073224, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the CSF-1R tyrosine kinase inhibitor is 4-((2-(((1R,2R)-2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-yl)oxy)-N-methylpicolinamide (Compound A15) or a compound disclosed in PCT Publication No. WO 2005/073224. In one embodiment, the LNA-CDN compound is used in combination with 4-((2-(((1R,2R)-2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-yl)oxy)-N-methylpicolinamide (Compound A15) or a compound disclosed in PCT Publication No. WO 2005/073224, to treat a disorder such as cancer.

[00315] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an apoptosis inducer and/or an angiogenesis inhibitor, such as Imatinib mesylate (also known as GLEEVEC; Compound A16) or a compound disclosed in PCT Publication No. WO1999/003854, to treat a disorder, *e.g.*, a disorder described. In one embodiment, the apoptosis inducer and/or an angiogenesis inhibitor is Imatinib mesylate (Compound A16) or a compound disclosed in PCT Publication No. WO1999/003854. In one embodiment, the LNA-CDN compound is used in combination with Imatinib mesylate (Compound A16), or a compound disclosed in PCT Publication No. WO1999/003854, to treat a disorder such as a cancer, a multiple myeloma, a prostate cancer, a non-small cell lung cancer, a lymphoma, a gastric cancer, a melanoma, a breast cancer, a pancreatic cancer, a digestive/gastrointestinal cancer, a colorectal cancer, a glioblastoma multiforme, a liver cancer, a head and neck cancer, asthma, multiple sclerosis, allergy, Alzheimer's

dementia, amyotrophic lateral sclerosis, or rheumatoid arthritis. In certain embodiments, Imatinib mesylate (Compound A16) is administered at a dose of about 100 to 1000 mg, *e.g.*, about 200 mg to 800 mg, about 300 mg to 700 mg, or about 400 mg to 600 mg, *e.g.*, about 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, or 700 mg. The dosing schedule can vary from *e.g.*, every other day to daily, twice or three times a day. In one embodiment, Imatinib mesylate is administered at an oral dose from about 100 mg to 600 mg daily, *e.g.*, about 100 mg, 200 mg, 260 mg, 300 mg, 400 mg, or 600 mg daily.

[00316] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a JAK inhibitor, 2-fluoro-N-methyl-4-(7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2-yl)benzamide (Compound A17), or a dihydrochloric salt thereof, or a compound disclosed in PCT Publication No. WO 2007/070514, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the JAK inhibitor is 2-fluoro-N-methyl-4-(7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2-yl)benzamide (Compound A17), or a dihydrochloric salt thereof, or a compound disclosed in PCT Publication No. WO 2007/070514. In one embodiment, the LNA-CDN compound is used in combination with 2-fluoro-N-methyl-4-(7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2-yl)benzamide (Compound A17), or a dihydrochloric salt thereof, or a compound disclosed in PCT Publication No. WO 2007/070514, to treat a disorder such as colorectal cancer, myeloid leukemia, hematological cancer, autoimmune disease, non-Hodgkin lymphoma, or thrombocythemia. In one embodiment, the JAK inhibitor or a 2-fluoro-N-methyl-4-(7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2-yl)benzamide (Compound A17), or a dihydrochloric salt thereof is administered at a dose of about 400-600 mg (*e.g.*, per day), *e.g.*, about 400, 500, or 600 mg, or about 400-500 or 500-600 mg.

[00317] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a JAK inhibitor, Ruxolitinib Phosphate (also known as JAKAFI; Compound A18) or a compound disclosed in PCT Publication No. WO 2007/070514 to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the JAK inhibitor is Ruxolitinib Phosphate (Compound A18) or a compound disclosed in PCT Publication No. WO 2007/070514. In one embodiment, the LNA-CDN compound is used in combination with Ruxolitinib Phosphate (Compound A18), or a compound disclosed in PCT Publication No. WO

2007/070514, to treat a disorder such as a prostate cancer, a lymphocytic leukemia, a multiple myeloma, a lymphoma, a lung cancer, a leukemia, cachexia, a breast cancer, a pancreatic cancer, rheumatoid arthritis, psoriasis, a colorectal cancer, a myeloid leukemia, a hematological cancer, an autoimmune disease, a non-Hodgkin lymphoma, or thrombocythemia. In one embodiment, the JAK inhibitor or Ruxolitinib Phosphate (Compound A18) is administered at a dose of about 15-25 mg, *e.g.*, twice daily. In some embodiments, the dose is about 15, 20, or 25 mg, or about 15-20 or 20-25 mg.

[00318] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a histone deacetylase (HDAC) inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of panobinostat, vorinostat, romidepsin, chidamide, valproic acid, belinostat, pyroxamide, mocetinostat, abexinostat, entinostat, pracinostat, resminostat, givinostat, quisinostat, ricolinostat, CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, and CG200745. In some embodiments, the combination comprising a LNA-CDN compound as described herein, is used in combination with a histone deacetylase (HDAC) inhibitor, Panobinostat (Compound A19), or a compound disclosed in PCT Publication No. WO 2014/072493, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the HDAC inhibitor is Panobinostat (Compound A19) or a compound disclosed in PCT Publication No. WO 2014/072493. In one embodiment, the LNA-CDN compound is used in combination with Panobinostat (Compound A19), a compound disclosed in PCT Publication No. WO 2014/072493, to treat a disorder such as a small cell lung cancer, a respiratory/thoracic cancer, a prostate cancer, a multiple myeloma, myelodysplastic syndrome, a bone cancer, a non-small cell lung cancer, an endocrine cancer, a lymphoma, a neurologic cancer, a leukemia, HIV/AIDS, an immune disorder, transplant rejection, a gastric cancer, a melanoma, a breast cancer, a pancreatic cancer, a colorectal cancer, a glioblastoma multiforme, a myeloid leukemia, a hematological cancer, a renal cancer, a non-Hodgkin lymphoma, a head and neck cancer, a hematopoiesis disorders, or a liver cancer. In one embodiment, the HDAC inhibitor or Panobinostat (Compound A19) is administered at a dose of about 20 mg (*e.g.*, per day).

[00319] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an inhibitor of one or more of cytochrome P450 (*e.g.*, 11B2), aldosterone or angiogenesis, Osilodrostat

(Compound A20), or a compound disclosed in PCT Publication No. WO2007/024945, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the inhibitor of one or more of cytochrome P450 (*e.g.*, 11B2), aldosterone or angiogenesis is Osilodrostat (Compound A20) or a compound disclosed in PCT Publication No. WO2007/024945. In one embodiment, the LNA-CDN compound is used in combination with Osilodrostat (Compound A20), or a compound disclosed in PCT Publication No. WO2007/024945, to treat a disorder such as Cushing's syndrome, hypertension, or heart failure therapy.

[00320] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a IAP inhibitor, (S)-N-((S)-1-cyclohexyl-2-((S)-2-(4-(4-fluorobenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-2-(methylamino)propanamide (Compound A21) or a compound disclosed in US 8,552,003, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the IAP inhibitor is (S)-N-((S)-1-cyclohexyl-2-((S)-2-(4-(4-fluorobenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-2-(methylamino)propanamide (Compound A21) or a compound disclosed in US Patent 8,552,003. In one embodiment, the LNA-CDN compound is used in combination with (S)-N-((S)-1-cyclohexyl-2-((S)-2-(4-(4-fluorobenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-2-(methylamino)propanamide (Compound A21), or a compound disclosed in US Patent 8,552,003, to treat a disorder such as a multiple myeloma, a breast cancer, an ovarian cancer, a pancreatic cancer, or a hematopoiesis disorder. In one embodiment, the IAP inhibitor or (S)-N-((S)-1-cyclohexyl-2-((S)-2-(4-(4-fluorobenzoyl)thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-2-(methylamino)propanamide (Compound A21) or a compound disclosed in US 8,552,003 is administered at a dose of approximately 1800 mg, *e.g.*, once weekly.

[00321] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination a Smoothened (SMO) inhibitor, Sonidegib phosphate (Compound A22), (R)-2-(5-(4-(6-benzyl-4,5-dimethylpyridazin-3-yl)-2-methylpiperazin-1-yl)pyrazin-2-yl)propan-2-ol (Compound A25), or a compound disclosed in PCT Publication No. WO 2007/131201 or WO 2010/007120, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the SMO inhibitor is Sonidegib phosphate (Compound A22), (R)-2-(5-(4-(6-benzyl-4,5-dimethylpyridazin-3-yl)-2-methylpiperazin-1-yl)pyrazin-2-yl)propan-2-ol (Compound A25), or a compound disclosed in PCT Publication No. WO 2007/131201 or WO

2010/007120. In one embodiment, the LNA-CDN compound is used in combination with Sonidegib phosphate (Compound A22), (R)-2-(5-(4-(6-benzyl-4,5-dimethylpyridazin-3-yl)-2-methylpiperazin-1-yl)pyrazin-2-yl)propan-2-ol (Compound A25), or a compound disclosed in PCT Publication No. WO 2007/131201 or WO 2010/007120 to treat a disorder such as a cancer, a medulloblastoma, a small cell lung cancer, a prostate cancer, a basal cell carcinoma, a pancreatic cancer, or an inflammation. In certain embodiments, Sonidegib phosphate (Compound A22) is administered at a dose of about 20 to 500 mg, *e.g.*, about 40 mg to 400 mg, about 50 mg to 300 mg, or about 100 mg to 200 mg, *e.g.*, about 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, or 300 mg. The dosing schedule can vary from *e.g.*, every other day to daily, twice or three times a day.

[00322] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an Alk inhibitor, ceritinib (also known as ZYKADIA; Compound A23) or a compound disclosed in PCT Publication No. WO 2007/131201, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the Alk inhibitor is ceritinib (Compound A23) or a compound disclosed in PCT Publication No. WO 2007/131201. In one embodiment, the LNA-CDN compound is used in combination with ceritinib (Compound A23), or a compound disclosed in PCT Publication No. WO 2007/131201, to treat a disorder such as non-small cell lung cancer or solid tumors. In one embodiment, the Alk inhibitor or ceritinib (Compound A23) is administered at a dose of approximately 750 mg, *e.g.*, once daily.

[00323] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a JAK and/or CDK4/6 inhibitor, 7-cyclopentyl-N,N-dimethyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A24), or a compound disclosed in US Patent 8,415,355 or US Patent 8,685,980, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the JAK and/or CDK4/6 inhibitor is 7-cyclopentyl-N,N-dimethyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A24) or a compound disclosed in US Patent 8,415,355 or US Patent 8,685,980. In one embodiment, the LNA-CDN compound is used in combination with 7-cyclopentyl-N,N-dimethyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A24), or a compound disclosed in US 8,415,355 or US 8,685,980, to treat a disorder such as a lymphoma, a

neurologic cancer, a melanoma, a breast cancer, or a solid tumor. In one embodiment, the JAK and/or CDK4/6 inhibitor or 7-cyclopentyl-N,N-dimethyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A24) is administered at a dose of approximately 200-600 mg, *e.g.*, per day. In one embodiment, the compound is administered at a dose of about 200, 300, 400, 500, or 600 mg, or about 200-300, 300-400, 400-500, or 500-600 mg.

[00324] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a PIM Kinase inhibitor, N-(4-((1R,3S,5S)-3-amino-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide (Compound A27) or a compound disclosed in PCT Publication No. WO 2010/026124, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the PIM Kinase inhibitor is N-(4-((1R,3S,5S)-3-amino-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide (Compound A27) or a compound disclosed in PCT Publication No. WO 2010/026124. In one embodiment, the LNA-CDN compound is used in combination with N-(4-((1R,3S,5S)-3-amino-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide (Compound A27), or a compound disclosed in PCT Publication No. WO 2010/026124, to treat a disorder such as a multiple myeloma, myelodysplastic syndrome, a myeloid leukemia, or a non-Hodgkin lymphoma.

[00325] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination a Wnt signaling inhibitor, 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28) or a compound disclosed in PCT publication No. WO 2010/101849, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the Wnt signaling inhibitor is 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28) or a compound disclosed in PCT publication No. WO 2010/101849. In one embodiment, the Wnt signaling inhibitor is 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28). In one embodiment, the LNA-CDN compound is used in combination with 2-(2',3-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28), or a compound disclosed in PCT publication No. WO 2010/101849, to treat a disorder such as a solid tumor (*e.g.*, a head and neck cancer, a squamous cell carcinoma, a

breast cancer, a pancreatic cancer, or a colon cancer). In certain embodiments, 2-(2',3'-dimethyl-[2,4'-bipyridin]-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (Compound A28) is administered at a dose of about 1 to 50 mg, *e.g.*, about 2 mg to 45 mg, about 3 mg to 40 mg, about 5 mg to 35 mg, 5 mg to 10 mg, or about 10 mg to 30 mg, *e.g.*, about 2 mg, 5 mg, 10 mg, 20 mg, 30 mg, or 40 mg. The dosing schedule can vary from *e.g.*, every other day to daily, twice or three times a day.

[00326] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a BRAF inhibitor, Encorafenib (Compound A29), or a compound disclosed in PCT Publication No. WO 2011/025927, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the BRAF inhibitor is Encorafenib (Compound A29) or a compound disclosed in PCT Publication No. WO 2011/025927. In one embodiment, the LNA-CDN compound is used in combination with Encorafenib (Compound A29), or a compound disclosed in PCT Publication No. WO 2011/025927, to treat a disorder such as a non-small cell lung cancer, a melanoma, or a colorectal cancer. In one embodiment, the BRAF inhibitor or Encorafenib (Compound A29) is administered at a dose of about 200-300, 200-400, or 300-400 mg, *e.g.*, per day. In one embodiment, the compound is administered at a dose of about 200, about 300 or about 400 mg.

[00327] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination a CDK4/6 inhibitor, 7-cyclopentyl-N,N-dimethyl-2-((5-((1R,6S)-9-methyl-4-oxo-3,9-diazabicyclo[4.2.1]nonan-3-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A30), or a compound disclosed in PCT publication No. WO 2011/101409, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the CDK4/6 inhibitor is 7-cyclopentyl-N,N-dimethyl-2-((5-((1R,6S)-9-methyl-4-oxo-3,9-diazabicyclo[4.2.1]nonan-3-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A30) or a compound disclosed in PCT publication No. WO 2011/101409. In one embodiment, the LNA-CDN compound is used in combination with 7-cyclopentyl-N,N-dimethyl-2-((5-((1R,6S)-9-methyl-4-oxo-3,9-diazabicyclo[4.2.1]nonan-3-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (Compound A30), or a compound disclosed in PCT publication No. WO 2011/101409, to treat a disorder such as a cancer, a mantle cell

lymphoma, a liposarcoma, a non-small cell lung cancer, a melanoma, a squamous cell esophageal cancer, or a breast cancer.

[00328] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a MEK inhibitor, Binimetinib (Compound A34), or a compound disclosed in PCT Publication No. WO 2003/077914, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the MEK inhibitor is Binimetinib (Compound A34), or a compound disclosed in PCT Publication No. WO 2003/077914. In one embodiment, the LNA-CDN compound is used in combination with Binimetinib (Compound A34), or a compound disclosed in PCT Publication No. WO 2003/077914, to treat a disorder such as a non-small cell lung cancer, a multisystem genetic disorder, a melanoma, an ovarian cancer, a digestive/gastrointestinal cancer, a rheumatoid arthritis, or a colorectal cancer. In one embodiment, the MEK inhibitor or Binimetinib (Compound A34) is administered at a dose of about 45 mg, *e.g.*, twice daily.

[00329] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination an inhibitor of one or more of c-KIT, histamine release, Flt3 (*e.g.*, FLK2/STK1) or PKC, Midostaurin (Compound A35) or a compound disclosed in PCT Publication No. WO 2003/037347, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the inhibitor is Midostaurin (Compound A35) or compound disclosed in PCT Publication No. WO 2003/037347. In one embodiment, the inhibitor of one or more of c-KIT, histamine release, Flt3 (*e.g.*, FLK2/STK1) or PKC is Midostaurin. In one embodiment, the LNA-CDN compound is used in combination with Midostaurin (Compound A35), or compound disclosed in PCT Publication No. WO 2003/037347, to treat a disorder such as a cancer, a colorectal cancer, a myeloid leukemia, myelodysplastic syndrome, an age-related muscular degeneration, a diabetic complication, or a dermatologic disorder.

[00330] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a TOR inhibitor (*e.g.*, mTOR inhibitor), Everolimus (also known as AFINITOR; Compound A36) or a Compound disclosed in PCT Publication No. WO 2014/085318, to treat a disorder, *e.g.*, a disorder described herein). In one embodiment, the TOR inhibitor is Everolimus (Compound A36) or a Compound disclosed in PCT Publication No. WO 2014/085318.

In one embodiment, the LNA-CDN compound is used in combination with Everolimus (Compound A36) to treat a disorder such as an interstitial lung disease, a small cell lung cancer, a respiratory/thoracic cancer, a prostate cancer, a multiple myeloma, a sarcoma, an age-related macular degeneration, a bone cancer, tuberous sclerosis, a non-small cell lung cancer, an endocrine cancer, a lymphoma, a neurologic disorders, an astrocytoma, a cervical cancer, a neurologic cancer, a leukemia, an immune disorders, transplant rejection, a gastric cancer, a melanoma, epilepsy, a breast cancer, or a bladder cancer. In one embodiment, the TOR inhibitor or Everolimus is (Compound A36) administered at a dose of about 2.5-20 mg/day. In one embodiment, the compound is administered at a dose of about 2.5, 5, 10, or 20 mg/day, *e.g.*, about 2.5-5, 5-10, or 10-20 mg/day.

[00331] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination an inhibitor of one or more of VEGFR-2, PDGFRbeta, KIT or Raf kinase C, 1-methyl-5-((2-(5-(trifluoromethyl)-1H-imidazol-2-yl)pyridin-4-yl)oxy)-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-2-amine (Compound A37) or a compound disclosed in PCT Publication No. WO 2007/030377, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the inhibitor of one or more of VEGFR-2, PDGFRbeta, KIT or Raf kinase C is 1-methyl-5-((2-(5-(trifluoromethyl)-1H-imidazol-2-yl)pyridin-4-yl)oxy)-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-2-amine (Compound A37) or a compound disclosed in PCT Publication No. WO 2007/030377. In one embodiment, the LNA-CDN compound is used in combination with 1-methyl-5-((2-(5-(trifluoromethyl)-1H-imidazol-2-yl)pyridin-4-yl)oxy)-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-2-amine (Compound A37), or a compound disclosed in PCT Publication No. WO 2007/030377, to treat a disorder such as a cancer, a melanoma, or a solid tumor.

[00332] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination a somatostatin agonist and/or growth hormone release inhibitor, Pasireotide diaspartate (also known as SIGNIFOR; Compound A38) or a compound disclosed in PCT Publication No. WO2002/010192 or US Patent No. 7,473,761, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the somatostatin agonist and/or growth hormone release inhibitor is Pasireotide diaspartate (Compound A38) or a compound disclosed in PCT Publication No. WO2002/010192 or US Patent No. 7,473,761. In one embodiment,

the LNA-CDN compound is used in combination with Pasireotide diaspertate (Compound A38), or a compound disclosed in PCT Publication No. WO2002/010192 or US Patent No. 7,473,761, to treat a disorder such as a prostate cancer, an endocrine cancer, a neurologic cancer, a skin cancer (*e.g.*, a melanoma), a pancreatic cancer, a liver cancer, Cushing's syndrome, a gastrointestinal disorder, acromegaly, a liver and biliary tract disorder, or liver cirrhosis.

[00333] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination a signal transduction modulator and/or angiogenesis inhibitor, Dovitinib (Compound A39) or a compound disclosed in PCT Publication No. WO 2009/115562, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the signal transduction modulator and/or angiogenesis inhibitor is Dovitinib (Compound A39) or a compound disclosed in PCT Publication No. WO 2009/115562. In one embodiment, the LNA-CDN compound is used in combination with Dovitinib (Compound A39), or a compound disclosed in PCT Publication No. WO 2009/115562, to treat a disorder such as a cancer, a respiratory/thoracic cancer, a multiple myeloma, a prostate cancer, a non-small cell lung cancer, an endocrine cancer, or a neurological genetic disorder.

[00334] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an EGFR inhibitor, (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40) or a compound disclosed in PCT Publication No. WO 2013/184757, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the EGFR inhibitor is (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino) but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40) or a compound disclosed in PCT Publication No. WO 2013/184757. In one embodiment, the LNA-CDN compound is used in combination with (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40), or a compound disclosed in PCT Publication No. WO 2013/184757, to treat a disorder such as a cancer, *e.g.*, a solid tumor. In one embodiment, the EGFR inhibitor or (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide (Compound A40) is administered at a dose of 150-250 mg, *e.g.*,

per day. In one embodiment, the compound is administered at a dose of about 150, 200, or 250 mg, or about 150-200 or 200-250 mg.

[00335] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination an ALK inhibitor, N⁶-(2-isopropoxy-5-methyl-4-(1-methylpiperidin-4-yl)phenyl)-N⁴-(2-(isopropylsulfonyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine (Compound A42) or a compound disclosed in PCT Publication No. WO 2008/073687, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the ALK inhibitor is N⁶-(2-isopropoxy-5-methyl-4-(1-methylpiperidin-4-yl)phenyl)-N⁴-(2-(isopropylsulfonyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine (Compound A42) or a compound disclosed in PCT Publication No. WO 2008/073687. In one embodiment, the LNA-CDN compound is used in combination with N⁶-(2-isopropoxy-5-methyl-4-(1-methylpiperidin-4-yl)phenyl)-N⁴-(2-(isopropylsulfonyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine (Compound A42), or a compound disclosed in PCT Publication No. WO 2008/073687, to treat a disorder such as a cancer, an anaplastic large-cell lymphoma (ALCL), a non-small cell lung carcinoma (NSCLC), or a neuroblastoma.

[00336] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination an IGF-1R inhibitor, 3-(4-(4-((5-chloro-4-((5-methyl-1H-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)-5-fluoro-2-methylphenyl)piperidin-1-yl)thietane 1,1-dioxide (Compound A43), 5-chloro-N²-(2-fluoro-5-methyl-4-(1-(tetrahydro-2H-pyran-4-yl)piperidin-4-yl)phenyl)-N⁴-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A44), or 5-chloro-N²-(4-(1-ethylpiperidin-4-yl)-2-fluoro-5-methylphenyl)-N⁴-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A45) or a compound disclosed in PCT Publication No. WO 2010/002655, to treat a disorder, *e.g.*, a disorder described. In one embodiment, the IGF-1R inhibitor is 3-(4-(4-((5-chloro-4-((5-methyl-1H-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)-5-fluoro-2-methylphenyl)piperidin-1-yl)thietane 1,1-dioxide (Compound A43), 5-chloro-N²-(2-fluoro-5-methyl-4-(1-(tetrahydro-2H-pyran-4-yl)piperidin-4-yl)phenyl)-N⁴-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A44), 5-chloro-N²-(4-(1-ethylpiperidin-4-yl)-2-fluoro-5-methylphenyl)-N⁴-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A45), or a compound disclosed in PCT Publication No. WO 2010/002655. In one embodiment, the LNA-CDN compound is

used in combination with 3-(4-(4-((5-chloro-4-((5-methyl-1H-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)-5-fluoro-2-methylphenyl)piperidin-1-yl)thietane 1,1-dioxide (Compound A43), 5-chloro-N²-(2-fluoro-5-methyl-4-(1-(tetrahydro-2H-pyran-4-yl)piperidin-4-yl)phenyl)-N⁴-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A44), 5-chloro-N²-(4-(1-ethylpiperidin-4-yl)-2-fluoro-5-methylphenyl)-N⁴-(5-methyl-1H-pyrazol-3-yl)pyrimidine-2,4-diamine (Compound A45), or a compound disclosed in PCT Publication No. WO 2010/002655, to treat a disorder such as a cancer or a sarcoma.

[00337] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination a P-Glycoprotein 1 inhibitor, Valspodar (also known as AMDRAY; Compound A46) or a compound disclosed in EP 296122, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the P-Glycoprotein 1 inhibitor is Valspodar (Compound A46) or a compound disclosed in EP 296122. In one embodiment, the LNA-CDN compound is used in combination with Valspodar (Compound A46), or a compound disclosed in EP 296122, to treat a disorder such as a cancer or a drug-resistant tumor.

[00338] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination one or more of a VEGFR inhibitor, Vatalanib succinate (Compound A47) or a compound disclosed in WO 98/35958, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the VEGFR inhibitor is Vatalanib succinate (Compound A47) or a compound disclosed in WO 98/35958. In one embodiment, the LNA-CDN compound is used in combination with Vatalanib succinate (Compound A47), or a compound disclosed in EP 296122, to treat cancer.

[00339] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an IDH inhibitor or a compound disclosed in WO2014/141104, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the IDH inhibitor is a compound disclosed in PCT Publication No. WO2014/141104. In one embodiment, the LNA-CDN compound is used in combination with a compound disclosed in WO2014/141104 to treat a disorder such as a cancer.

[00340] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a BCL-ABL inhibitor or a compound disclosed in PCT Publication No. WO2013/171639, WO2013/171640, WO2013/171641, or WO2013/171642, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the BCL-ABL inhibitor is a compound disclosed in PCT Publication No. WO2013/171639, WO2013/171640, WO2013/171641, or WO2013/171642. In one embodiment, the LNA-CDN compound is used in combination with a compound disclosed in PCT Publication No. WO2013/171639, WO2013/171640, WO2013/171641, or WO2013/171642 to treat a disorder such as a cancer.

[00341] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with a c-RAF inhibitor or a compound disclosed in PCT Publication No. WO2014/151616, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the c-RAF inhibitor is Compound A50 or a compound disclosed in PCT Publication No. WO2014/151616. In one embodiment, the LNA-CDN compound is used in combination with a compound disclosed in PCT Publication No. WO2014/151616 to treat a disorder such as a cancer.

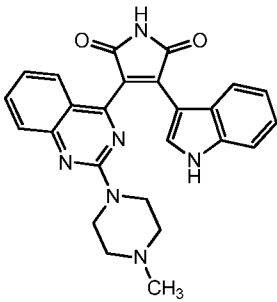
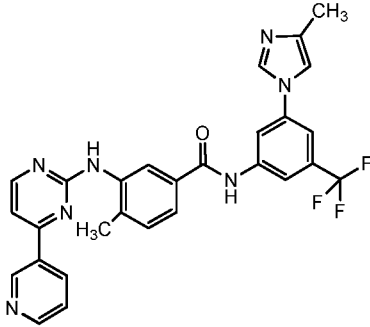
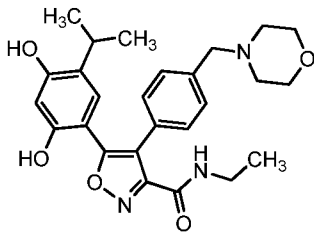
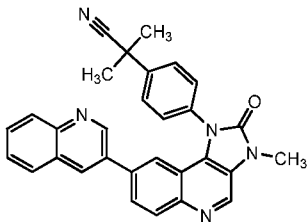
[00342] In another embodiment, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is used in combination with an ERK1/2 ATP competitive inhibitor or a compound disclosed in PCT Publication No. WO2015/066188, to treat a disorder, *e.g.*, a disorder described herein. In one embodiment, the ERK1/2 ATP competitive inhibitor is a compound disclosed in PCT Publication No. WO2015/066188. In one embodiment, the LNA-CDN compound is used in combination with Compound A51 or a compound disclosed in PCT Publication No. WO2015/066188 to treat a disorder such as a cancer. In some embodiments, the combination, *e.g.*, a combination comprising a LNA-CDN compound described herein, and Compound A51 or a compound disclosed in PCT Publication No. WO2015/066188, is administered in combination with one or more agents selected from, Compound A8, Compound A17, Compound A23, Compound A24, Compound A27, Compound A29, and Compound A33.

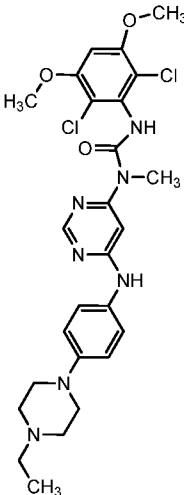
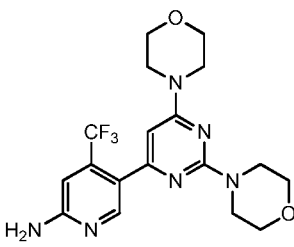
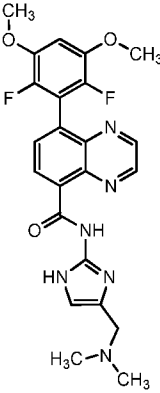
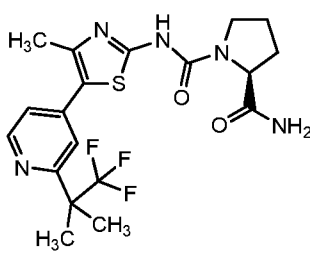
[00343] In some embodiments, the combination, *e.g.*, a combination comprising a LNA-CDN compound as described herein, is administered in combination with an anti-cancer agent having a known activity in an immune cell assay, *e.g.*, in one or more of a

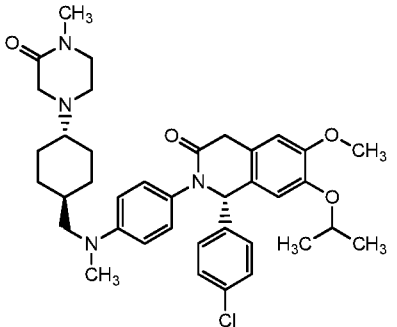
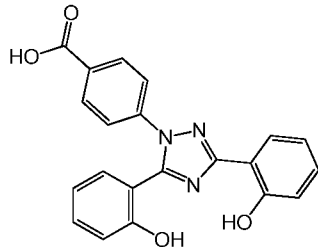
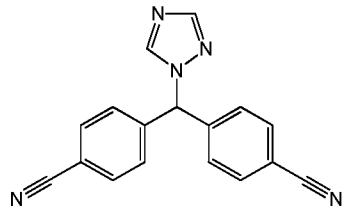
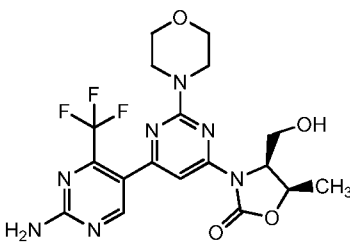
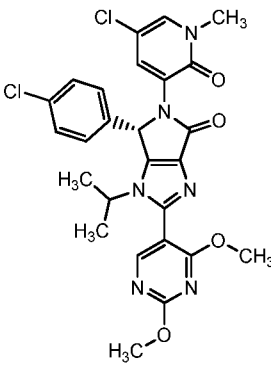
huMLR assay, a T cell proliferation assay, and a B-cell proliferation assay, where such assays are known in the art, and can be used to demonstrate the compounds will not inhibit an immune response (i.e. demonstrate little or no inhibition in such assays). An IC50 in such assays can be determined for the compounds to be used in combination with the LNA-CDN compound. In embodiments, the anti-cancer agent has an IC50 of, *e.g.*, >1 μ M, 1-4 μ M, or greater than 4 μ M, *e.g.*, 4-10 μ M or 4-20 μ M, or greater than 20 μ M. In embodiments, the second therapeutic agent is chosen from one or more of: Compound A9, Compound A16, Compound A17, Compound A21, Compound A22, Compound A25, Compound A28, Compound A48, and Compound A49.

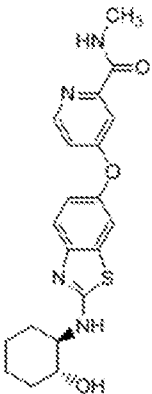
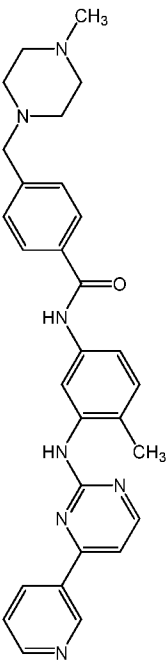
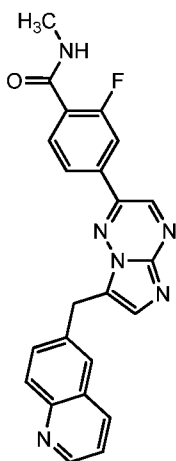
[00344] In some embodiments, the Compound A28 (or a compound related to Compound A28) is administered at a dose of approximately 5-10 or 10-30 mg. In some embodiments, the Compound A22 (or compound related to Compound A22) is administered at a dose of about 200 mg. In some embodiments, the Compound A17 (or compound related to Compound A17) is administered at a dose of approximately 400-600 mg. In some embodiments, the Compound A16 (or compound related to Compound A16) is administered at a dose of approximately 400-600 mg PO qDay. In some embodiments, the Compound A29 (or compound related to Compound A29) is administered at a dose of approximately 200-400 or 300-400 mg. In some embodiments, the Compound A24 (or compound related to Compound A24) is administered at a dose of approximately 200-600 mg. In some embodiments, the Compound A23 (ceritinib) (or compound related to ceritinib) is administered at a dose of approximately 750 mg once daily. In some embodiments, the Compound A8 (or compound related to Compound A8) is administered at a dose of approximately 200-400 or 300-400 mg. In some embodiments, the Compound A5 (or compound related to Compound A5) is administered at a dose of approximately 100-125 mg. In some embodiments, the Compound A6 (or compound related to Compound A6) is administered at a dose of about 100 mg. In some embodiments, the Compound A1 (or compound related to Compound A1) is administered at a dose of approximately 200-300 or 200-600 mg. In some embodiments, the Compound A40 (or compound related to Compound A40) is administered at a dose of approximately 150-250 mg. In embodiments, the Compound A10 (or compound related to Compound A10) is administered at a dose of approximately 400 to 700 mg, *e.g.*, administered three times weekly, 2 weeks on and one week off. In embodiments, the BCR-ABL inhibitor is administered at a dose of approximately 20 mg bid-80 mg bid.

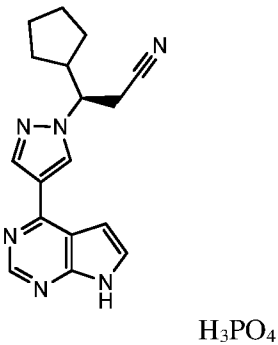
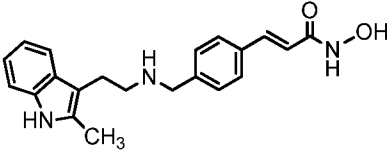
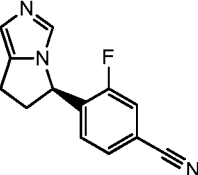
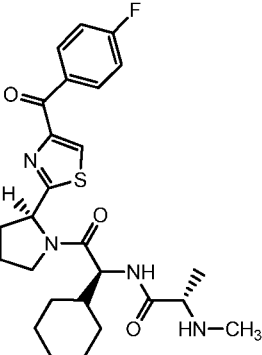
[00345] Table 2. Therapeutic agents that can be administered in combination with the LNA-CDN compounds as described herein.

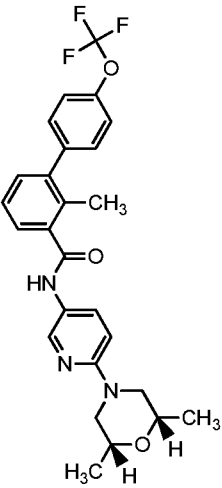
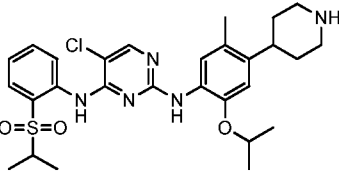
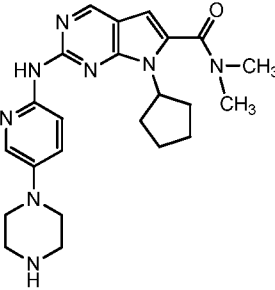
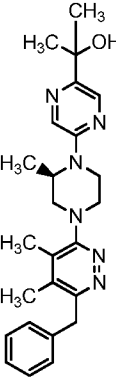
Compound designation/ generic name	Patent / Reference	Compound Structure
A1 Sotrastaurin	EP 1682103 US 2007/142401 WO 2005/039549	
A2 Nilotinib HCl monohydrate TASIGNA®	WO 2004/005281 US 7,169,791	 HCl • H ₂ O
A3	WO 2010/060937 WO 2004/072051 EP 1611112 US 8,450,310	
A4 Dactolisib	WO 2006/122806	

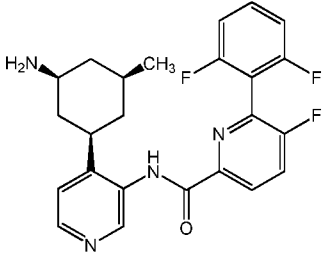
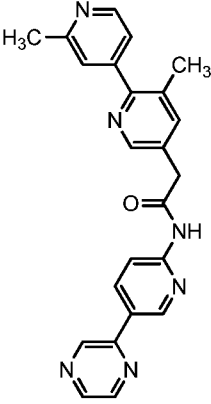
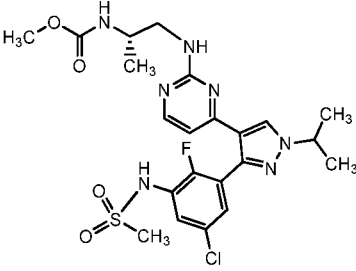
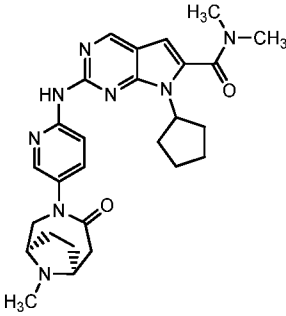
Compound designation/ generic name	Patent / Reference	Compound Structure
A5	US 8,552,002	
A6 Buparlisib	WO 2007/084786	
A7	WO 2009/141386 US 2010/0105667	
A8	WO 2010/029082	
A9 CYP17 inhibitor	WO 2010/149755 US 8,263,635 B2 EP 2445903 B1	

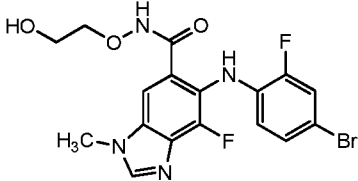
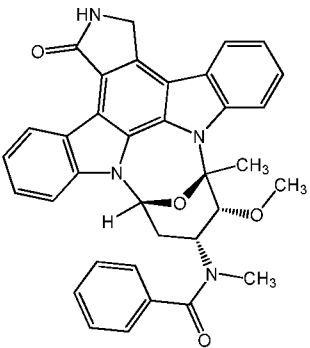
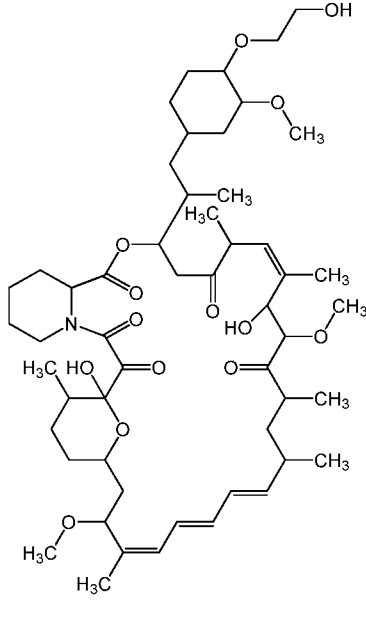
Compound designation/ generic name	Patent / Reference	Compound Structure
A10	WO 2011/076786	
A11 Deferasirox EXJADE®	WO 1997/049395	
A12 Letrozole FEMARA®	US 4,978,672	
A13	WO 2013/124826 US 2013/0225574	
A14	WO 2013/111105	

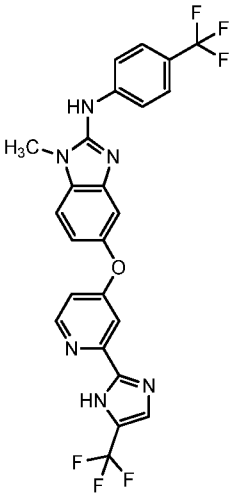
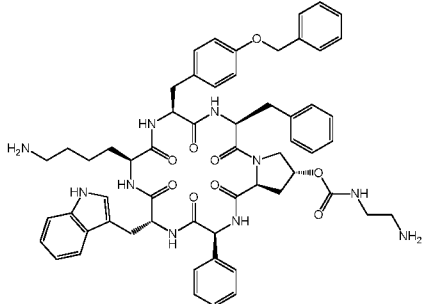
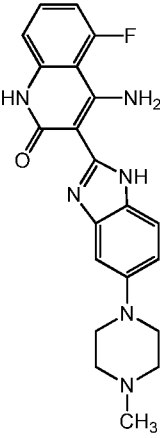
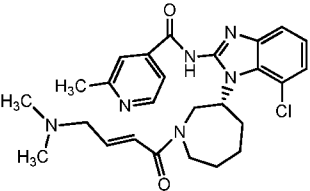
Compound designation/ generic name	Patent / Reference	Compound Structure
A15	WO 2005/073224	
A16 Imatinib mesylate GLEEVEC®	WO 1999/003854	 mesylate
A17	EP 2099447 US 7,767,675 US 8,420,645	 dihydrochloric salt

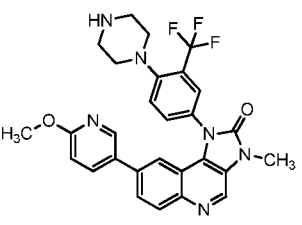
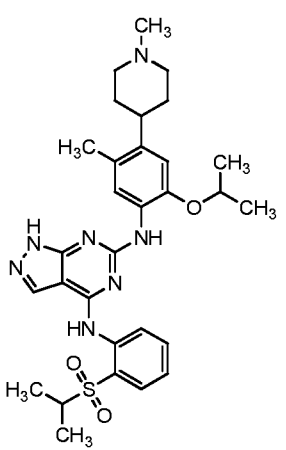
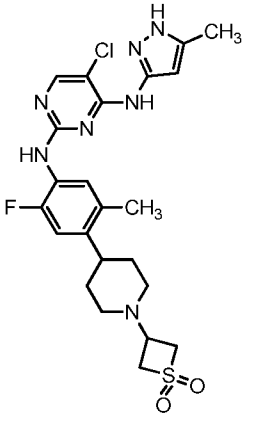
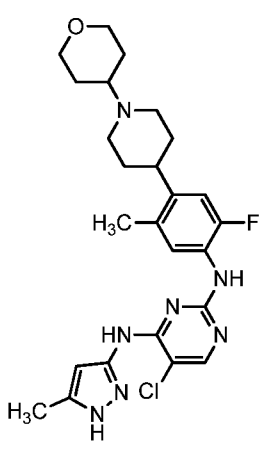
Compound designation/ generic name	Patent / Reference	Compound Structure
A18 Ruxolitinib phosphate JAKAFI®	WO 2007/070514 EP 2474545 US 7,598,257 WO 2014/018632	 H₃PO₄
A19 Panobinostat	WO 2014/072493 WO 2002/022577 EP 1870399	
A20 Osilodrostat	WO 2007/024945	
A21	WO 2008/016893 EP 2051990 US 8,546,336	

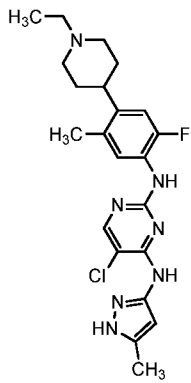
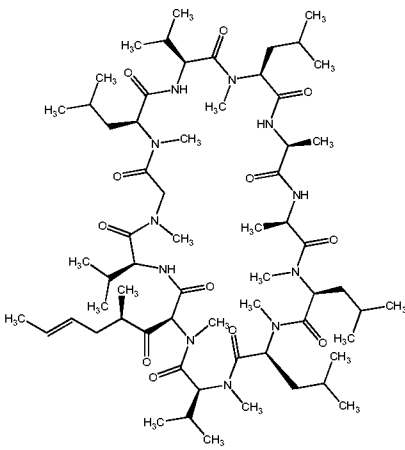
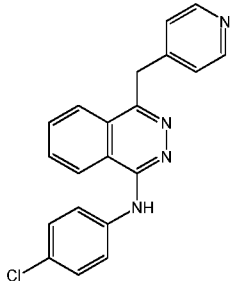
Compound designation/ generic name	Patent / Reference	Compound Structure
A22 Sonidegib phosphate	WO 2007/131201 EP 2021328 US 8,178,563	
A23 Ceritinib ZYKADIA™	WO 2008/073687 US 8,039,479	
A24	US 8,415,355 US 8,685,980	
A25	WO 2010/007120	
A26	US 7,867,493	Human monoclonal antibody to PRLR

Compound designation/ generic name	Patent / Reference	Compound Structure
A27	WO 2010/026124 EP 2344474 US 2010/0056576 WO2008/106692	
A28	WO 2010/101849	
A29 Encorafenib	WO 2011/025927	
A30	WO 2011/101409	
A31	WO 2012/022814 EP 2606070 US 8,735,551	Human monoclonal antibody to HER3
A32	WO 2014/160160 Ab: 12425 (see Table 1, paragraph [00191]) Linker: SMCC (see	Antibody Drug Conjugate (ADC)

Compound designation/ generic name	Patent / Reference	Compound Structure
	paragraph [00117] Payload: DM1 (see paragraph [00111]) See also Claim 29	
A33	WO 2004/045532	Monoclonal antibody or Fab to M-CSF
A34 Binimetinib	WO 2003/077914	 The chemical structure of Binimetinib consists of a 4-methyl-1H-imidazole ring substituted at the 2-position with a 4-bromo-3-fluorophenylamino group and at the 5-position with a 2-(2-hydroxyethoxy)carbamoyl group.
A35 Midostaurin	WO 2003/037347 EP 1441737 US 2012/252785	 The chemical structure of Midostaurin is a complex polycyclic molecule. It features a central indole-like core with multiple fused and linked rings, including a benzodioxane system. It has several substituents, including a 2-methyl-2-phenylacetamido group, a 2-methoxypropyl group, and a 2-oxo-1,2,3,4-tetrahydronaphthalen-1-yl group.
A36 Everolimus AFINITOR®	WO 2014/085318	 The chemical structure of Everolimus (sirolimus) is a large, complex macrocyclic molecule. It contains a 14-membered ring with multiple ester, ether, and ketone functional groups. It also features several side chains, including a 2-methoxypropyl group, a 2-hydroxypropyl group, and a 2-methyl-2-phenylacetamido group.

Compound designation/ generic name	Patent / Reference	Compound Structure
A37	WO 2007/030377 US 7,482,367	
A38 Pasireotide diaspertate SIGNIFOR®	WO2002/010192 US 7,473,761	
A39 Dovitinib	WO 2009/115562 US 8,563,556	
A40	WO 2013/184757	

Compound designation/ generic name	Patent / Reference	Compound Structure
A41	WO 2006/122806	
A42	WO 2008/073687 US 8,372,858	
A43	WO 2010/002655 US 8,519,129	
A44	WO 2010/002655 US 8,519,129	

Compound designation/ generic name	Patent / Reference	Compound Structure
A45	WO 2010/002655	
A46 Valspodar AMDRAY™	EP 296122	
A47 Vatalanib succinate	WO 98/35958	 succinate
A48	WO2014/141104	IDH inhibitor
A49	WO2013/171639 WO2013/171640 WO2013/171641 WO2013/171642	BCR-ABL inhibitor
A50	WO2014/151616	cRAF inhibitor
A51	WO2015/066188	ERK1/2 ATP competitive inhibitor

Immunomodulatory Cell Lines

[00346] By “inactivated tumor cell” is meant a tumor cell (either “autologous” or “allogeneic” to the patient) which has been treated to prevent division of the cells. For purposes of the present invention, such cells preserve their immunogenicity and their metabolic activity. Such tumor cells are genetically modified to express a transgene which is expressed within a patient as part of cancer therapy. Thus, a composition or vaccine of the invention comprises neoplastic (e.g., tumor) cells that are autologous or allogeneic to the patient undergoing treatment and is most preferably the same general type of tumor cell as is afflicting the patient. For example, a patient suffering from melanoma will typically be administered a genetically modified cell derived from a melanoma. Methods for inactivating tumor cells for use in the present invention, such as the use of irradiation, are well known in the art.

[00347] In some embodiments, the inactivated tumor cells of the present invention are modified to express and secrete one or more heat shock proteins. For example, gp96-Ig fusion proteins can be expressed and secreted to stimulate an immune response (Yamazaki et al., *The Journal of Immunology*, 1999, 163:5178-5182; Strbo et al., *Immunol Res.* 2013 Dec;57(1-3):311-25). In some embodiments the inactivated tumor cells are modified to express and secrete a gp96-Ig fusion protein.

[00348] The inactivated tumor cells of the present invention are administered to the patient together with one or more costimulatory molecules or agents. A preferred costimulatory agent comprises one or more cytokines which stimulate dendritic cell induction, recruitment, and/or maturation. Methods for assessing such costimulatory agents are well known in the literature. Induction and maturation of DCs is typically assessed by increased expression of certain membrane molecules such as CD80 and CD86, and/or secretion of pro-inflammatory cytokines, such as IL-12 and type I interferons following stimulation.

[00349] In preferred embodiments, the inactivated tumor cells themselves are modified to express and secrete one or more cytokines which stimulate dendritic cell induction, recruitment, and/or maturation. The present invention is described in exemplary terms with regard to the use of GM-CSF. Thus, by way of example, the tumor cell may express a transgene encoding GM-CSF as described in U.S. Pat. Nos. 5,637,483, 5,904,920, 6,277,368 and 6,350,445, as well as in US Patent Publication No. 20100150946. A form of GM-CSF-expressing genetically modified cancer cells or a “cytokine-expressing

cellular vaccine" for the treatment of pancreatic cancer is described in U.S. Pat. Nos. 6,033,674 and 5,985,290.

[00350] Other suitable cytokines which may be expressed by such inactivated tumor cells and/or bystander cells instead of, or together with, GM-CSF include, but are not limited to, one or more of CD40 ligand, FLT-3 ligand, IL-12, CCL3, CCL20, and CCL21. This list is not meant to be limiting.

[00351] While it is preferred that the inactivated tumor cells administered to the subject express one or more cytokines of interest, the tumor cell line may be accompanied by an inactivated bystander cell line which expresses and secretes one or more cytokines which stimulate dendritic cell induction, recruitment, and/or maturation. The bystander cell line may provide all of the cytokines which stimulate dendritic cell induction, recruitment, and/or maturation, or may supplement cytokines which stimulate dendritic cell induction, recruitment, and/or maturation expressed and secreted by the inactivated tumor cells. By way of example, immunomodulatory cytokine-expressing bystander cell lines are disclosed in U.S. Pat. Nos. 6,464,973, and 8,012,469, Dessureault et al., Ann. Surg. Oncol. 14: 869-84, 2007, and Eager and Nemunaitis, Mol. Ther. 12: 18-27, 2005.

[00352] By "Granulocyte-macrophage colony stimulating factor (GM-CSF) polypeptide" is meant a cytokine or fragment thereof having immunomodulatory activity and having at least about 85% amino acid sequence identity to GenBank Accession No. AAA52122.1.

Vaccines

[00353] In certain embodiments, the CDN compositions are administered in conjunction with one or more vaccines intended to stimulate an immune response to one or more predetermined antigens. Examples of target antigens that may find use in the invention are listed in the following table. The target antigen may also be a fragment or fusion polypeptide comprising an immunologically active portion of the antigens listed in the table. This list is not meant to be limiting.

[00354] Table 1. List of antigens for use in combination with the LNA-CDN compounds as described herein.

Antigen	Reference
Tumor antigens	
Mesothelin	GenBank Acc. No. NM_005823; U40434; NM_013404; BC003512 (see also, e.g., Hassan, <i>et al.</i> (2004) Clin. Cancer Res. 10:3937-3942; Muminova, <i>et al.</i> (2004) BMC Cancer 4:19; Iacobuzio-Donahue, <i>et al.</i> (2003) Cancer Res. 63:8614-8622).
Wilms' tumor-1 associated protein (Wt-1), including isoform A; isoform B; isoform C; isoform D.	WT-1 isoform A (GenBank Acc. Nos. <u>NM_000378</u> ; NP_000369). WT-1 isoform B (GenBank Acc. Nos. <u>NM_024424</u> ; NP_077742). WT-1 isoform C (GenBank Acc. Nos. <u>NM_024425</u> ; NP_077743). WT-1 isoform D (GenBank Acc. Nos. <u>NM_024426</u> ; NP_077744).
Stratum corneum chymotryptic enzyme (SCCE), and variants thereof.	GenBank Acc. No. NM_005046; NM_139277; AF332583. See also, e.g., Bondurant, <i>et al.</i> (2005) Clin. Cancer Res. 11:3446-3454; Santin, <i>et al.</i> (2004) Gynecol. Oncol. 94:283-288; Shigemasa, <i>et al.</i> (2001) Int. J. Gynecol. Cancer 11:454-461; Sepehr, <i>et al.</i> (2001) Oncogene 20:7368-7374.
MHC class I chain-related protein A (MICA); MHC class I chain-related protein B (MICB).	See, e.g., Groh, <i>et al.</i> (2005) Proc. Natl. Acad. Sci. USA 102:6461-6466; GenBank Acc. Nos. NM_000247; BC_016929; AY750850; NM_005931.
Gastrin and peptides derived from gastrin; gastrin/CCK-2 receptor (also known as CCK-B).	Harris, <i>et al.</i> (2004) Cancer Res. 64:5624-5631; Gilliam, <i>et al.</i> (2004) Eur. J. Surg. Oncol. 30:536-543; Laheru and Jaffee (2005) Nature Reviews Cancer 5:459-467.

Antigen	Reference
Glypican-3 (an antigen of, e.g., hepatocellular carcinoma and melanoma).	GenBank Acc. No. <u>NM_004484</u> . Nakatsura, <i>et al.</i> (2003) Biochem. Biophys. Res. Commun. 306:16-25; Capurro, <i>et al.</i> (2003) Gastroenterol. 125:89-97; Nakatsura, <i>et al.</i> (2004) Clin. Cancer Res. 10:6612-6621).
Coactosin-like protein.	Nakatsura, <i>et al.</i> (2002) Eur. J. Immunol. 32:826-836; Laheru and Jaffee (2005) Nature Reviews Cancer 5:459-467.
Prostate stem cell antigen (PSCA).	GenBank Acc. No. AF043498; AR026974; AR302232 (see also, e.g., Argani, <i>et al.</i> (2001) Cancer Res. 61:4320-4324; Christiansen, <i>et al.</i> (2003) Prostate 55:9-19; Fuessel, <i>et al.</i> (2003) 23:221-228).
Prostate acid phosphatase (PAP); prostate-specific antigen (PSA); PSM; PSMA.	Small, <i>et al.</i> (2000) J. Clin. Oncol. 18:3894-3903; Altwein and Luboldt (1999) Urol. Int. 63:62-71; Chan, <i>et al.</i> (1999) Prostate 41:99-109; Ito, <i>et al.</i> (2005) Cancer 103:242-250; Schmittgen, <i>et al.</i> (2003) Int. J. Cancer 107:323-329; Millon, <i>et al.</i> (1999) Eur. Urol. 36:278-285.
Six-transmembrane epithelial antigen of prostate (STEAP).	See, e.g., Machlenkin, <i>et al.</i> (2005) Cancer Res. 65:6435-6442; GenBank Acc. No. NM_018234; NM_001008410; NM_182915; NM_024636; NM_012449; BC011802.
Prostate carcinoma tumor antigen-1 (PCTA-1).	See, e.g., Machlenkin, <i>et al.</i> (2005) Cancer Res. 65:6435-6442; GenBank Acc. No. L78132.
Prostate tumor-inducing gene-1 (PTI-1).	See, e.g., Machlenkin, <i>et al.</i> (2005) Cancer Res. 65:6435-6442).
Prostate-specific gene with homology to G protein-coupled	See, e.g., Machlenkin, <i>et al.</i> (2005) Cancer Res. 65:6435-6442).

Antigen	Reference
receptor.	
Protease (an antrophen regulated serine protease).	See, e.g., Machlenkin, <i>et al.</i> (2005) Cancer Res. 65:6435-6442; GenBank Acc. No. BC096178; BC096176; BC096175.
Proteinase 3.	GenBank Acc. No. X55668.
Cancer-testis antigens, e.g., NY-ESO-1; SCP-1; SSX-1; SSX-2; SSX-4; GAGE, CT7; CT8; CT10; MAGE-1; MAGE-2; MAGE-3; MAGE-4; MAGE-6; LAGE-1.	GenBank Acc. No. NM_001327 (NY-ESO-1) (see also, e.g., Li, <i>et al.</i> (2005) Clin. Cancer Res. 11:1809-1814; Chen, <i>et al.</i> (2004) Proc. Natl. Acad. Sci. U S A. 101(25):9363-9368; Kubuschok, <i>et al.</i> (2004) Int. J. Cancer. 109:568-575; Scanlan, <i>et al.</i> (2004) Cancer Immun. 4:1; Scanlan, <i>et al.</i> (2002) Cancer Res. 62:4041-4047; Scanlan, <i>et al.</i> (2000) Cancer Lett. 150:155-164; Dalerba, <i>et al.</i> (2001) Int. J. Cancer 93:85-90; Ries, <i>et al.</i> (2005) Int. J. Oncol. 26:817-824.
MAGE-A1, MAGE-A2; MAGE-A3; MAGE-A4; MAGE-A6; MAGE-A9; MAGE-A10; MAGE-A12; GAGE-3/6; NT-SAR-35; BAGE; CA125.	Otte, <i>et al.</i> (2001) Cancer Res. 61:6682-6687; Lee, <i>et al.</i> (2003) Proc. Natl. Acad. Sci. USA 100:2651-2656; Sarcevic, <i>et al.</i> (2003) Oncology 64:443-449; Lin, <i>et al.</i> (2004) Clin. Cancer Res. 10:5708-5716.
GAGE-1; GAGE-2; GAGE-3; GAGE-4; GAGE-5; GAGE-6; GAGE-7; GAGE-8; GAGE-65; GAGE-11;	De Backer, <i>et al.</i> (1999) Cancer Res. 59:3157-3165; Scarcella, <i>et al.</i> (1999) Clin. Cancer Res. 5:335-341.

Antigen	Reference
GAGE-13; GAGE-7B.	
HIP1R; LMNA; KIAA1416; Seb4D; KNSL6; TRIP4; MBD2; HCAC5; MAGEA3.	Scanlan, <i>et al.</i> (2002) Cancer Res. 62:4041-4047.
DAM family of genes, e.g., DAM-1; DAM-6.	Fleishhauer, <i>et al.</i> (1998) Cancer Res. 58:2969-2972.
RCAS1.	Enjoji, <i>et al.</i> (2004) Dig. Dis. Sci. 49:1654-1656.
RU2.	Van Den Eynde, <i>et al.</i> (1999) J. Exp. Med. 190:1793-1800.
CAMEL.	Slager, <i>et al.</i> (2004) J. Immunol. 172:5095-5102; Slager, <i>et al.</i> (2004) Cancer Gene Ther. 11:227-236.
Colon cancer associated antigens, e.g., NY-CO-8; NY-CO-9; NY-CO-13; NY-CO-16; NY-CO-20; NY-CO-38; NY-CO-45; NY-CO-9/HDAC5; NY-CO-41/MBD2; NY-CO-42/TRIP4; NY-CO-95/KIAA1416;	Scanlan, <i>et al.</i> (2002) Cancer Res. 62:4041-4047.

Antigen	Reference
KNSL6; seb4D.	
N-Acetylglucosaminyl-tranferase V (GnT-V).	Dosaka-Akita, <i>et al.</i> (2004) Clin. Cancer Res. 10:1773-1779.
Elongation factor 2 mutated (ELF2M).	Renkvist, <i>et al.</i> (2001) Cancer Immunol Immunother. 50:3-15.
HOM-MEL-40/SSX2	Neumann, <i>et al.</i> (2004) Int. J. Cancer 112:661-668; Scanlan, <i>et al.</i> (2000) Cancer Lett. 150:155-164.
BRDT.	Scanlan, <i>et al.</i> (2000) Cancer Lett. 150:155-164.
SAGE; HAGE.	Sasaki, <i>et al.</i> (2003) Eur. J. Surg. Oncol. 29:900-903.
RAGE.	See, e.g., Li, <i>et al.</i> (2004) Am. J. Pathol. 164:1389-1397; Shirasawa, <i>et al.</i> (2004) Genes to Cells 9:165-174.
MUM-1 (melanoma ubiquitous mutated); MUM-2; MUM-2 Arg-Gly mutation; MUM-3.	Gueguen, <i>et al.</i> (1998) J. Immunol. 160:6188-6194; Hirose, <i>et al.</i> (2005) Int. J. Hematol. 81:48-57; Baurain, <i>et al.</i> (2000) J. Immunol. 164:6057-6066; Chiari, <i>et al.</i> (1999) Cancer Res. 59:5785-5792.
LDLR/FUT fusion protein antigen of	Wang, <i>et al.</i> (1999) J. Exp. Med. 189:1659-1667.

Antigen	Reference
melanoma.	
NY-REN series of renal cancer antigens.	Scanlan, <i>et al.</i> (2002) Cancer Res. 62:4041-4047; Scanlan, <i>et al.</i> (1999) Cancer Res. 83:456-464.
NY-BR series of breast cancer antigens, e.g., NY-BR-62; NY-BR-75; NY-BR-85; NY-BR-62; NY-BR-85.	Scanlan, <i>et al.</i> (2002) Cancer Res. 62:4041-4047; Scanlan, <i>et al.</i> (2001) Cancer Immunity 1:4.
BRCA-1; BRCA-2.	Stolier, <i>et al.</i> (2004) Breast J. 10:475-480; Nicoletto, <i>et al.</i> (2001) Cancer Treat Rev. 27:295-304.
DEK/CAN fusion protein.	Von Lindern, <i>et al.</i> (1992) Mol. Cell. Biol. 12:1687-1697.
Ras, e.g., wild type ras, ras with mutations at codon 12, 13, 59, or 61, e.g., mutations G12C; G12D; G12R; G12S; G12V; G13D; A59T; Q61H. K-RAS; H-RAS; N-RAS.	GenBank Acc. Nos. P01112; P01116; M54969; M54968; P01111; P01112; K00654. See also, e.g., GenBank Acc. Nos. M26261; M34904; K01519; K01520; BC006499; NM_006270; NM_002890; NM_004985; NM_033360; NM_176795; NM_005343.

Antigen	Reference
BRAF (an isoform of RAF).	Tannapfel, <i>et al.</i> (2005) Am. J. Clin. Pathol. 123:256-260l; Tsao and Sober (2005) Dermatol. Clin. 23:323-333.
Melanoma antigens, including HST-2 melanoma cell antigens.	GenBank Acc. No. NM_206956; NM_206955; NM_206954; NM_206953; NM_006115; NM_005367; NM_004988; AY148486; U10340; U10339; M77481. See, e.g., Suzuki, <i>et al.</i> (1999) J. Immunol. 163:2783-2791.
Survivin	GenBank Acc. No. AB028869; U75285 (see also, e.g., Tsuruma, <i>et al.</i> (2004) J. Translational Med. 2:19 (11 pages); Pisarev, <i>et al.</i> (2003) Clin. Cancer Res. 9:6523-6533; Siegel, <i>et al.</i> (2003) Br. J. Haematol. 122:911-914; Andersen, <i>et al.</i> (2002) Histol. Histopathol. 17:669-675).
MDM-2	<u>NM_002392</u> ; <u>NM_006878</u> (see also, e.g., Mayo, <i>et al.</i> (1997) Cancer Res. 57:5013-5016; Demidenko and Blagosklonny (2004) Cancer Res. 64:3653-3660).
Methyl-CpG-binding proteins (MeCP2; MBD2).	Muller, <i>et al.</i> (2003) Br. J. Cancer 89:1934-1939; Fang, <i>et al.</i> (2004) World J. Gastroenterol. 10:3394-3398.
NA88-A.	Moreau-Aubry, <i>et al.</i> (2000) J. Exp. Med. 191:1617-1624.
Histone deacetylases (HDAC), e.g., HDAC5.	Waltregny, <i>et al.</i> (2004) Eur. J. Histochem. 48:273-290; Scanlan, <i>et al.</i> (2002) Cancer Res. 62:4041-4047.

Antigen	Reference
Cyclophilin B (Cyp-B).	Tamura, <i>et al.</i> (2001) Jpn. J. Cancer Res. 92:762-767.
CA 15-3; CA 27.29.	Clinton, <i>et al.</i> (2003) Biomed. Sci. Instrum. 39:408-414.
Heat shock protein Hsp70.	Faure, <i>et al.</i> (2004) Int. J. Cancer 108:863-870.
GAGE/PAGE family, e.g., PAGE-1; PAGE-2; PAGE-3; PAGE-4; XAGE-1; XAGE-2; XAGE-3.	Brinkmann, <i>et al.</i> (1999) Cancer Res. 59:1445-1448.
MAGE-A, B, C, and D families. MAGE-B5; MAGE-B6; MAGE-C2; MAGE-C3; MAGE-3; MAGE-6.	Lucas, <i>et al.</i> (2000) Int. J. Cancer 87:55-60; Scanlan, <i>et al.</i> (2001) Cancer Immun. 1:4.
Kinesin 2; TATA element modulatory factor 1; tumor protein D53; NY	Scanlan, <i>et al.</i> (2001) Cancer Immun. 30:1-4.

Antigen	Reference
Alpha-fetoprotein (AFP)	Grimm, <i>et al.</i> (2000) Gastroenterol. 119:1104-1112.
SART1; SART2; SART3; ART4.	Kumamuru, <i>et al.</i> (2004) Int. J. Cancer 108:686-695; Sasatomi, <i>et al.</i> (2002) Cancer 94:1636-1641; Matsumoto, <i>et al.</i> (1998) Jpn. J. Cancer Res. 89:1292-1295; Tanaka, <i>et al.</i> (2000) Jpn. J. Cancer Res. 91:1177-1184.
Preferentially expressed antigen of melanoma (PRAME).	Matsushita, <i>et al.</i> (2003) Leuk. Lymphoma 44:439-444; Oberthuer, <i>et al.</i> (2004) Clin. Cancer Res. 10:4307-4313.
Carcinoembryonic antigen (CEA), CAP1-6D enhancer agonist peptide.	GenBank Acc. No. <u>M29540</u> ; E03352; X98311; M17303 (see also, e.g., Zaremba (1997) Cancer Res. 57:4570-4577; Sarobe, <i>et al.</i> (2004) Curr. Cancer Drug Targets 4:443-454; Tsang, <i>et al.</i> (1997) Clin. Cancer Res. 3:2439-2449; Fong, <i>et al.</i> (2001) Proc. Natl. Acad. Sci. USA 98:8809-8814).
HER-2/neu.	Disis, <i>et al.</i> (2004) J. Clin. Immunol. 24:571-578; Disis and Cheever (1997) Adv. Cancer Res. 71:343-371.
Cdk4; cdk6; p16 (INK4); Rb protein.	Ghazizadeh, <i>et al.</i> (2005) Respiration 72:68-73; Ericson, <i>et al.</i> (2003) Mol. Cancer Res. 1:654-664.
TEL; AML1; TEL/AML1.	Stams, <i>et al.</i> (2005) Clin. Cancer Res. 11:2974-2980.
Telomerase (TERT).	Nair, <i>et al.</i> (2000) Nat. Med. 6:1011-1017.
707-AP.	Takahashi, <i>et al.</i> (1997) Clin. Cancer Res. 3:1363-1370.
Annexin, e.g., Annexin II.	Zimmerman, <i>et al.</i> (2004) Virchows Arch. 445:368-374.

Antigen	Reference
BCR/ABL; BCR/ABL p210; BCR/ABL p190; CML-66; CML-28.	Cobalddda, <i>et al.</i> (2000) Blood 95:1007-1013; Hakansson, <i>et al.</i> (2004) Leukemia 18:538-547; Schwartz, <i>et al.</i> (2003) Semin. Hematol. 40:87-96; Lim, <i>et al.</i> (1999) Int. J. Mol. Med. 4:665-667.
BCL2; BLC6; CD10 protein.	Iqbal, <i>et al.</i> (2004) Am. J. Pathol. 165:159-166.
CDC27 (this is a melanoma antigen).	Wang, <i>et al.</i> (1999) Science 284:1351-1354.
Sperm protein 17 (SP17); 14-3-3-zeta; MEMD; KIAA0471; TC21.	Arora, <i>et al.</i> (2005) Mol. Carcinog. 42:97-108.
Tyrosinase-related proteins 1 and 2 (TRP-1 and TRP-2).	GenBank Acc. No. NM_001922. (see also, e.g., Bronte, <i>et al.</i> (2000) Cancer Res. 60:253-258).
Gp100/pmel-17.	GenBank Acc. Nos. AH003567 ; U31798 ; U31799 ; U31807 ; U31799 (see also, e.g., Bronte, <i>et al.</i> (2000) Cancer Res. 60:253-258).
TARP.	See, e.g., Clifton, <i>et al.</i> (2004) Proc. Natl. Acad. Sci. USA 101:10166-10171; Virok, <i>et al.</i> (2005) Infection Immunity 73:1939-1946.
Tyrosinase-related proteins 1 and 2 (TRP-1 and TRP-2).	GenBank Acc. No. NM_001922. (see also, e.g., Bronte, <i>et al.</i> (2000) Cancer Res. 60:253-258).
Melanocortin 1 receptor (MC1R); MAGE-3; gp100; tyrosinase; dopachrome	Salazar-Onfray, <i>et al.</i> (1997) Cancer Res. 57:4348-4355; Reynolds, <i>et al.</i> (1998) J. Immunol. 161:6970-6976; Chang, <i>et al.</i> (2002) Clin. Cancer Res. 8:1021-1032.

Antigen	Reference
tautomerase (TRP-2); MART-1.	
MUC-1; MUC-2.	See, e.g., Davies, <i>et al.</i> (1994) <i>Cancer Lett.</i> 82:179-184; Gambus, <i>et al.</i> (1995) <i>Int. J. Cancer</i> 60:146-148; McCool, <i>et al.</i> (1999) <i>Biochem. J.</i> 341:593-600.
Spas-1.	U.S. Published Pat. Appl. No. 20020150588 of Allison, <i>et al.</i>
CASP-8; FLICE; MACH.	Mandruzzato, <i>et al.</i> (1997) <i>J. Exp. Med.</i> 186:785-793.
CEACAM6; CAP-1.	Duxbury, <i>et al.</i> (2004) <i>Biochem. Biophys. Res. Commun.</i> 317:837-843; Morse, <i>et al.</i> (1999) <i>Clin. Cancer Res.</i> 5:1331-1338.
HMGB1 (a DNA binding protein and cytokine).	Brezniceanu, <i>et al.</i> (2003) <i>FASEB J.</i> 17:1295-1297.
ETV6/AML1.	Codrington, <i>et al.</i> (2000) <i>Br. J. Haematol.</i> 111:1071-1079.
Mutant and wild type forms of adenomatous polyposis coli (APC); beta-catenin; c-met; p53; E-cadherin; cyclooxygenase-2 (COX-2).	Clements, <i>et al.</i> (2003) <i>Clin. Colorectal Cancer</i> 3:113-120; Gulmann, <i>et al.</i> (2003) <i>Appl. Immunohistochem. Mol. Morphol.</i> 11:230-237; Jungck, <i>et al.</i> (2004) <i>Int. J. Colorectal. Dis.</i> 19:438-445; Wang, <i>et al.</i> (2004) <i>J. Surg. Res.</i> 120:242-248; Abutaily, <i>et al.</i> (2003) <i>J. Pathol.</i> 201:355-362; Liang, <i>et al.</i> (2004) <i>Br. J. Surg.</i> 91:355-361; Shirakawa, <i>et al.</i> (2004) <i>Clin. Cancer Res.</i> 10:4342-4348.
Renal cell carcinoma antigen bound by mAB	Mulders, <i>et al.</i> (2003) <i>Urol. Clin. North Am.</i> 30:455-465; Steffens, <i>et al.</i> (1999) <i>Anticancer Res.</i> 19:1197-1200.

Antigen	Reference
G250.	
EphA2	See, e.g., U.S. Patent Publication No. 2005/0281783 A1; Genbank Accession No. NM_004431 (human); Genbank Accession No. NM_010139 (Mouse); Genbank Accession No. AB038986 (Chicken, partial sequence); GenBank Accession Nos. NP_004422, AAH37166, and AAA53375 (human); GenBank Accession Nos. NP_034269 (mouse), AAH06954 (mouse), XP_345597 (rat), and BAB63910 (chicken).
EGFRvIII	See, e.g., WO/2012/068360
<i>Francisella tularensis</i> antigens	
<i>Francisella tularensis</i> A and B.	Complete genome of subspecies Schu S4 (GenBank Acc. No. AJ749949); of subspecies Schu 4 (GenBank Acc. No. NC_006570). Outer membrane protein (43 kDa) Bevanger, <i>et al.</i> (1988) J. Clin. Microbiol. 27:922-926; Porsch-Ozcurumez, <i>et al.</i> (2004) Clin. Diagnostic. Lab. Immunol. 11:1008-1015). Antigenic components of <i>F. tularensis</i> include, e.g., 80 antigens, including 10 kDa and 60 kDa chaperonins (Havlasova, <i>et al.</i> (2002) Proteomics 2:857-86), nucleoside diphosphate kinase, isocitrate dehydrogenase, RNA-binding protein Hfq, the chaperone ClpB (Havlasova, <i>et al.</i> (2005) Proteomics 5:2090-2103). See also, e.g., Oyston and Quarry (2005) Antonie Van Leeuwenhoek 87:277-281; Isherwood, <i>et al.</i> (2005) Adv. Drug Deliv. Rev. 57:1403-1414; Biagini, <i>et al.</i> (2005) Anal. Bioanal. Chem. 382:1027-1034.
Malarial antigens	
Circumsporozoite protein (CSP); SSP2; HEP17; Exp-1 orthologs found in	See, e.g., Haddad, <i>et al.</i> (2004) Infection Immunity 72:1594-1602; Hoffman, <i>et al.</i> (1997) Vaccine 15:842-845; Oliveira-Ferreira and Daniel-Ribeiro (2001) Mem. Inst. Oswaldo Cruz, Rio de Janeiro 96:221-227. CSP (see, e.g., GenBank Acc. No. AB121024). SSP2

Antigen	Reference
P. falciparum; and LSA-1.	(see, e.g., GenBank Acc. No. AF249739). LSA-1 (see, e.g., GenBank Acc. No. Z30319).
Ring-infected erythrocyte surface protein (RESA); merozoite surface protein 2 (MSP2); Spf66; merozoite surface protein 1(MSP1); 195A; BVp42.	See, e.g., Stirnadel, <i>et al.</i> (2000) Int. J. Epidemiol. 29:579-586; Krzych, <i>et al.</i> (1995) J. Immunol. 155:4072-4077. See also, Good, <i>et al.</i> (2004) Immunol. Rev. 201:254-267; Good, <i>et al.</i> (2004) Ann. Rev. Immunol. 23:69-99. MSP2 (see, e.g., GenBank Acc. No. X96399; X96397). MSP1 (see, e.g., GenBank Acc. No. X03371). RESA (see, e.g., GenBank Acc. No. X05181; X05182).
Apical membrane antigen 1 (AMA1).	See, e.g., Gupta, <i>et al.</i> (2005) Protein Expr. Purif. 41:186-198. AMA1 (see, e.g., GenBank Acc. No. A`13; AJ494905; AJ490565).
Viruses and viral antigens	
Hepatitis A	GenBank Acc. Nos., e.g., NC_001489; AY644670; X83302; K02990; M14707.
Hepatitis B	Complete genome (see, e.g., GenBank Acc. Nos. AB214516; NC_003977; AB205192; AB205191; AB205190; AJ748098; AB198079; AB198078; AB198076; AB074756).
Hepatitis C	Complete genome (see, e.g., GenBank Acc. Nos. NC_004102; AJ238800; AJ238799; AJ132997; AJ132996; AJ000009; D84263).
Hepatitis D	GenBank Acc. Nos, e.g. NC_001653; AB118847; AY261457.
Human papillomavirus, including all 200+ subtypes (classed in 16 groups), such as the high risk subtypes 16,	See, e.g., Trimble, <i>et al.</i> (2003) Vaccine 21:4036-4042; Kim, <i>et al.</i> (2004) Gene Ther. 11:1011-1018; Simon, <i>et al.</i> (2003) Eur. J. Obstet. Gynecol. Reprod. Biol. 109:219-223; Jung, <i>et al.</i> (2004) J. Microbiol. 42:255-266; Damasus-Awatai and Freeman-Wang (2003) Curr. Opin. Obstet. Gynecol. 15:473-477; Jansen and Shaw (2004) Annu. Rev.

Antigen	Reference
18, 30, 31, 33, 45.	Med. 55:319-331; Roden and Wu (2003) Expert Rev. Vaccines 2:495-516; de Villiers, <i>et al.</i> (2004) Virology 324:17-24; Hussain and Paterson (2005) Cancer Immunol. Immunother. 54:577-586; Molijn, <i>et al.</i> (2005) J. Clin. Virol. 32 (Suppl. 1) S43-S51. GenBank Acc. Nos. AY686584; AY686583; AY686582; NC_006169; NC_006168; NC_006164; NC_001355; NC_001349; NC_005351; NC_001596).
Human T-cell lymphotropic virus (HTLV) types I and II, including the HTLV type I subtypes Cosmopolitan, Central African, and Austro-Melanesian, and the HTLV type II subtypes Iia, Iib, Iic, and Iid.	See, e.g., Capdepon, <i>et al.</i> (2005) AIDS Res. Hum. Retrovirus 21:28-42; Bhigjee, <i>et al.</i> (1999) AIDS Res. Hum. Restrovirus 15:1229-1233; Vandamme, <i>et al.</i> (1998) J. Virol. 72:4327-4340; Vallejo, <i>et al.</i> (1996) J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 13:384-391. HTLV type I (see, e.g., GenBank Acc. Nos. AY563954; AY563953. HTLV type II (see, e.g., GenBank Acc. Nos. L03561; Y13051; AF139382).
Coronaviridae, including Coronaviruses, such as SARS-coronavirus (SARS-CoV), and Toroviruses.	See, e.g., Brian and Baric (2005) Curr. Top. Microbiol. Immunol. 287:1-30; Gonzalez, <i>et al.</i> (2003) Arch. Virol. 148:2207-2235; Smits, <i>et al.</i> (2003) J. Virol. 77:9567-9577; Jamieson, <i>et al.</i> (1998) J. Infect. Dis. 178:1263-1269 (GenBank Acc. Nos. AY348314; NC_004718; AY394850).
Rubella virus.	GenBank Acc. Nos. NC_001545; AF435866.
Mumps virus, including the genotypes A, C, D, G, H, and I.	See, e.g., Orvell, eta l. (2002) J. Gen. Virol. 83:2489-2496. See, e.g., GenBank Acc. Nos. AY681495; NC_002200; AY685921; AF201473.

Antigen	Reference
Coxsackie virus A including the serotypes 1, 11, 13, 15, 17, 18, 19, 20, 21, 22, and 24 (also known as Human enterovirus C; HEV-C).	See, e.g., Brown, <i>et al.</i> (2003) J. Virol. 77:8973-8984. GenBank Acc. Nos. AY421768; AY790926: X67706.
Coxsackie virus B, including subtypes 1-6.	See, e.g., Ahn, <i>et al.</i> (2005) J. Med. Virol. 75:290-294; Patel, <i>et al.</i> (2004) J. Virol. Methods 120:167-172; Rezig, <i>et al.</i> (2004) J. Med. Virol. 72:268-274. GenBank Acc. No. X05690.
Human enteroviruses including, e.g., human enterovirus A (HEV-A, CAV2 to CAV8, CAV10, CAV12, CAV14, CAV16, and EV71) and also including HEV-B (CAV9, CBV1 to CBV6, E1 to E7, E9, E11 to E21, E24 to E27, E29 to E33, and EV69 and E73), as well as HEV.	See, e.g., Oberste, <i>et al.</i> (2004) J. Virol. 78:855-867. Human enterovirus A (GenBank Acc. Nos. NC_001612); human enterovirus B (NC_001472); human enterovirus C (NC_001428); human enterovirus D (NC_001430). Simian enterovirus A (GenBank Acc. No. NC_003988).
Polioviruses including PV1, PV2, and PV3.	See, e.g., He, <i>et al.</i> (2003) J. Virol. 77:4827-4835; Hahsido, <i>et al.</i> (1999) Microbiol. Immunol. 43:73-77. GenBank Acc. No. AJ132961 (type 1); AY278550 (type 2); X04468 (type 3).
Viral encephalitides viruses, including equine encephalitis,	See, e.g., Hoke (2005) Mil. Med. 170:92-105; Estrada-Franco, <i>et al.</i> (2004) Emerg. Infect. Dis. 10:2113-2121; Das, <i>et al.</i> (2004) Antiviral Res. 64:85-92; Aguilar, <i>et al.</i> (2004) Emerg. Infect. Dis. 10:880-888;

Antigen	Reference
Venezuelan equine encephalitis (VEE) (including subtypes IA, IB, IC, ID, IIC, IID), Eastern equine encephalitis (EEE), Western equine encephalitis (WEE), St. Louis encephalitis, Murray Valley (Australian) encephalitis, Japanese encephalitis, and tick-born encephalitis.	Weaver, <i>et al.</i> (2004) Arch. Virol. Suppl. 18:43-64; Weaver, <i>et al.</i> (2004) Annu. Rev. Entomol. 49:141-174. Eastern equine encephalitis (GenBank Acc. No. NC_003899; AY722102); Western equine encephalitis (NC_003908).
Human herpesviruses, including cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus-1 (HHV-1), HHV-2, HHV-3, HHV-4, HHV-5, HHV-6, HHV-7, HHV-8, herpes B virus, herpes simplex virus types 1 and 2 (HSV-1, HSV-2), and varicella zoster virus (VZV).	See, e.g., Studahl, <i>et al.</i> (2000) Scand. J. Infect. Dis. 32:237-248; Padilla, <i>et al.</i> (2003) J. Med. Virol. 70 (Suppl. 1) S103-S110; Jainkittivong and Langlais (1998) Oral Surg. Oral Med. 85:399-403. GenBank Nos. NC_001806 (herpesvirus 1); NC_001798 (herpesvirus 2); X04370 and NC_001348 (herpesvirus 3); NC_001345 (herpesvirus 4); NC_001347 (herpesvirus 5); X83413 and NC_000898 (herpesvirus 6); NC_001716 (herpesvirus 7). Human herpesviruses types 6 and 7 (HHV-6; HHV-7) are disclosed by, e.g., Padilla, <i>et al.</i> (2003) J. Med. Virol. 70 (Suppl. 1)S103-S110. Human herpesvirus 8 (HHV-8), including subtypes A-E, are disclosed in, e.g., Treurnicht, <i>et al.</i> (2002) J. Med. Virul. 66:235-240.
HIV-1 including group M (including subtypes	See, e.g., Smith, <i>et al.</i> (1998) J. Med. Virol. 56:264-268. See also, e.g., GenBank Acc. Nos. DQ054367; NC_001802; AY968312;

Antigen	Reference
A to J) and group O (including any distinguishable subtypes) (HIV-2, including subtypes A-E.	DQ011180; DQ011179; DQ011178; DQ011177; AY588971; AY588970; AY781127; AY781126; AY970950; AY970949; AY970948; X61240; AJ006287; AJ508597; and AJ508596.
Epstein-Barr virus (EBV), including subtypes A and B.	See, e.g., Peh, <i>et al.</i> (2002) Pathology 34:446-450. Epstein-Barr virus strain B95-8 (GenBank Acc. No. V01555).
Reovirus, including serotypes and strains 1, 2, and 3, type 1 Lang, type 2 Jones, and type 3 Dearing.	See, e.g., Barthold, <i>et al.</i> (1993) Lab. Anim. Sci. 43:425-430; Roner, <i>et al.</i> (1995) Proc. Natl. Acad. Sci. USA 92:12362-12366; Kedl, <i>et al.</i> (1995) J. Virol. 69:552-559. GenBank Acc. No. K02739 (sigma-3 gene surface protein).
Cytomegalovirus (CMV) subtypes include CMV subtypes I-VII.	See, e.g., Chern, <i>et al.</i> (1998) J. Infect. Dis. 178:1149-1153; Vilas Boas, <i>et al.</i> (2003) J. Med. Virol. 71:404-407; Trincado, <i>et al.</i> (2000) J. Med. Virol. 61:481-487. GenBank Acc. No. X17403.
Rhinovirus, including all serotypes.	Human rhinovirus 2 (GenBank Acc. No. X02316); Human rhinovirus B (GenBank Acc. No. NC_001490); Human rhinovirus 89 (GenBank Acc. No. NC_001617); Human rhinovirus 39 (GenBank Acc. No. AY751783).
Adenovirus, including all serotypes.	AY803294; NC_004001; AC_000019; AC_000018; AC_000017; AC_000015; AC_000008; AC_000007; AC_000006; AC_000005; AY737798; AY737797; NC_003266; NC_002067; AY594256; AY594254; AY875648; AJ854486; AY163756; AY594255; AY594253; NC_001460; NC_001405; AY598970; AY458656;

Antigen	Reference
	AY487947; NC_001454; AF534906; AY45969; AY128640; L19443; AY339865; AF532578.
Filoviruses, including Marburg virus and Ebola virus, and strains such as Ebola-Sudan (EBO-S), Ebola-Zaire (EBO-Z), and Ebola-Reston (EBO-R).	See, e.g., Geisbert and Jahrling (1995) <i>Virus Res.</i> 39:129-150; Hutchinson, <i>et al.</i> (2001) <i>J. Med. Virol.</i> 65:561-566. Marburg virus (see, e.g., GenBank Acc. No. NC_001608). Ebola virus (see, e.g., GenBank Acc. Nos. NC_006432; AY769362; NC_002549; AF272001; AF086833).
Arenaviruses, including lymphocytic choriomeningitis (LCM) virus, Lassa virus, Junin virus, and Machupo virus.	Junin virus, segment S (GenBank Acc. No. NC_005081); Junin virus, segment L (GenBank Acc. No. NC_005080).
Rabies virus.	See, e.g., GenBank Acc. Nos. NC_001542; AY956319; AY705373; AF499686; AB128149; AB085828; AB009663.
Arboviruses, including West Nile virus, Dengue viruses 1 to 4, Colorado tick fever virus, Sindbis virus, Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae, Rhabdoviridae, Orthomyxoviridae, and the like.	Dengue virus type 1 (see, e.g., GenBank Acc. Nos. AB195673; AY762084). Dengue virus type 2 (see, e.g., GenBank Acc. Nos. NC_001474; AY702040; AY702039; AY702037). Dengue virus type 3 (see, e.g., GenBank Acc. Nos. AY923865; AT858043). Dengue virus type 4 (see, e.g., GenBank Acc. Nos. AY947539; AY947539; AF326573). Sindbis virus (see, e.g., GenBank Acc. Nos. NC_001547; AF429428; J02363; AF103728). West Nile virus (see, e.g., GenBank Acc. Nos. NC_001563; AY603654).

Antigen	Reference
Poxvirus including orthopoxvirus (variola virus, monkeypox virus, vaccinia virus, cowpox virus), yatapoxvirus (tanapox virus, Yaba monkey tumor virus), parapoxvirus, and molluscipoxvirus.	Viriola virus (see, e.g., GenBank Acc. Nos. NC_001611; Y16780; X72086; X69198).
Yellow fever.	See, e.g., GenBank Acc. No. NC_002031; AY640589; X03700.
Hantaviruses, including serotypes Hantaan (HTN), Seoul (SEO), Dobrava (DOB), Sin Nombre (SN), Puumala (PUU), and Dobrava-like Saaremaa (SAAV).	See, e.g., Elgh, <i>et al.</i> (1997) J. Clin. Microbiol. 35:1122-1130; Sjolander, <i>et al.</i> (2002) Epidemiol. Infect. 128:99-103; Zeier, <i>et al.</i> (2005) Virus Genes 30:157-180. GenBank Acc. No. NC_005222 and NC_005219 (Hantavirus). See also, e.g., GenBank Acc. Nos. NC_005218; NC_005222; NC_005219.
Flaviviruses, including Dengue virus, Japanese encephalitis virus, West Nile virus, and yellow fever virus.	See, e.g., Mukhopadhyay, <i>et al.</i> (2005) Nature Rev. Microbiol. 3:13-22. GenBank Acc. Nos NC_001474 and AY702040 (Dengue). GenBank Acc. Nos. NC_001563 and AY603654.
Measles virus.	See, e.g., GenBank Acc. Nos. AB040874 and AY486084.
Human parainfluenzaviruses (HPV), including HPV	Human parainfluenza virus 2 (see, e.g., GenBank Acc. Nos. AB176531; NC003443). Human parainfluenza virus 3 (see, e.g., GenBank Acc. No. NC_001796).

Antigen	Reference
types 1-56.	
Influenza virus, including influenza virus types A, B, and C.	Influenza nucleocapsid (see, e.g., GenBank Acc. No. AY626145). Influenza hemagglutinin (see, e.g., GenBank Acc. Nos. AY627885; AY555153). Influenza neuraminidase (see, e.g., GenBank Acc. Nos. AY555151; AY577316). Influenza matrix protein 2 (see, e.g., GenBank Acc. Nos. AY626144). Influenza basic protein 1 (see, e.g., GenBank Acc. No. AY627897). Influenza polymerase acid protein (see, e.g., GenBank Acc. No. AY627896). Influenza nucleoprotein (see, e.g., GenBank Acc. Nno. AY627895).
Influenza A virus subtypes, e.g., swine viruses (SIV): H1N1 influenzaA and swine influenza virus.	Hemagglutinin of H1N1 (GenBank Acc. No. S67220). Influenza A virus matrix protein (GenBank Acc. No. AY700216). Influenza virus A H5H1 nucleoprotein (GenBank Acc. No. AY646426). H1N1 haemagglutinin (GenBank Acc. No. D00837). See also, GenBank Acc. Nos. BD006058; BD006055; BD006052. See also, e.g., Wentworth, <i>et al.</i> (1994) J. Virol. 68:2051-2058; Wells, <i>et al.</i> (1991) J.A.M.A. 265:478-481.
Respiratory syncytial virus (RSV), including subgroup A and subgroup B.	Respiratory syncytial virus (RSV) (see, e.g., GenBank Acc. Nos. AY353550; NC_001803; NC001781).
Rotaviruses, including human rotaviruses A to E, bovine rotavirus, rhesus monkey rotavirus, and human-RVV reassortments.	Human rotavirus C segment 8 (GenBank Acc. No. AJ549087); Human rotavirus G9 strain outer capsid protein (see, e.g., GenBank Acc. No. DQ056300); Human rotavirus B strain non-structural protein 4 (see, e.g., GenBank Acc. No. AY548957); human rotavirus A strain major inner capsid protein (see, e.g., GenBank Acc. No. AY601554).

Antigen	Reference
Polyomavirus, including simian virus 40 (SV40), JC virus (JCV) and BK virus (BKV).	See, e.g., Engels, <i>et al.</i> (2004) <i>J. Infect. Dis.</i> 190:2065-2069; Vilchez and Butel (2004) <i>Clin. Microbiol. Rev.</i> 17:495-508; Shivapurkar, <i>et al.</i> (2004) <i>Cancer Res.</i> 64:3757-3760; Carbone, <i>et al.</i> (2003) <i>Oncogene</i> 2:5173-5180; Barbanti-Brodano, <i>et al.</i> (2004) <i>Virology</i> 318:1-9 (SV40 complete genome in, e.g., GenBank Acc. Nos. NC_001669; AF168994; AY271817; AY271816; AY120890; AF345344; AF332562).
Coltivirus, including Colorado tick fever virus, Eyach virus.	Attoui, <i>et al.</i> (1998) <i>J. Gen. Virol.</i> 79:2481-2489. Segments of Eyach virus (see, e.g., GenBank Acc. Nos. <u>AF282475</u> ; <u>AF282472</u> ; <u>AF282473</u> ; <u>AF282478</u> ; <u>AF282476</u> ; <u>NC_003707</u> ; <u>NC_003702</u> ; <u>NC_003703</u> ; <u>NC_003704</u> ; <u>NC_003705</u> ; <u>NC_003696</u> ; <u>NC_003697</u> ; <u>NC_003698</u> ; <u>NC_003699</u> ; <u>NC_003701</u> ; <u>NC_003706</u> ; <u>NC_003700</u> ; <u>AF282471</u> ; <u>AF282477</u>).
Calciviruses, including the genogroups Norwalk, Snow Mountain group (SMA), and Saaporo.	Snow Mountain virus (see, e.g., GenBank Acc. No. AY134748).
Parvoviridae, including dependovirus, parvovirus (including parvovirus B19), and erythrovirus.	See, e.g., Brown (2004) <i>Dev. Biol. (Basel)</i> 118:71-77; Alvarez-Lafuente, <i>et al.</i> (2005) <i>Ann. Rheum. Dis.</i> 64:780-782; Ziyaeyan, <i>et al.</i> (2005) <i>Jpn. J. Infect. Dis.</i> 58:95-97; Kaufman, <i>et al.</i> (2005) <i>Virology</i> 332:189-198.

Other organisms for which suitable antigens are known in the art include, but are not limited to, *Chlamydia trachomatis*, *Streptococcus pyogenes* (Group A Strep), *Streptococcus agalactia* (Group B Strep), *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Vibrio cholerae*, *Salmonella* species (including *typhi*, *typhimurium*), enterica

(including *Helicobacter pylori*, *Shigella flexneri* and other Group D *shigella* species), *Burkholderia mallei*, *Burkholderia pseudomallei*, *Klebsiella pneumoniae*, *Clostridium* species (including *C. difficile*), *Vibrio parahaemolyticus* and *V. vulnificus*. This list is not meant to be limiting.

Pharmaceutical Compositions

[00355] The term “pharmaceutical” as used herein refers to a chemical substance intended for use in the cure, treatment, or prevention of disease and which is subject to an approval process by the U.S. Food and Drug Administration (or a non-U.S. equivalent thereof) as a prescription or over-the-counter drug product. Details on techniques for formulation and administration of such compositions may be found in Remington, The Science and Practice of Pharmacy 21st Edition (Mack Publishing Co., Easton, PA) and Nielloud and Marti-Mestres, *Pharmaceutical Emulsions and Suspensions: 2nd Edition* (Marcel Dekker, Inc, New York).

[00356] For the purposes of this disclosure, the pharmaceutical compositions may be administered by a variety of means including non-parenterally, parenterally, by inhalation spray, topically, or rectally in formulations containing pharmaceutically acceptable carriers, adjuvants and vehicles. "Non-parenteral administration" encompasses oral, buccal, sublingual, topical, transdermal, ophthalmic, otic, nasal, rectal, cervical, pulmonary, mucosal, and vaginal routes. The term parenteral as used here includes but is not limited to subcutaneous, intravenous, intramuscular, intraarterial, intradermal, intrathecal and epidural injections with a variety of infusion techniques. Intraarterial and intravenous injection as used herein includes administration through catheters. Administration via intracoronary stents and intracoronary reservoirs is also contemplated. Intra-tumoral (directly into the tumor mass) or peri-tumoral (around the tumor mass) administration of the compounds of the present invention may directly activate locally infiltrating DC, directly promote tumor cell apoptosis or sensitize tumor cells to cytotoxic agents. The term oral as used herein includes, but is not limited to oral ingestion, or delivery by a sublingual or buccal route. Oral administration includes fluid drinks, energy bars, as well as pill formulations.

[00357] Pharmaceutical compositions may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges,

aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing a drug compound in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as starch, gelatin or acacia; and lubricating agents; such as magnesium stearate, stearic acid or talc. Tablets may be uncoated, or may be coated by known techniques including enteric coating, colonic coating, or microencapsulation to delay disintegration and adsorption in the gastrointestinal tract and/or provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

[00358] Formulations for oral use may be also presented as hard gelatin capsules where the drug compound is mixed with an inert solid diluent, for example calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

[00359] Pharmaceutical compositions may be formulated as aqueous suspensions in admixture with excipients suitable for the manufacture of aqueous-suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxy-benzoate, one or

more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose or saccharin.

[00360] Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

[00361] Dispersible powders and granules of the disclosure suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

[00362] The pharmaceutical compositions of the disclosure may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents.

[00363] Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

[00364] The pharmaceutical compositions of the disclosure may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent such as a solution in

1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

[00365] The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 20 to 500 mg of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions. It is preferred that the pharmaceutical composition be prepared which provides easily measurable amounts for administration. Typically, an effective amount to be administered systemically is about 0.1 mg/kg to about 100 mg/kg and depends upon a number of factors including, for example, the age and weight of the subject (e.g., a mammal such as a human), the precise condition requiring treatment and its severity, the route of administration, and will ultimately be at the discretion of the attendant physician or veterinarian. It will be understood, however, that the specific dose level for any particular patient will depend on a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex and diet of the individual being treated; the time and route of administration; the rate of excretion; other drugs which have previously been administered; and the severity of the particular condition undergoing therapy, as is well understood by those skilled in the art.

[00366] As noted above, formulations of the disclosure suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient, as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The pharmaceutical compositions may also be administered as a bolus, electuary or paste.

[00367] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a

suitable machine the active ingredient in a free flowing form such as a powder or granules, optionally mixed with a binder (e.g., povidone, gelatin, hydroxypropyl ethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface active or dispersing agent. Molded tablets may be made in a suitable machine using a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropyl methylcellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric or colonic coating to provide release in parts of the gut other than the stomach. This is particularly advantageous with the LNA-CDN compounds as described herein when such compounds are susceptible to acid hydrolysis.

[00368] Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

[00369] Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

[00370] Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

[00371] Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, 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. Injection solutions and

suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

[00372] When a disclosed compound or its salt is named or depicted by structure, it is to be understood that the compound or salt, including solvates (particularly, hydrates) thereof, may exist in crystalline forms, non-crystalline forms or a mixture thereof. The compound or salt, or solvates (particularly, hydrates) thereof, may also exhibit polymorphism (i.e. the capacity to occur in different crystalline forms). These different crystalline forms are typically known as “polymorphs.” It is to be understood that when named or depicted by structure, the disclosed compound, or solvates (particularly, hydrates) thereof, also include all polymorphs thereof. Polymorphs have the same chemical composition but differ in packing, geometrical arrangement, and other descriptive properties of the crystalline solid state. Polymorphs may have different physical properties such as density, shape, hardness, stability, and dissolution properties. Polymorphs typically exhibit different melting points, IR spectra, and X-ray powder diffraction patterns, which may be used for identification. One of ordinary skill in the art will appreciate that different polymorphs may be produced, for example, by changing or adjust the conditions used during the crystallization or recrystallization of the compound.

[00373] For solvates of compounds of this invention, or salts thereof, that are in crystalline form, the skilled artisan will appreciate that pharmaceutically acceptable solvates may be formed wherein solvent molecules are incorporated into the crystalline lattice during crystallization. Solvates may involve nonaqueous solvents such as ethanol, isopropanol, dimethyl sulfoxide, acetic acid, ethanolamine, and ethyl acetate, or they may involve water as the solvent that is incorporated into the crystalline lattice. Solvates wherein water is the solvent that is incorporated into the crystalline lattice are typically referred to as “hydrates.” Hydrates include stoichiometric hydrates as well as compositions containing variable amounts of water. The invention includes all such solvates.

[00374] Because of their potential use in medicine, the salts of the compounds of this invention are preferably pharmaceutically acceptable. Suitable pharmaceutically acceptable salts include those described by P. Heinrich Stahl and Camille G. Wermuth in Handbook of Pharmaceutical Salts: Properties, Selection, and Use, 2nd ed. (Wiley-VCH: 2011) and also Remington's Pharmaceutical Sciences, 18th ed. (Mack Publishing, Easton

PA: 1990) and also Remington: The Science and Practice of Pharmacy, 19th ed. (Mack Publishing, Easton PA: 1995). Salt encompassed within the term “pharmaceutically acceptable salts” refer to non-toxic salts of the compounds in this invention.

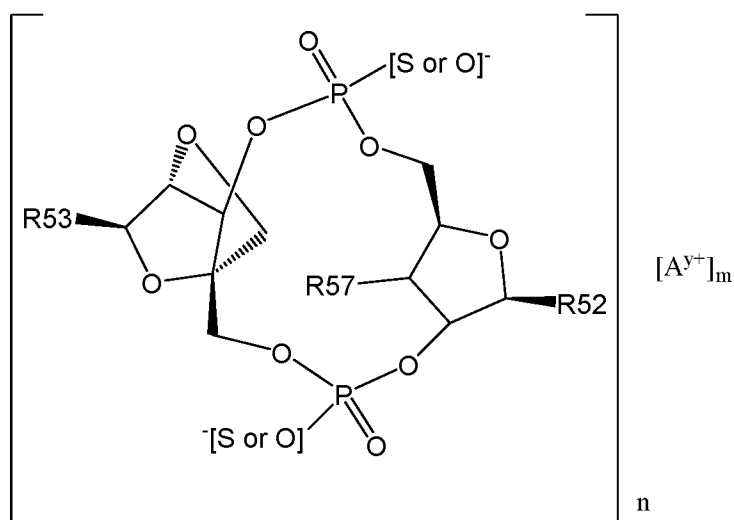
[00375] Salts of the compounds of this invention containing a basic amine or other basic functional group may be prepared by any suitable method known in the art, including treatment of the free bases with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like, or with an organic acid, such as acetic acid, trifluoroacetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, formic acid, alginic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, pyranosildyl acid, such as glucuronic acid or galacturonic acid, alphahydroxy acid, such as citric acid or tartaric acid, amino acid, such as aspartic acid or glutamic acid, aromatic acid, such as benzoic acid or cinnamic acid, sulfonic acid, such as p-toluenesulfonic acid, methanesulfonic acid, ethanesulfonic acid or the like. Examples of pharmaceutically acceptable salts include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrate, caproates, heptanoates, propiolates, oxalates, malonates succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, phenylacetates, phenylpropionates, phenylbutrates, citrates, lactates, glycolate, resinate, lactates, camsylates, tartrates, mandelates, and sulfonates, such as xylenesulfonates, methanesulfonates, propanesulfonates, naphthalene-1-sulfonates and naphthalene-2-sulfonates.

[00376] Salts of the compounds of this invention containing a phosphate diester, phosphorothioate diester or other acidic functional group can be prepared by reacting with a suitable base. Pharmaceutically acceptable salts include, but are not limited to: pyridine, ammonium, piperazine, diethylamine, nicotinamide, formic, urea, sodium, potassium, calcium, magnesium, zinc, lithium, cinnamic, methylamino, methanesulfonic, picric, tartaric, triethylamino, dimethylamino, and tris(hydroxymethyl)aminomethane. Additional pharmaceutically acceptable salts are known to those skilled in the art.

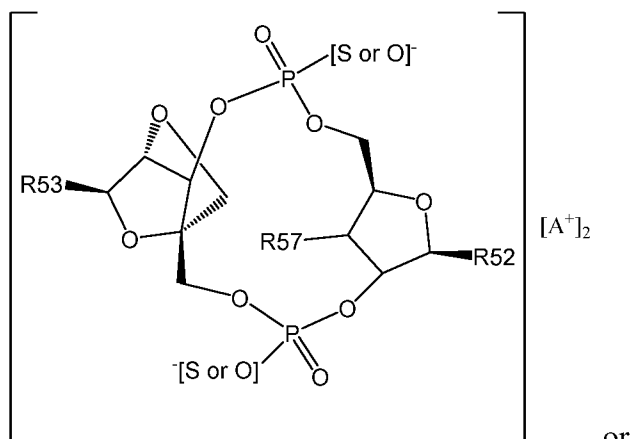
[00377] Such a pharmaceutically acceptable salt may be made with a base which affords a pharmaceutically acceptable cation, which includes alkali metal salts (especially

sodium and potassium), alkaline earth metal salts (especially calcium and magnesium), aluminum salts and ammonium salts, zinc, as well as salts made from physiologically acceptable organic bases such as diethylamine, isopropylamine, olamine, benzathine, benethamine, tromethamine (2-amino-2-(hydroxymethyl)propane-1,3-diol), morpholine, epolamine, piperidine, piperazine, picoline, dicyclohexylamine, N,N'-dibenzylethylenediamine, 2-hydroxyethylamine, tri-(2-hydroxyethyl)amine, chloroprocaine, choline, deanol, imidazole, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), procaine, dibenzylpiperidine, dehydroabietylamine, glucamine, collidine, quinine, quinolone, erbumine and basic amino acids such as lysine and arginine.

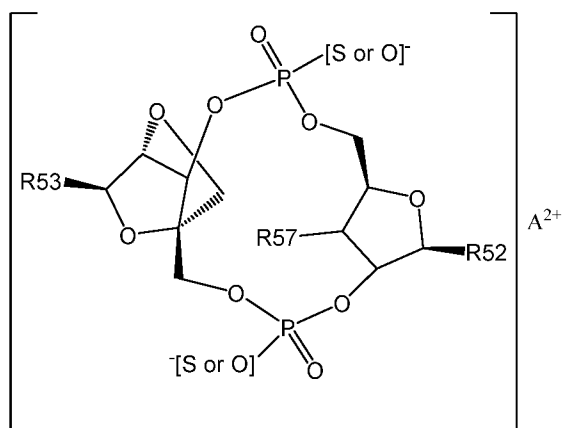
[00378] The LNA-CDN compounds as described herein that include salts thereof can be described by structures wherein the -SH or -OH in the phosphate or thiophosphate bond (i.e. R3 or R4 of the compounds of Formula I as described herein) are represented as -S⁻ or -O⁻ with a corresponding cation to form salts of the compounds as described herein. For example, salts of compounds of Formula IVa as described herein where R54 and R55 are -OH or -SH can be represented by the following structures:



wherein A^{y+} represents a mono or polyvalent salt cation, and n and m are the lowest possible whole number for a given y . For example when A^{y+} is monovalent, i.e. when y is 1, such as Na^+ , K^+ , NH_4^+ , $TEAH^+$ or the like, n is 1 and m is 2; when y is 2, such as Ca^{2+} , Mg^{2+} and the like, n is 1 and m is 1; when y is 3, e.g. Al^{3+} or the like, n is 3 and m is 2. For example, salts of a monovalent or divalent salt cation can be represented as

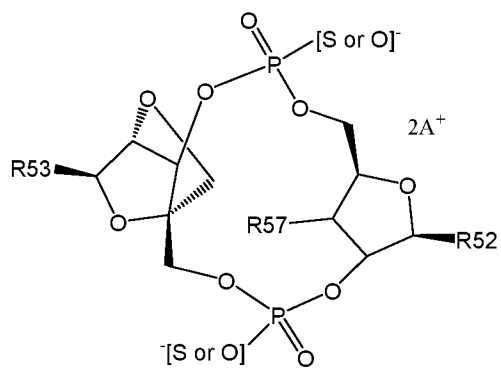


or

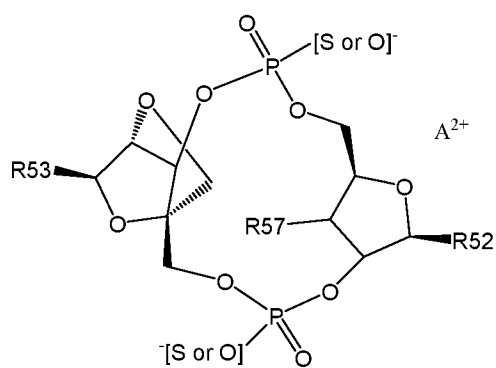


, respectively, or in cases where n = 1, these

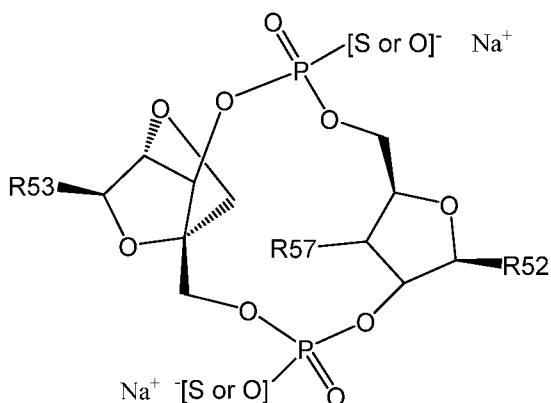
can be represented without brackets, e.g. as



or

Alternatively, monovalent salts can be depicted with A⁺ adjacent each of the -S⁻ or -O⁻.

For example, the sodium salt of above compounds of Formula IVa can be depicted as



[00379] Other non-pharmaceutically acceptable salts, e.g. trifluoroacetate or triethylammonium may be used, for example in the isolation of compounds of the invention, and are included within the scope of this invention.

[00380] The invention includes within its scope all possible stoichiometric and non-stoichiometric forms of the salts of the compounds of this invention.

[00381] If a compound of this invention containing a basic amine or other basic functional group is isolated as a salt, the corresponding free base form of that compound may be prepared by any suitable method known to the art, including treatment of the salt with an inorganic or organic base, suitably an inorganic or organic base having a higher pK_a than the free base form of the compound. Similarly, if a compound of this invention containing a phosphate diester, phosphorothioate diester or other acidic functional group is isolated as a salt, the corresponding free acid form of that compound may be prepared by any suitable method known to the art, including treatment of the salt with an inorganic or organic acid, suitably an inorganic or organic acid having a lower pK_a than the free acid form of the compound.

[00382] An effective amount of a LNA-CDN compound, or a pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof as described herein, for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the route and dose of administration and the severity of side effects. Guidance for methods of treatment and diagnosis is available (see, e.g., Maynard, et al. (1996) A Handbook of SOPs for Good

Clinical Practice, Interpharm Press, Boca Raton, FL; Dent (2001) Good Laboratory and Good Clinical Practice, Urch Publ., London, UK).

[00383] An effective amount may be given in one dose, but is not restricted to one dose. Thus, the administration can be two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more, administrations of a pharmaceutical composition comprising the LNA-CDN compound, or a pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof as described herein. Where there is more than one administration of a pharmaceutical composition in the present methods, the administrations can be spaced by time intervals of one minute, two minutes, three, four, five, six, seven, eight, nine, ten, or more minutes, by intervals of about one hour, two hours, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, and so on. In the context of hours, the term “about” means plus or minus any time interval within 30 minutes. The administrations can also be spaced by time intervals of one day, two days, three days, four days, five days, six days, seven days, eight days, nine days, ten days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, and combinations thereof. The invention is not limited to dosing intervals that are spaced equally in time, but encompass doses at non-equal intervals.

[00384] A dosing schedule of, for example, once/week, twice/week, three times/week, four times/week, five times/week, six times/week, seven times/week, once every two weeks, once every three weeks, once every four weeks, once every five weeks, and the like, is available for the invention. The dosing schedules encompass dosing for a total period of time of, for example, one week, two weeks, three weeks, four weeks, five weeks, six weeks, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, and twelve months.

[00385] Provided are cycles of the above dosing schedules. The cycle can be repeated about, e.g., every seven days; every 14 days; every 21 days; every 28 days; every 35 days; 42 days; every 49 days; every 56 days; every 63 days; every 70 days; and the like. An interval of non dosing can occur between a cycle, where the interval can be about, e.g., seven days; 14 days; 21 days; 28 days; 35 days; 42 days; 49 days; 56 days; 63 days; 70 days; and the like. In this context, the term “about” means plus or minus one day, plus or

minus two days, plus or minus three days, plus or minus four days, plus or minus five days, plus or minus six days, or plus or minus seven days.

[00386] Methods for co-administration with an additional therapeutic agent are well known in the art (Hardman, et al. (eds.) (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill, New York, NY; Poole and Peterson (eds.) (2001) Pharmacotherapeutics for Advanced Practice: A Practical Approach, Lippincott, Williams & Wilkins, Phila., PA; Chabner and Longo (eds.) (2001) Cancer Chemotherapy and Biotherapy, Lippincott, Williams & Wilkins, Phila., PA). Generally, co-administration or administration together indicates treating a subject with two or more agents, where the agents can be administered simultaneously or at different times. For example, such agents may be delivered to a single subject as separate administrations, which may be at essentially the same time or different times, and which may be by the same route or different routes of administration. Such agents may be delivered to a single subject in the same administration (e.g. same formulation) such that they are administered at the same time by the same route of administration.

[00387] As noted, the compositions of the present invention are preferably formulated as pharmaceutical compositions for parenteral or enteral delivery. A typical pharmaceutical composition for administration to an animal subject comprises a pharmaceutically acceptable vehicle such as aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like. See, e.g., Remington's Pharmaceutical Sciences, 15th Ed., Easton ed. , Mack Publishing Co., pp 1405-1412 and 1461- 1487 (1975); The National Formulary XIV, 14th Ed., American Pharmaceutical Association, Washington, DC (1975) . Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, *etc.* Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial agents, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components the pharmaceutical composition are adjusted according to routine skills in the art.

[00388] Repeated administrations of a particular vaccine (homologous boosting) have proven effective for boosting humoral responses. Such an approach may not be effective

at boosting cellular immunity because prior immunity to the vector tends to impair robust antigen presentation and the generation of appropriate inflammatory signals. One approach to circumvent this problem has been the sequential administration of vaccines that use different antigen-delivery systems (heterologous boosting). In a heterologous boosting regimen, at least one prime or boost delivery comprises delivery of the inactivated tumor cell/LNA-CDN compound or compositions thereof described herein. The heterologous arm of the regimen may comprise delivery of antigen using one or more of the following strategies:

inactivated or attenuated bacteria or viruses comprising the antigen of interest, which are particles that have been treated with some denaturing condition to render them ineffective or inefficient in mounting a pathogenic invasion;

purified antigens, which are typically naturally-produced antigens purified from a cell culture of the pathogen or a tissue sample containing the pathogen, or a recombinant version thereof;

live viral or bacterial delivery vectors recombinantly engineered to express and/or secrete antigens in the host cells of the subject. These strategies rely on attenuating (e.g., via genetic engineering) the viral or bacterial vectors to be non-pathogenic and non-toxic;

antigen presenting cell (APC) vectors, such as a dendritic cell (DC) vector, which comprise cells that are loaded with an antigen, or transfected with a composition comprising a nucleic acid encoding the antigen (e.g., Provenge® (Dendreon Corporation) for the treatment of castration-resistant metastatic prostate cancer);

liposomal antigen delivery vehicles; and

naked DNA vectors and naked RNA vectors which may be administered by a gene gun, electroporation, bacterial ghosts, microspheres, microparticles, liposomes, polycationic nanoparticles, and the like.

[00389] A prime vaccine and a boost vaccine can be administered by any one or combination of the following routes. In one aspect, the prime vaccine and boost vaccine are administered by the same route. In another aspect, the prime vaccine and boost

vaccine are administered by different routes. The term “different routes” encompasses, but is not limited to, different sites on the body, for example, a site that is oral, non-oral, enteral, parenteral, rectal, intranode (lymph node), intravenous, arterial, subcutaneous, intramuscular, intratumor, peritumor, intratumor, infusion, mucosal, nasal, in the cerebrospinal space or cerebrospinal fluid, and so on, as well as by different modes, for example, oral, intravenous, and intramuscular.

[00390] An effective amount of a prime or boost vaccine may be given in one dose, but is not restricted to one dose. Thus, the administration can be two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more, administrations of the vaccine. Where there is more than one administration of a vaccine the administrations can be spaced by time intervals of one minute, two minutes, three, four, five, six, seven, eight, nine, ten, or more minutes, by intervals of about one hour, two hours, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, and so on. In the context of hours, the term “about” means plus or minus any time interval within 30 minutes. The administrations can also be spaced by time intervals of one day, two days, three days, four days, five days, six days, seven days, eight days, nine days, ten days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, and combinations thereof. The invention is not limited to dosing intervals that are spaced equally in time, but encompass doses at non-equal intervals, such as a priming schedule consisting of administration at 1 day, 4 days, 7 days, and 25 days, just to provide a non-limiting example.

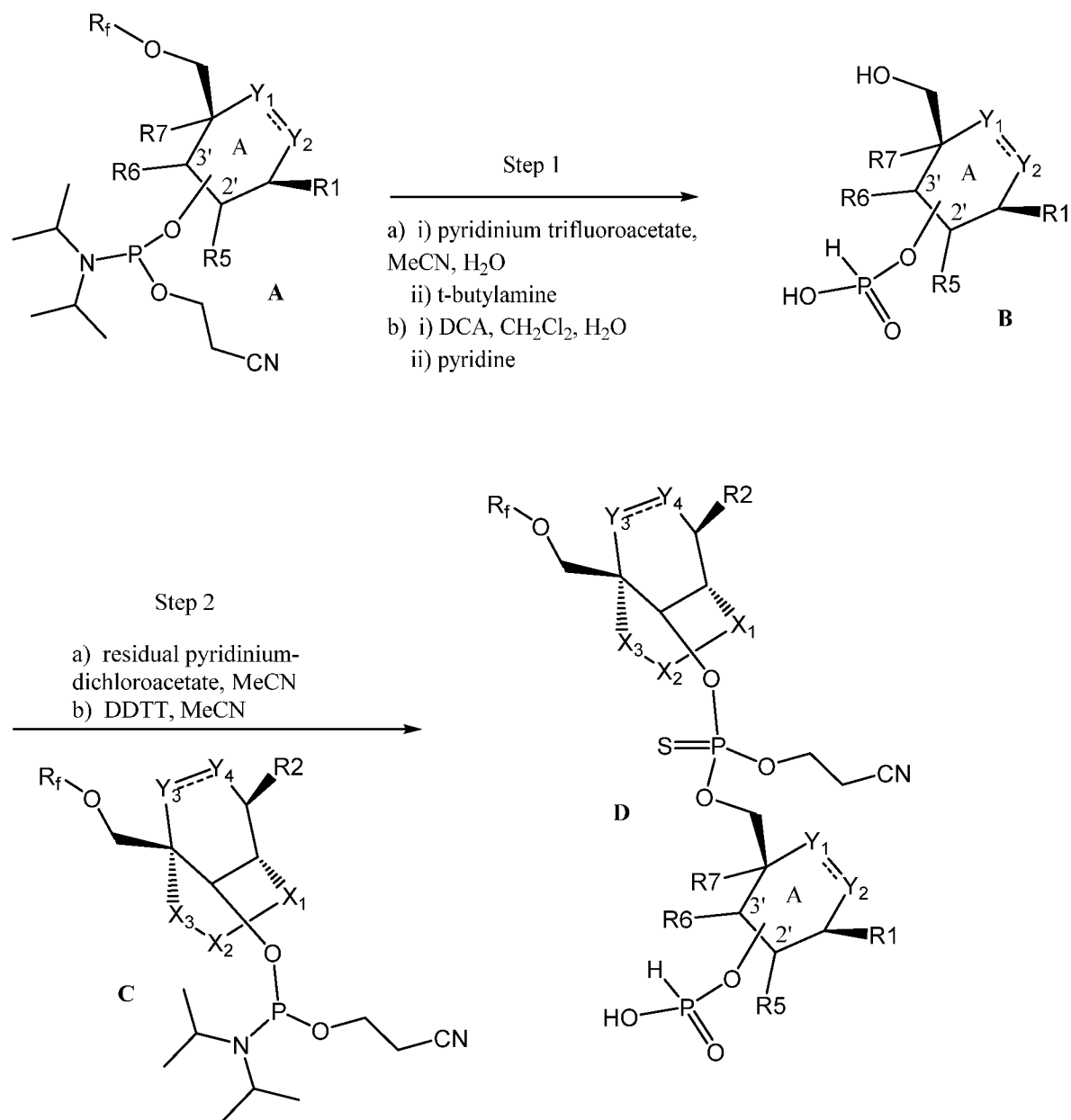
Synthesis of Compounds of Formula I

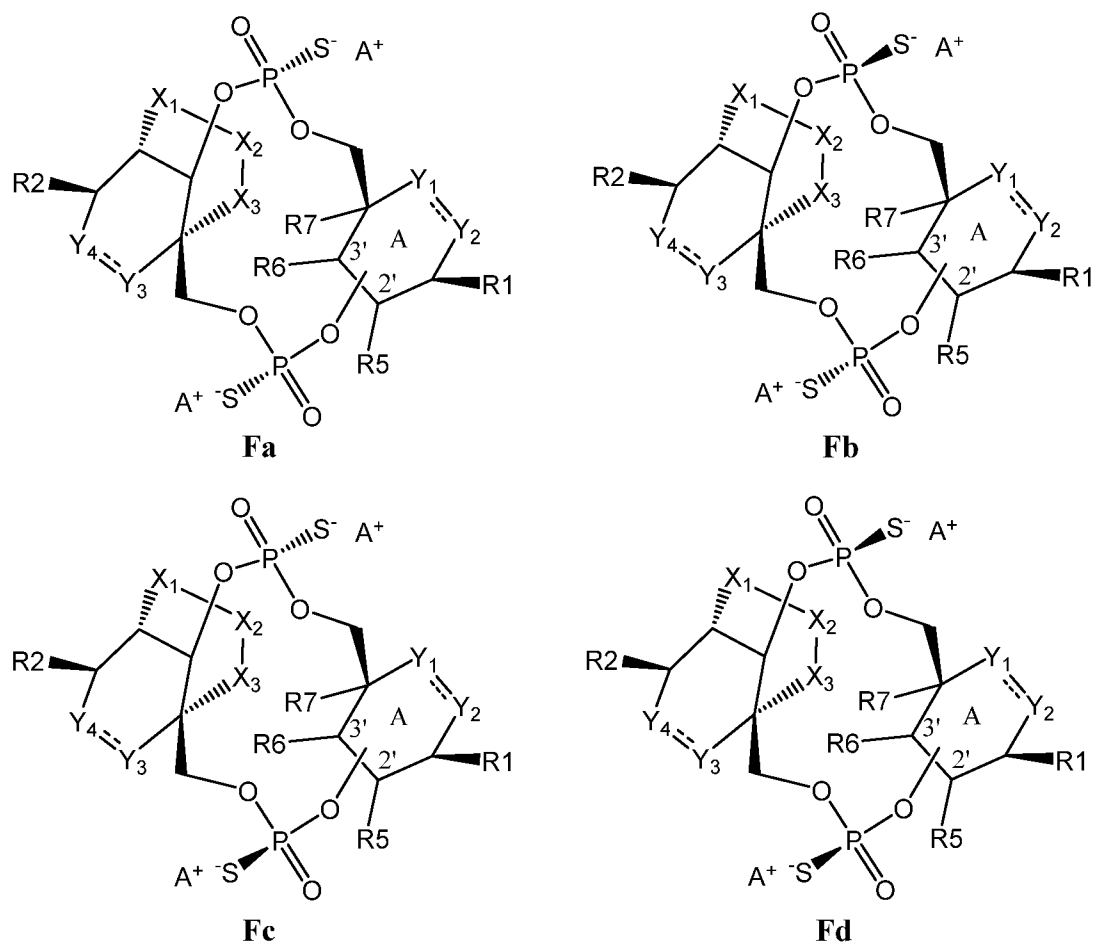
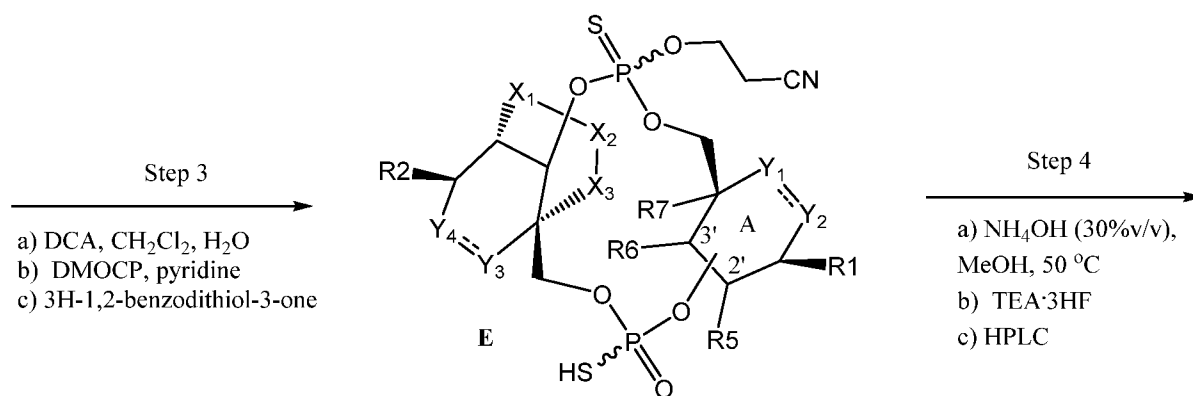
[00391] The compounds of Formula I, and sub-generic formulae thereof, can be prepared by methods readily available to those of skill in the chemical arts, for example, without limitation, as described in the following generic synthetic Scheme I, and for the specific compounds as described in Examples 1-6 below.

[00392] The following Scheme I provides methods for the synthesis of compounds of Formula I wherein R₃ and R₄ are SH, and X₄ is O, represented by compounds Fa, Fb, Fc and Fd in Scheme I, wherein ring A, Y₁, Y₂, Y₃, Y₄, X₁, X₂, X₃, R₁, R₂, R₅, R₆, and R₇ are as defined for Compounds of Formula I, and R_f is a suitable oxygen protecting group (e.g. dimethoxytrityl). It is understood that the compounds represented in Scheme I have

suitable protecting groups as needed, e.g. R1 and R2 include suitably protected bases (e.g. adenine with a nitrogen protecting group on the 6-position amine, guanine with a nitrogen protecting group on the 2-position amine, and the like), or R5 and R6 as OH is a suitably protected (e.g. OTBS).

Scheme I





[00393] Scheme I, Step 1: Compound **B**: Compound **A** (where the oxygen can be bound to either the 2' or 3' carbon of ring A) is dissolved in e.g. MeCN with H₂O (e.g. ~0.6% v/v) and pyridinium trifluoroacetate is added. The reaction mixture is stirred for e.g. 15 min at room temperature and *tert*-butyl amine is added. After e.g. 15 min, the reaction solution is concentrated *in vacuo* and water is removed as an azeotrope after concentration *in vacuo* 3x with MeCN. The resulting material is dissolved in e.g. CH₂Cl₂ and to the resulting solution is added water (e.g. ~1% v/v) and 8 % (v/v) solution of DCA in solvent CH₂Cl₂. After e.g. 10 min of stirring at room temperature, the solution is charged with pyridine. The resulting solution is concentrated *in vacuo* and water is removed as an azeotrope after concentration *in vacuo* with 3x MeCN to provide Compound **B**, which can be left in MeCN for the next step.

[00394] Scheme I, Step 2: Compound **D**: A solution of compound **C** in e.g. MeCN is dried through concentration *in vacuo*. This process is repeated two more times to remove water as an azeotrope. On the last azeotrope, to this solution of compound **C** in MeCN is introduced 3Å molecular sieves and the solution is stored under an atmosphere of nitrogen. To a stirred mixture of compound **B** with residual pyridinium dichloroacetate in MeCN is added the solution of compound **C** in MeCN. After e.g. 1 h, to the stirred mixture is added DDTT, and after e.g. 2 h, the mixture is concentrated *in vacuo* to give compound **D**.

[00395] Scheme I, Step 3: Compound **E**: To a solution of compound **D** in e.g. CH₂Cl₂ is added water (e.g. ~0.6% v/v) and 15 % (v/v) solution of DCA in CH₂Cl₂. After e.g. 10 min at room temperature pyridine is added to the solution, and the resulting solution is concentrated *in vacuo* to a volume of approximately 20 mL. Pyridine is added and the solution is concentrated 2x *in vacuo*, leaving the mixture in approximately 10 mL. To the stirred mixture is added pyridine and DMOCP. After e.g. 20 min, to the solution is added water (e.g. ~1.25 % v/v), followed immediately by the introduction of 3*H*-1,2-benzodithiol-3-one. After e.g. 45 min, the solution is poured into a 1N aqueous NaHCO₃ solution. After e.g. 30 min, the biphasic mixture is extracted with EtOAc. After separation, the aqueous layer is back extracted 2x with EtOAc. The organic extracts are combined and concentrated *in vacuo*. To the concentrated material is added toluene and the mixture is evaporated *in vacuo* to remove residual pyridine, and repeated 2x. The resulting material is purified by silica gel chromatography (e.g. 0% to 60% MeOH in

CH₂Cl₂) to provide compound **E** as a mixture of stereoisomers. The newly formed phosphorothioate bond is shown as a mixture of stereoisomers on the phosphorus, while in some instances, this reaction may result in primarily the R isomer at this phosphorus.

[00396] Scheme I, Step 4: Compound **F** (including **Fa**, **Fb**, **Fc**, **Fd**): To a stirred solution of compound **E** in e.g. EtOH is added AMA and the resulting solution is heated at 50 °C. After e.g. 2 h, the solution is allowed to cool and concentrated *in vacuo*. To the residual solid is introduced triethylamine trihydrofluoride and the yellow solution is heated to 40 °C. After e.g. 3 h, the solution is allowed to cool to room temperature, and the solution is slowly added to a cooled solution of 1M TEAB and triethylamine. The mixture is allowed to stir for e.g. 1 h. The resulting residue is purified by reverse phase silica gel chromatography (e.g. 0% to 50% MeCN in 10 mM aqueous TEAA) to isolate the RR, RS, SR and SS isomers (at the two phosphorous atoms) Compound **Fa**, Compound **Fb**, Compound **Fc**, and Compound **Fd** as a white bis-triethylammonium salt after lyophilization. In instances where the phosphorothioate bond formed in Step 3 is primarily the R isomer, primarily compounds **Fa** and **Fb** will be isolated.

[00397] The methods of Scheme I can be modified as necessary by one skilled in the chemical arts, e.g. in terms of relative amounts of compounds and solvents, temperatures, reaction conditions, and the like. Similar methods as described in Examples 1-6 below may also be used to provide the LNA-CDN compounds as described herein.

[00398] The starting materials, i.e. compounds **A** and **C**, are readily available to one of skill in the chemical arts, including modifications that are readily available to one of skill in the chemical arts to prepare compounds of Formula I. For example, compounds as described in Examples 1-6 below, or suitable modifications thereof, can be used as compound **A** or **C**. In addition, compounds of Formula **A** or **C**, or compounds that can be readily converted to a compound of Formula **A** or **C** are described in, for example, PCT Publication Number WO 2010/091308 and WO 2009/143369, and US Patent No. 8,022,193, 8,278,283 and 9,005,906, and Seth et al., J. Org. Chem., 2012, 77: 5074-5085, or Migawa et al., Org. Letters, 2013, 15(17): 4316-4319 (Y₁-Y₂ is -O-CH₂- or -CH=CH-, R₅ is F and the phosphoramidite in **A** is linked to the 3' carbon, or where R₅ and R₇ and/or -X₁-X₂-X₃- form -O-CH₂-; or Y₁ is O, Y₂ is absent, and R₅ and R₇ and/or -X₁-X₂-X₃- form -O-CRR- or -O-C(=CH₂)-, where each R is independently e.g. H or CH₃, wherein the methyl is optionally substituted with one F or one methoxy); US Patent No.

7,569,686, 7,427,672 and US Patent Publication No. 2012/071646 (Y_1 is O, Y_2 is absent, and R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-O-NR-CH_2-$, R is e.g. H, CH_3 , or a nitrogen protecting group such as $-C(=O)-CH_2-O-phenyl$); US Patent No. 8,541,562 (Y_1 is O, Y_2 is absent, and R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-O-NR-C(=O)-$, $-NR-C(=O)-$ or $-NR-C(=O)-CH_2-$, R is e.g. H, Me or a nitrogen protecting group); PCT Publication Number WO 2015/142735 and WO 2014/145356 (Y_1 is O, Y_2 is absent, and R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-CH_2-NR-CH_2-$, R is e.g. H or a nitrogen protecting group such as $-C(=O)-CF_3$); PCT Publication Number WO 99/60855, WO 2013/036868, US Patent Publication No. 2003/0028013, and US Patent 6,403,556 and 6,833,361 (Y_1 is O, Y_2 is absent, and R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-CH_2-O-CH_2-$); PCT Publication No. WO 2008/154401, US Patent No. 8,461,124, 8,278,426, and Xu et al., J. Org. Chem. 2009, 74, 6534-6554 (Y_1 is O, Y_2 is absent, and R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-CRR-CR'R'-$, each R is independently e.g. H, F, NH_2 or CH_3 or forms $=CH_2$ optionally substituted with F, or forms $=O$ and each R' is independently e.g. H, F, OH, OCH_3 , CH_3 optionally substituted with F, forms $=CH_2$ optionally substituted with F, or forms $=O$; or R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-CRR-CH_2-CH_2-$, R is e.g. H, CH_3); PCT Publication No. WO 2016/017422 (Y_1 is O, Y_2 is absent, and R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-CRR-CH_2-O-$, each R is independently e.g. H, CH_3 or forms $=CH_2$); US Patent No. 8,278,425, PCT Publication No. WO 2007/145593 (Y_1 is O, Y_2 is absent, and R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-NR-CH_2-$, R is e.g. OR' , R' is e.g. $C_{1-6}alkyl$ (e.g. methyl, ethyl) optionally substituted with e.g. F or OCH_3), or R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-NH-CH_2-CH_2-$); US Patent Publication No. US 2015/0337002 (Y_1 is O, Y_2 is absent, and R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-NR-S(=O)_2-$, $-NR-S(=O)_2-CH_2-$ or $-S(=O)_2-NR-CH_2-$, R is e.g. H or $C_{1-3}alkyl$); and PCT Publication No. WO 2015/125783, WO 2011/085102, WO 2009/006478, US Patent No. 7,217,805, 7,335,765, 8,278,283, and US Patent Publication No. 2015/0152132 (Y_1 is O, Y_2 is absent, and R_5 and R_7 and/or $-X_1-X_2-X_3-$ form $-O-CH_2-CH_2-$ or $-O-CRR-$, each R is independently e.g. H, CH_3 , forms $=CH_2$, or together with the carbon atom to which they are bound form a 3-6 membered heterocyclyl or $C_{3-6}cycloalkyl$ (e.g. cyclopropyl), US 7,217,805 includes compounds where X_4 and/or X_4 are NH). Additional references describing non-LNA starting materials and synthesis of cyclic dinucleotides include, for example, PCT Publication No. WO 2015/185565 (including compounds where e.g. Y_1 is $-CH_2-$ and Y_2 is absent), WO2007/054279, WO2014/093936, WO2014/189805, U.S. Patents 7,709,458 and

7,592,326; Yan et al., Bioorg. Med. Chem Lett. 2008, 18: 5631; and Kovács et al., 1995, Nucleosides & Nucleotides, 14(6): 1259-1267 (including compounds where Y1 is O, Y2 is absent, R6 is F and the phosphoramidite is linked to the 2' carbon). It is understood in discussing these references, where Y₁, Y₂, R5 and R7 of Compound **A** are discussed, this also applies to Compound **C**, i.e. to Y₃, Y₄, X₁, X₂ and X₃ of Compound **C**. These references are incorporated by reference herein as it relates to the compounds and methods described herein, e.g. compounds of Formula **A** or **C**.

EXAMPLES

[00399] The following examples serve to illustrate the present invention. These examples are in no way intended to limit the scope of the invention.

General Methods

[00400] Anhydrous solvents and reagents suitable for solution phase oligonucleotide synthesis were purchased from commercial suppliers (Aldrich, ChemGenes Corporation, Wilmington, MA, USA) and handled under dry argon or nitrogen using anhydrous technique. Phosphoramidite coupling reactions and H-phosphonate cyclizations were carried out in anhydrous acetonitrile or pyridine under dry argon or nitrogen. The starting materials for all reactions in dry pyridine were dried by concentration (three times) from pyridine, unless indicated otherwise. Chromatography conditions were as follows unless indicated otherwise in the examples below. Preparative silica gel flash chromatography was carried out under medium pressure chromatography (MPLC) using RediSep Rf silica columns (Teledyne Isco, Lincoln, NE) on a Combiflash Rf+ UV-Vis (Teledyne Isco) using gradients of methanol in dichloromethane. Reverse phase preparative chromatography was executed under MPLC conditions using RediSep Rf C18 Aq columns (Teledyne Isco) on a Combiflash Rf+ UV-Vis using gradients of acetonitrile in aqueous 10 mM TEAA solution. Analytical high pressure liquid chromatography (HPLC) was performed on a Shimadzu Prominence HPLC system with two LC-20AD pumps and a SPD-M30A photodiode array detector monitoring at 254 nm. Gradients of 10 mM TEAA in acetonitrile or 20 mM NH₄OAc in acetonitrile were used with either a 5 micron (Thermo Scientific Acclaim 120) C-18 column (4.6 x 250 mm) or a 10 micron (Thermo Scientific Hypersil) C-18 column (4.0 x 250 mm) at room temperature. Ultra-Performance analytical high pressure liquid chromatography (UPLC) was performed on a

Shimadzu Nexera X2 LCMS system with two LC-30AD pumps and a SPD-M30A photodiode array detector monitoring at 254 nm. Gradients of 20 mM NH₄OAc in acetonitrile were used with a 1.7 micron (Acquity UPLC ® BEH) C-18 column (2.1 x 30 mm) at room temperature. Preparative HPLC was carried out on a Shimadzu preparative LC20-AP HPLC system, equipped with a SPD-20A UV/Vis detector monitoring at 254nm on a Varian Microsorb 60-8 C-18 41.6 x 250 mm column using gradients of 10 mM TEAA and acetonitrile at a flow rate of 50 ml/min. Solid phase extractions using C-18 Sep-Pak (Waters) were carried out at loadings of 3% (wt/wt). Analytical LCMS were recorded using a Shimadzu LCMS system featuring a Prominence HPLC coupled to a Shimadzu LCMS-2020 single quadrupole mass spectrometer, using an electrospray ionization source (ESI). Additional or alternative instrumentation and methods may also be provided in the examples that follow.

[00401] ¹H NMR, ¹⁹F NMR, ³¹P NMR and ¹³C NMR spectra were recorded in CDCl₃, d₆-DMSO, CD₃OD or D₂O as solvent, using a Bruker 400 MHz spectrometer with a broad band channel and variable temperature probe. The operating frequency for ¹H was 400 MHz, ¹⁹F was 376 MHz, ³¹P was 162 MHz, and ¹³C was 100 MHz. All spectra were recorded at ambient temperature (20-25 °C) unless otherwise noted. The temperature for variable-temperature experiments was calibrated monthly or bimonthly using the ethylene glycol method described in C. Amman, P. Meier and A. E. Merbach, *J. Magn. Reson.* **1982**, 46, 319-321.

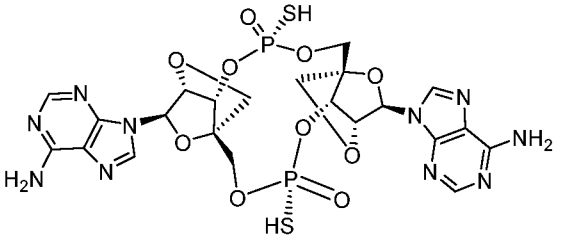
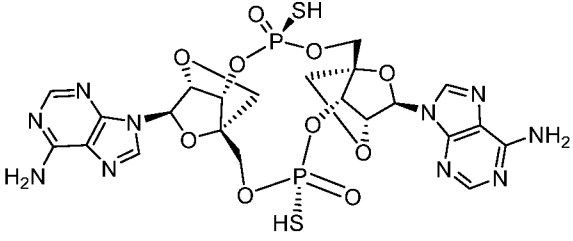
[00402] The final compounds may exist as the triethylammonium (TEAH⁺ or Et₃NH⁺) salt, which can be converted to other salt forms (including but not limited to sodium (Na⁺) or ammonium (NH₄⁺)) using standard ion exchange techniques or other well known methods.

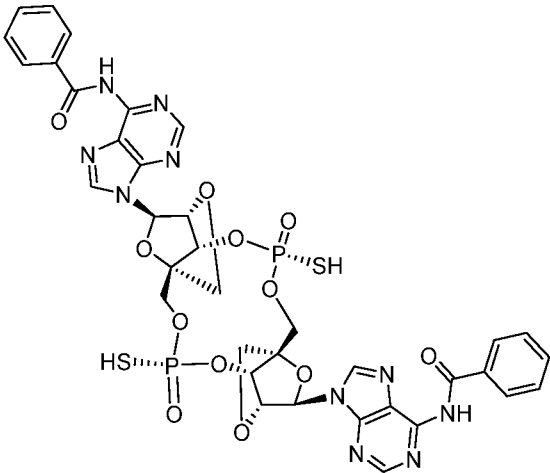
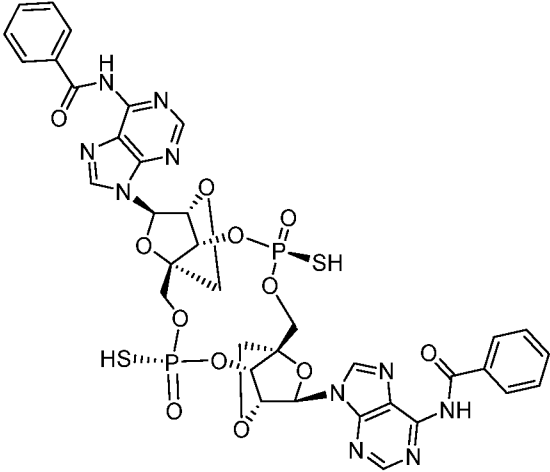
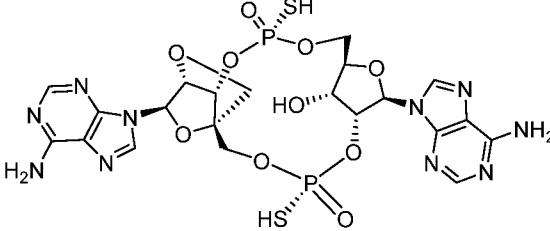
[00403] Assignments of Stereochemistry at the phosphorus were made in analogy to literature methods (Zhao et al. Nucleosides, Nucleotides, and Nucleic Acid 289:352-378, 2009) or as discussed in the examples below.

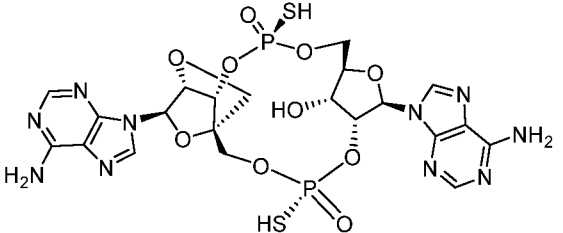
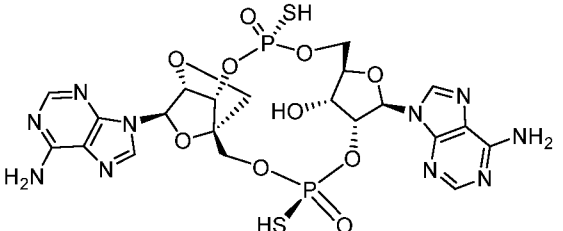
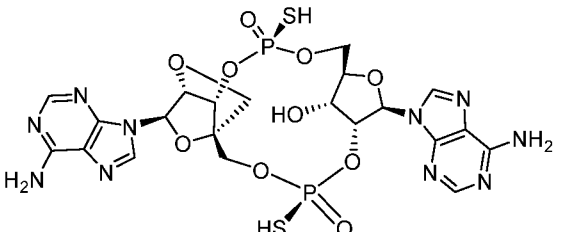
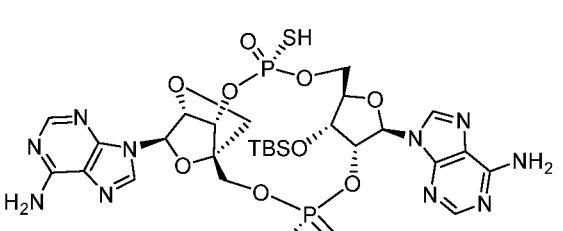
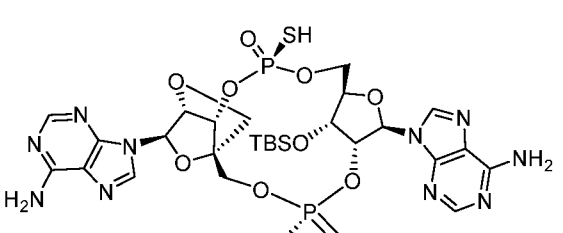
[00404] Compound names were generated using the software program ChemBioDraw Ultra V 14.0 available from CambridgeSoft Corporation, 100 CambridgePark Drive, Cambridge, MA 02140 USA (www.cambridgesoft.com). Abridged names of compounds for which a name could not be generated by ChemBioDraw, or reference compounds used in the examples, are provided in the following Table 3. Reference compounds 2'3'-RR-

(G)(A) and 2'3'-RR-(A)(A) were prepared as described in PCT Publication No. WO2014/189805, incorporated by reference with respect to such syntheses. Structures in the examples may also be represented as salts, e.g. $-O^- A^+$ or $-S^- A^+$, where A^+ is the salt cation.

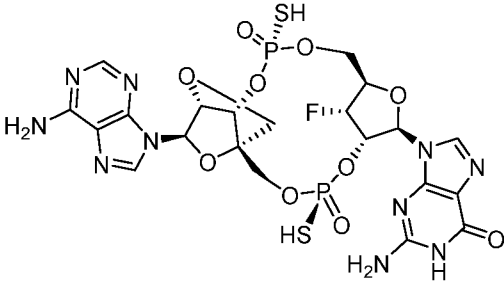
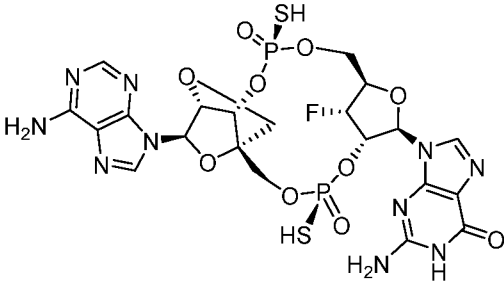
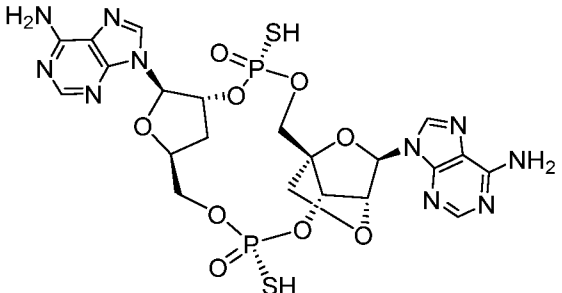
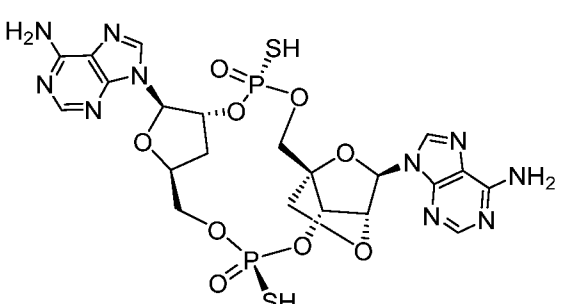
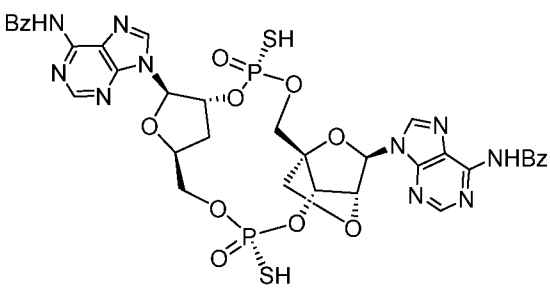
[00405] Table 3: Abridged compound names and structures.

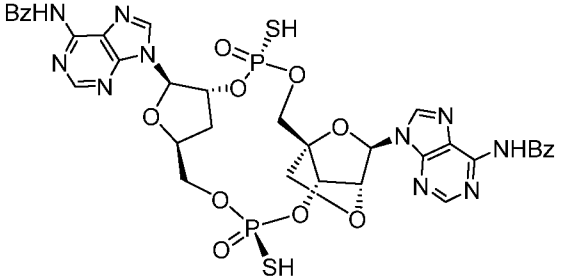
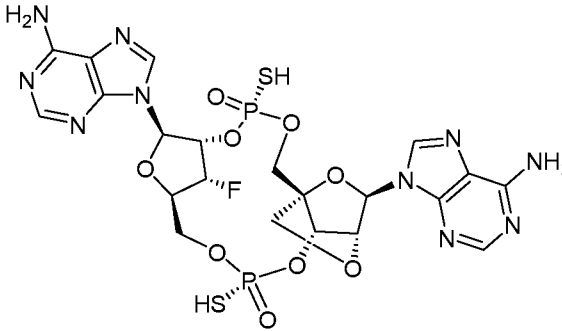
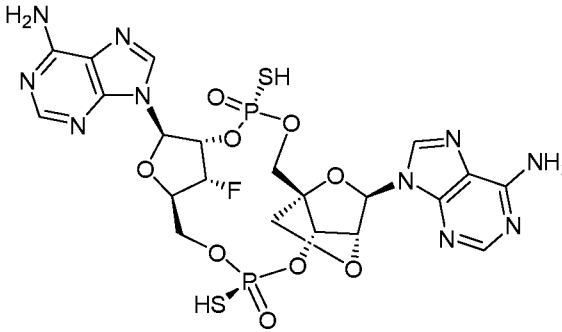
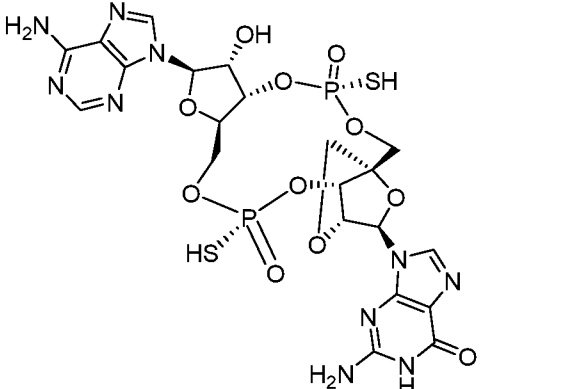
Example number and abridged Compound names	Structure
Example 2 Compound 5a 3'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) dithio-(Rp,Rp)-cyclic-[2'O,4'C-LNA-A(3',5')p-2'O,4'C-LNA-A(3',5')p] (2R,3R,3aS,5R,7aR,9S,10R,10aS,12R,14aR)-2,9-bis(6-amino-9H-purin-9-yl)-5,12-dimercaptotetrahydro-2H,7H,9H,14H-3,14a:10,7a-bis(epoxymethano)difuro[3,2-d:3',2'-j][1,3,7,9]tetraoxa[2,8]diphosphacyclododecine 5,12-dioxide	
Example 2 Compound 5b 3'3'-RS-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) dithio-(Rp,Sp)-cyclic-[2'O,4'C-LNA-A(3',5')p-2'O,4'C-LNA-A(3',5')p] (2R,3R,3aS,5R,7aR,9S,10R,10aS,12S,14aR)-2,9-bis(6-amino-9H-purin-9-yl)-5,12-dimercaptotetrahydro-2H,7H,9H,14H-3,14a:10,7a-bis(epoxymethano)difuro[3,2-d:3',2'-j][1,3,7,9]tetraoxa[2,8]diphosphacyclododecine 5,12-dioxide	

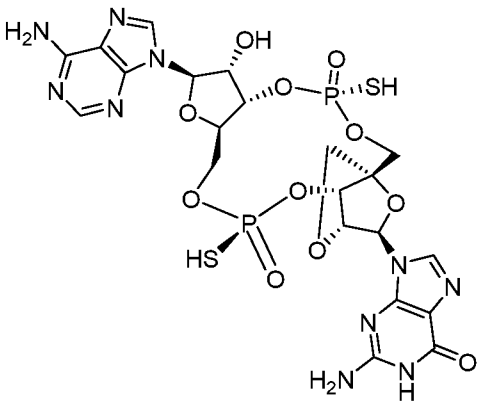
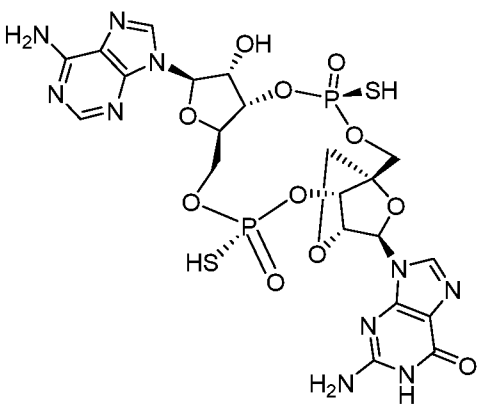
Example number and abridged Compound names	Structure
Example 2 Compound 4a 3'3'-RR-(2'O,4'C-LNA-BzA)(2'O,4'C-LNA-BzA) dithio-(Rp,Rp)-cyclic-[2'O,4'C-LNA-BzA(3',5')p-2'O,4'C-LNA-BzA(3',5')p] <i>N,N'</i>-(((2<i>R</i>,3<i>R</i>,3<i>aS</i>,5<i>R</i>,7<i>aR</i>,9<i>R</i>,10<i>R</i>,10<i>aS</i>,12<i>R</i>,14<i>aR</i>)-5,12-dimercapto-5,12-dioxidotetrahydro-2<i>H</i>,7<i>H</i>,9<i>H</i>,14<i>H</i>-3,14<i>a</i>:10,7<i>a</i>-bis(epoxymethano)difuro[3,2-<i>d</i>:3',2'-<i>j</i>][1,3,7,9]tetraoxa[2,8]diphosphacyclododecine-2,9-diyl)bis(9<i>H</i>-purine-9,6-diyl))dibenzamide	
Example 2 Compound 4b 3'3'-RS-(2'O,4'C-LNA-BzA)(2'O,4'C-LNA-BzA) dithio-(Rp,Sp)-cyclic-[2'O,4'C-LNA-BzA(3',5')p-2'O,4'C-LNA-BzA(3',5')p] <i>N,N'</i>-(((2<i>R</i>,3<i>R</i>,3<i>aS</i>,5<i>R</i>,7<i>aR</i>,9<i>R</i>,10<i>R</i>,10<i>aS</i>,12<i>S</i>,14<i>aR</i>)-5,12-dimercapto-5,12-dioxidotetrahydro-2<i>H</i>,7<i>H</i>,9<i>H</i>,14<i>H</i>-3,14<i>a</i>:10,7<i>a</i>-bis(epoxymethano)difuro[3,2-<i>d</i>:3',2'-<i>j</i>][1,3,7,9]tetraoxa[2,8]diphosphacyclododecine-2,9-diyl)bis(9<i>H</i>-purine-9,6-diyl))dibenzamide	
Example 3 Compound 10a 2'3'-RR-(A)(2'O,4'C-LNA-A) dithio-(Rp,Rp)-cyclic-[A(2',5')p-2'O,4'C-LNA-A(3',5')p]	

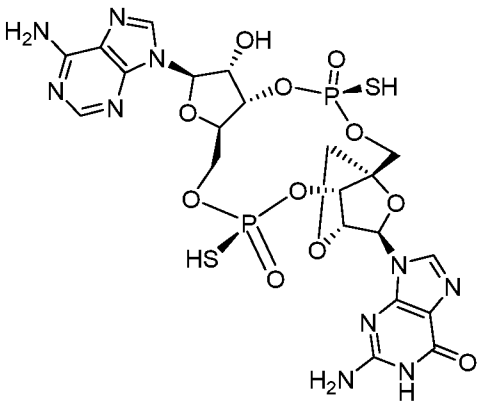
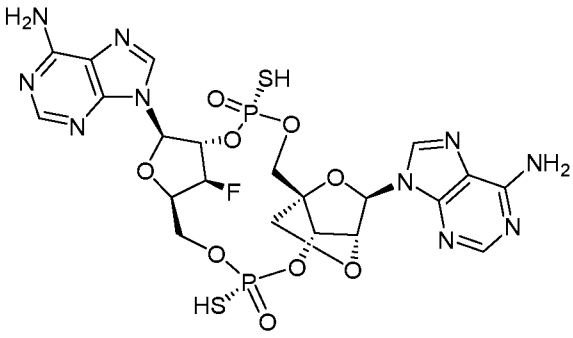
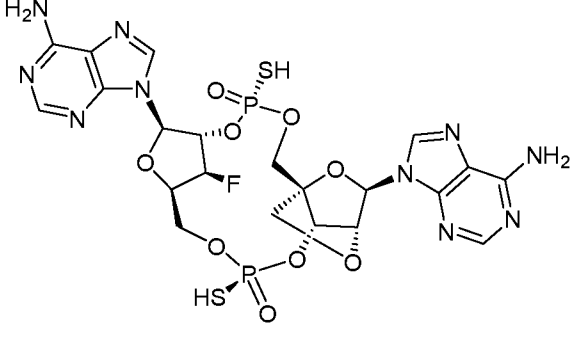
Example number and abridged Compound names	Structure
Example 3 Compound 10b 2'3'-RS-(A)(2'O,4'C-LNA-A) dithio-(Rp,Sp)-cyclic-[A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 3 Compound 10c 2'3'-SR-(A)(2'O,4'C-LNA-A) dithio-(Sp,Rp)-cyclic-[A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 3 Compound 10d 2'3'-SS-(A)(2'O,4'C-LNA-A) dithio-(Sp,Sp)-cyclic-[A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 3 Compound 11a 2'3'-RR-(3'-OTBS-A)(2'O,4'C-LNA-A) dithio-(Rp,Rp)-cyclic-[3'-OTBS-A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 3 Compound 11b 2'3'-RS-(3'-OTBS-A)(2'O,4'C-LNA-A) dithio-(Rp,Sp)-cyclic-[3'-OTBS-A(2',5')p-2'O,4'C-LNA-A(3',5')p]	

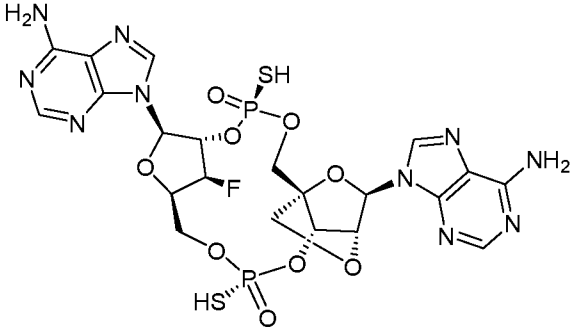
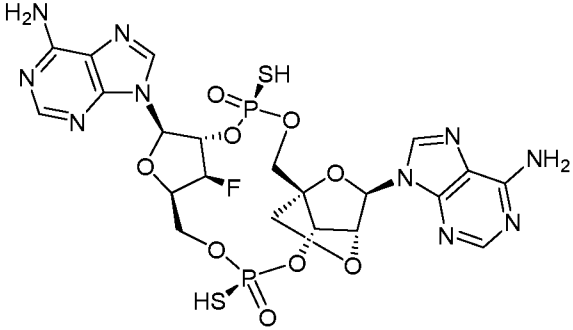
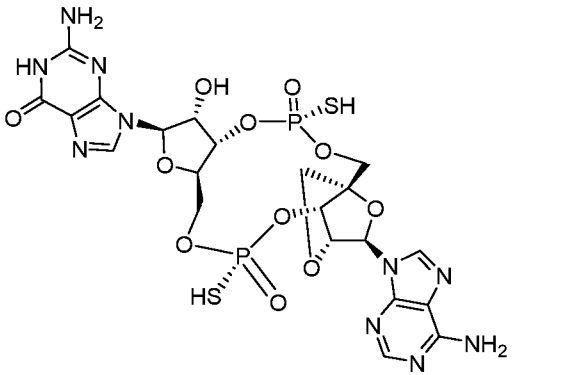
Example number and abridged Compound names	Structure
Example 3 Compound 11c 2'3'-SS-(3'-OTBS-A)(2'O,4'C-LNA-A) dithio-(<i>Sp,Sp</i>)-cyclic-[3'- OTBS-A(2',5')p-2'O,4'C-LNA- A(3',5')p]	
Example 3 Compound 11d 2'3'-RR-(BzA)(2'O,4'C-LNA-BzA) dithio-(<i>Rp,Rp</i>)-cyclic-[3'-BzA(2',5')p- 2'O,4'C-LNA-BzA(3',5')p]	
Example 3 Compound 11e 2'3'-RS-(BzA)(2'O,4'C-LNA-BzA) dithio-(<i>Rp,Sp</i>)-cyclic-[3'-BzA(2',5')p- 2'O,4'C-LNA-BzA(3',5')p]	
Example 3 Compound 11f 2'3'-SR-(BzA)(2'O,4'C-LNA-BzA) dithio-(<i>Sp,Rp</i>)-cyclic-[3'-BzA(2',5')p- 2'O,4'C-LNA-BzA(3',5')p]	
Example 4 Compound 15a 2'3'-RR-(3'F-G)(2'O,4'C-LNA-A) dithio-(<i>Rp,Rp</i>)-cyclic-[3'F-G(2',5')p- 2'O,4'C-LNA-A(3',5')p]	
Example 4 Compound 15b 2'3'-RS-(3'F-G)(2'O,4'C-LNA-A) dithio-(<i>Rp,Sp</i>)-cyclic-[3'F-G(2',5')p- 2'O,4'C-LNA-A(3',5')p]	

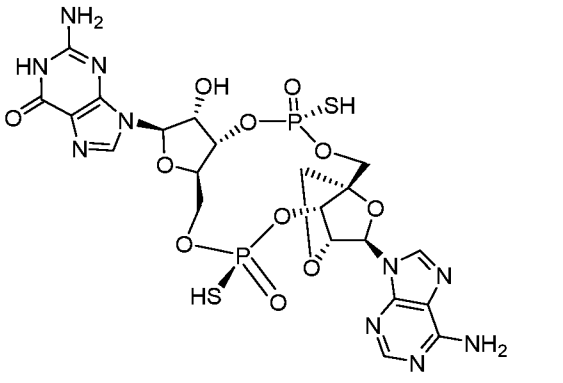
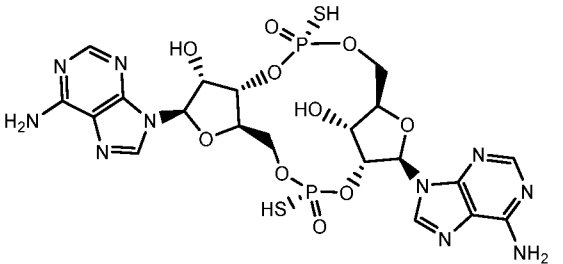
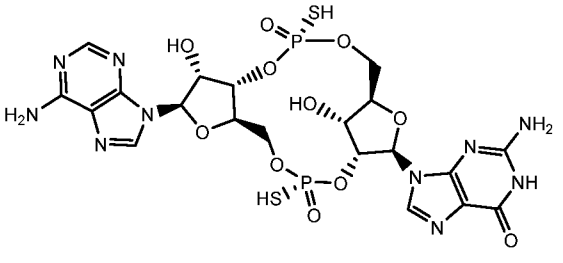
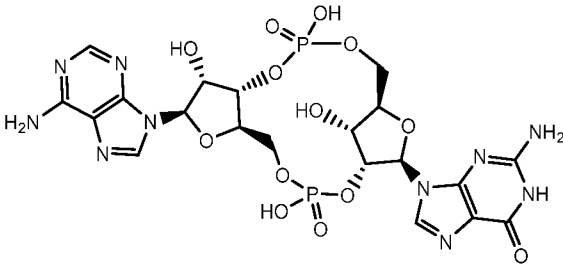
Example number and abridged Compound names	Structure
Example 4 Compound 15c 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A) dithio-(<i>Sp,Rp</i>)-cyclic-[3'F-G(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 4 Compound 15d 2'3'-SS-(3'F-G)(2'O,4'C-LNA-A) dithio-(<i>Sp,Sp</i>)-cyclic-[3'F-G(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 5 Compound 21a 2'3'-RR-(3'H-A)(2'O,4'C-LNA-A) dithio-(<i>Rp,Rp</i>)-cyclic-[3'H-A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 5 Compound 21b 2'3'-RS-(3'H-A)(2'O,4'C-LNA-A) dithio-(<i>Rp,Sp</i>)-cyclic-[3'H-A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 5 Compound 22a 2'3'-RR-(3'H-BzA)(2'O,4'C-LNA-BzA) dithio-(<i>Rp,Rp</i>)-cyclic-[3'H-BzA(2',5')p-2'O,4'C-LNA-BzA(3',5')p]	

Example number and abridged Compound names	Structure
Example 5 Compound 22b 2'3'-RS-(3'H-BzA)(2'O,4'C-LNA-BzA) dithio-(<i>R_P</i>,<i>S_P</i>)-cyclic-[3'H-BzA(2',5')p-2'O,4'C-LNA-BzA(3',5')p]	
Example 6 Compound 27a 2'3'-RR-(3'F-A)(2'O,4'C-LNA-A) dithio-(<i>R_P</i>,<i>R_P</i>)-cyclic-[3'F-A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 6 Compound 27b 2'3'-RS-(3'F-A)(2'O,4'C-LNA-A) dithio-(<i>R_P</i>,<i>S_P</i>)-cyclic-[3'F-A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 7 Compound 33a 3'3'-RR-(A)(2'O,4'C-LNA-G) dithio-(<i>R_P</i>,<i>R_P</i>)-cyclic-[A(3',5')p-2'O,4'C-LNA-G(3',5')p] 2-amino-9-((2<i>R</i>,3<i>R</i>,3<i>aS</i>,5<i>R</i>,7<i>aR</i>,9<i>R</i>,10<i>R</i>,10<i>aS</i>,12<i>R</i>,14<i>aR</i>)-9-(6-amino-9<i>H</i>-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2<i>H</i>,7<i>H</i>,14<i>H</i>-3,14<i>a</i>-(epoxymethano)difuro[3,2-<i>d</i>:3',2'-<i>j</i>][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-2-yl)-1,9-dihydro-6<i>H</i>-purin-6-one	

Example number and abridged Compound names	Structure
Example 7 Compound 33b 3'3'-RS-(A)(2'O,4'C-LNA-G) dithio-(<i>R_P</i>,<i>S_P</i>)-cyclic-[A(3',5')p-2'O,4'C-LNA-G(3',5')p] 2-amino-9-((2<i>R</i>,3<i>R</i>,3<i>aS</i>,5<i>S</i>,7<i>aR</i>,9<i>R</i>,10<i>R</i>,10<i>aS</i>,12<i>R</i>,14<i>aR</i>)-9-(6-amino-9<i>H</i>-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2<i>H</i>,7<i>H</i>,14<i>H</i>-3,14<i>a</i>-(epoxymethano)difuro[3,2-<i>d</i>:3',2'-<i>j</i>][1,3,7,9]tetraoxa[2,8]diphosphacyclodecin-2-yl)-1,9-dihydro-6<i>H</i>-purin-6-one	
Example 7 Compound 33c 3'3'-SR-(A)(2'O,4'C-LNA-G) dithio-(<i>S_P</i>,<i>R_P</i>)-cyclic-[A(3',5')p-2'O,4'C-LNA-G(3',5')p] 2-amino-9-((2<i>R</i>,3<i>R</i>,3<i>aS</i>,5<i>R</i>,7<i>aR</i>,9<i>R</i>,10<i>R</i>,10<i>aS</i>,12<i>S</i>,14<i>aR</i>)-9-(6-amino-9<i>H</i>-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2<i>H</i>,7<i>H</i>,14<i>H</i>-3,14<i>a</i>-(epoxymethano)difuro[3,2-<i>d</i>:3',2'-<i>j</i>][1,3,7,9]tetraoxa[2,8]diphosphacyclodecin-2-yl)-1,9-dihydro-6<i>H</i>-purin-6-one	

Example number and abridged Compound names	Structure
Example 7 Compound 33d 3'3'-SS-(A)(2'O,4'C-LNA-G) dithio-(<i>S_P</i>,<i>S_P</i>)-cyclic-[A(3',5')p-2'O,4'C-LNA-G(3',5')p] 2-amino-9-((2<i>R</i>,3<i>R</i>,3<i>aS</i>,5<i>S</i>,7<i>aR</i>,9<i>R</i>,10<i>R</i>,10<i>aS</i>,12<i>S</i>,14<i>aR</i>)-9-(6-amino-9<i>H</i>-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2<i>H</i>,7<i>H</i>,14<i>H</i>-3,14<i>a</i>-(epoxymethano)difuro[3,2-<i>d</i>:3',2'-<i>j</i>][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-2-yl)-1,9-dihydro-6<i>H</i>-purin-6-one	
Example 8 Compound 37a 2'3'-RR-(3'βF-A)(2'O,4'LNA-A) dithio-(<i>R_P</i>,<i>R_P</i>)-cyclic-[3'βF-A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 8 Compound 37b 2'3'-RS-(3'βF-A)(2'O,4'LNA-A) dithio-(<i>R_P</i>,<i>S_P</i>)-cyclic-[3'βF-A(2',5')p-2'O,4'C-LNA-A(3',5')p]	

Example number and abridged Compound names	Structure
Example 8 Compound 37c 2'3'-SR-(3'βF-A)(2'O,4'LNA-A) dithio-(<i>S_P</i>,<i>R_P</i>)-cyclic-[3'βF-A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 8 Compound 37d 2'3'-SS-(3'βF-A)(2'O,4'LNA-A) dithio-(<i>S_P</i>,<i>S_P</i>)-cyclic-[3'βF-A(2',5')p-2'O,4'C-LNA-A(3',5')p]	
Example 9 Compound 45a 3'3'-RR-(G)(2'O,4'C-LNA-A) dithio-(<i>R_P</i>,<i>R_P</i>)-cyclic-[G(3',5')p-2'O,4'C-LNA-A(3',5')p] 2-amino-9- ((2<i>R</i>,3<i>R</i>,3<i>aS</i>,5<i>R</i>,7<i>aR</i>,9<i>R</i>,10<i>R</i>,10<i>aS</i>,12<i>R</i>,14<i>aR</i>)-2-(6-amino-9<i>H</i>-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2<i>H</i>,7<i>H</i>,14<i>H</i>-3,14<i>a</i>-(epoxymethano)difuro[3,2-<i>d</i>:3',2'-<i>j</i>][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-9-yl)-1,9-dihydro-6<i>H</i>-purin-6-one	

Example number and abridged Compound names	Structure
Example 9 Compound 45b 3'3'-RS-(G)(2'O,4'C-LNA-A) dithio-(<i>R_P</i>,<i>S_P</i>)-cyclic-[G(3',5')p-2'O,4'C-LNA-A(3',5')p] 2-amino-9-((2<i>R</i>,3<i>R</i>,3<i>aS</i>,5<i>S</i>,7<i>aR</i>,9<i>R</i>,10<i>R</i>,10<i>aS</i>,12<i>R</i>,14<i>aR</i>)-2-(6-amino-9<i>H</i>-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2<i>H</i>,7<i>H</i>,14<i>H</i>-3,14<i>a</i>-(epoxymethano)difuro[3,2-<i>d</i>:3',2'-<i>j</i>][1,3,7,9]tetraoxa[2,8]diphosphacyclodecin-9-yl)-1,9-dihydro-6<i>H</i>-purin-6-one	
2'3'-RR-(A)(A); dithio-[<i>R_P</i>, <i>R_P</i>]-cyclic-[A(2',5')pA(3',5')p]	
2'3'-RR-(G)(A); dithio-[<i>R_P</i>, <i>R_P</i>]-cyclic-[G(2',5')pA(3',5')p]	
2'3'-(G)(A); cyclic-[G(2',5')pA(3',5')p]	

[00406] Abbreviations and Acronyms. SalPCl = Salicyl chlorophosphite. DCA = dichloroacetic acid. DDTT = ((Dimethylamino-methylidene)amino)-3H-1,2,4-

dithiazaoline-3-thione. DMP = Dess-Martin Periodinane. DAST = diethylaminosulfur trifluoride. NaHCO_3 = sodium bicarbonate. DCM = CH_2Cl_2 = dichloromethane. IPA = isopropyl alcohol. EtOH = ethanol. EtOAc = ethyl acetate. AcOH = acetic acid. KOAc = potassium acetate. MeCN = acetonitrile. MeOH = methanol. NH_4OAc = ammonium acetate. NH_4OH = ammonium hydroxide. DMAP = N,N-dimethylpyridin-4-amine. DMOCP = 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane-2-oxide. DMTCl = 4,4'-dimethoxytrityl chloride. DMT = 4,4-dimethoxytrityl. N-phenyltriflamide = 1,1,1-trifluoro-N-phenyl-N-((trifluoromethyl)sulfonyl)methanesulfonamide. TBAF = tetrabutylammonium fluoride. TBS = *tert*-butyldimethylsilyl. TEAA = Triethylammonium acetate. TEA = triethylamine. TEAH^+ = triethylammonium. TEAB = triethylammonium bicarbonate. TFA = trifluoroacetic acid. TMSCl = trimethylsilyl chloride. HF = hydrofluoric acid. THF = tetrahydrofuran. MeTHF = 2-Methyltetrahydrofuran. G = Guanine. G^{ib} = isobutyryl guanine. A = adenine. A^{Bz} = benzoyl adenine. AMA = ammonium hydroxide/40% methylamine solution in water. Rt = retention time. rt = room temperature. min = minute(s). h = hour(s).

Example 1: Synthesis of Intermediate Compounds

[00407] The LCMS or HRMS data in this example, and where indicated in the following examples, were recorded using the indicated methods as follows. In all instances, masses reported are those of the protonated parent ions unless indicated otherwise.

Method A: LCMS data were recorded using a Waters System: Micromass ZQ mass spectrometer; Column: Sunfire C18 3.5 micron, 3.0 x 30 mm; gradient: 40-98% MeCN in water with 0.05% TFA over a 2.0 min period; flow rate 2 mL/min; column temperature 40 °C).

Method B: LCMS were recorded using a Waters System: Micromass SQ mass spectrometer; Column: Acquity UPLC BEH C18 1.7 micron, 2.1 x 30 mm; gradient 1% to 30% MeCN to 3.20 min then gradient: 30-98% MeCN in water with 5mM NH_4OH over a 1.55 min period before returning to 1% MeCN at 5.19 min – total run time 5.2 min; flow rate 1 mL/min; column temperature 50 °C.

Method C: LCMS were recorded using a Waters System: Micromass SQ mass spectrometer; Column: Acquity UPLC BEH C18 1.7 micron, 2.1 x 50 mm; gradient: 2-98% MeCN in water + 5mM NH_4OH over a 4.40 min period isocratic for 0.65 min before returning to 2% MeCN at 5.19 min – total run time 5.2 min; flow rate 1 mL/min;

column temperature 50 °C.

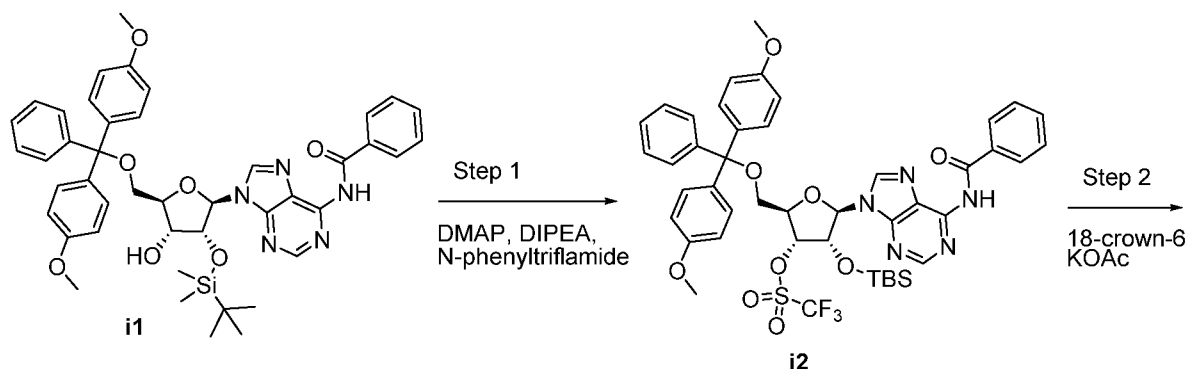
Method E: HRMS data were recorded using a Waters System: Acquity G2 Xevo QTof mass spectrometer; Column: Acquity BEH 1.7 micron, 2.1 x 50 mm; gradient: 40-98% MeCN in water with 0.1% Formic acid over a 3.4 min period, isocratic 98% MeCN for 1.75 mins returning to 40% at 5.2 mins; flow rate 1 mL/min; column temperature 50°C.

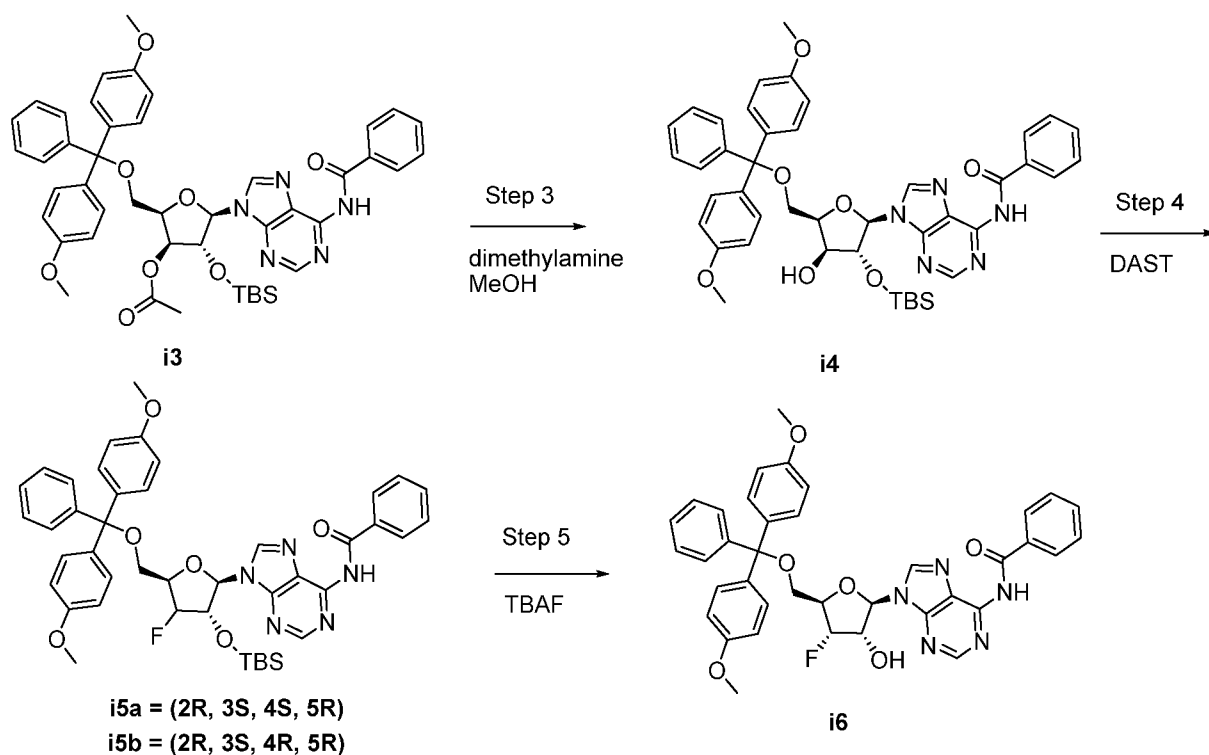
Method G: LCMS data were recorded using a Waters System: Micromass SQ mass spectrometer; Column: Acquity UPLC BEH C18 1.7 micron, 2.1 x 30 mm; gradient 1% to 30% MeCN to 1.20 mins then gradient: 30-98% MeCN in water with 5mM NH₄OAc over a 0.55 min period before returning to 1% MeCN at 2.19 mins – total run time 2.2 mins; flow rate 1 mL/min; column temperature 50 °C.

Method H: LCMS data were recorded using a Waters System: Micromass SQ mass spectrometer; Column: Acquity UPLC BEH C18 1.7 micron, 2.1 x 30 mm; gradient 2% to 98% MeCN to 1.76 mins then isocratic to 2.00 mins and then returning to 2% MeCN using gradient to 2.20 mins in water with 0.1% Formic acid; flow rate 1 mL/min; column temperature 50 °C.

Method I: LCMS data were recorded using a Waters System: Micromass SQ mass spectrometer; Column: Acquity UPLC BEH C18 1.7 micron, 2.1 x 30 mm; gradient 40% to 98% MeCN to 1.40 mins then isocratic to 2.05 mins and then returning to 40% MeCN using gradient to 2.20 mins in water with 0.1% Formic acid; flow rate 1 mL/min; column temperature 50 °C.

[00408] Intermediate **i6** (used in Example 6) was prepared according to the following Scheme 1A:





[00409] Step 1: Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-3-yl trifluoromethane-sulfonate (**i2**): A mixture of *N*-(9-((2*R*,3*R*,4*R*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-((tert-butyldimethylsilyl)oxy)-4-hydroxytetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (**i1**, 5.6 g, 7.11 mmol, ChemGenes) and DMAP (0.174 g, 1.42 mmol) was suspended in anhydrous THF (35 mL), addition of DIPEA (6.21 mL, 35.5 mmol) created a solution to which *N*-phenyltriflamide (5.08 g, 14.21 mmol), was added. The mixture was stirred for 3.5 h at rt, at which point it was poured into 5% brine (100 mL) and extracted with EtOAc (2 x 100 mL). The combined organic phases were dried (Na₂SO₄) the drying agent filtered-off and concentrated on silica gel (10g) *in vacuo*. The crude material was purified by chromatography on silica gel (gradient elution 25-100% EtOAc/heptane) to give the desired compound **i2** as a tan solid; 5.53 g; ¹H NMR (400 MHz, CDCl₃) δ 9.05 (s, 1H), 8.68 (s, 1H), 8.18 (s, 1H), 8.06 (d, *J* = 7.5 Hz, 2H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.61 – 7.48 (m, 4H), 7.48 – 7.25 (m, 7H), 6.88 (d, *J* = 8.8 Hz, 4H), 6.04 (d, *J* = 7.6 Hz, 1H), 5.50 (dd, *J* = 7.5, 4.7 Hz, 1H), 5.32 (d, *J* = 4.5 Hz, 1H), 4.50 (t, *J* = 4.1 Hz, 1H), 3.82 (s, 6H), 3.77 (dt, *J* = 10.8, 5.2 Hz, 1H), 3.41 (dd, *J* = 10.8, 3.7 Hz, 1H), 0.77 (s, 9H), -0.01 (s, 3H), -0.46 (s, 3H); LCMS (Method A) *R*_t = 1.65 min; *m/z* 920.5 [M+H]⁺.

[00410] Step 2: Preparation of (2*R*,3*S*,4*R*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((tert-butyltrimethylsilyl)oxy)tetrahydrofuran-3-yl acetate (**i3**): A mixture of compound **i2** (5.5 g, 5.98 mmol), KOAc (2.93 g, 29.9 mmol), and 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane, 0.79 g, 2.99 mmol) in toluene (40 mL) was heated at 110 °C for 4 h. The reaction mixture was then cooled to rt and silica gel (10g) added and the solvent was removed *in vacuo*. The crude material was purified by chromatography on silica gel (gradient elution 25-100% EtOAc/heptane) to give the desired compound **i3** as a tan solid: 3.3g; ¹H NMR (400 MHz, CDCl₃) δ 8.70 (s, 1H), 8.58 (s, 1H), 7.93 (s, 1H), 7.84 (d, *J* = 7.5 Hz, 2H), 7.44 (t, *J* = 7.4 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 2H), 7.28 (d, *J* = 7.2 Hz, 2H), 7.21 – 7.02 (m, 7H), 6.67 (dd, *J* = 8.9, 2.1 Hz, 4H), 5.98 (s, 1H), 4.97 (dd, *J* = 3.6, 1.4 Hz, 1H), 4.61 – 4.52 (m, 1H), 4.35 (s, 1H), 3.62 (s, 6H), 3.41 (dd, *J* = 9.8, 6.2 Hz, 1H), 3.18 (dd, *J* = 9.8, 5.6 Hz, 1H), 1.53 (s, 3H), 0.77 (s, 9H), 0.03 (s, 3H), 0.0 (s, 3H). LCMS (Method A) R_t 1.68 min; m/z 830.2 [M+H]⁺.

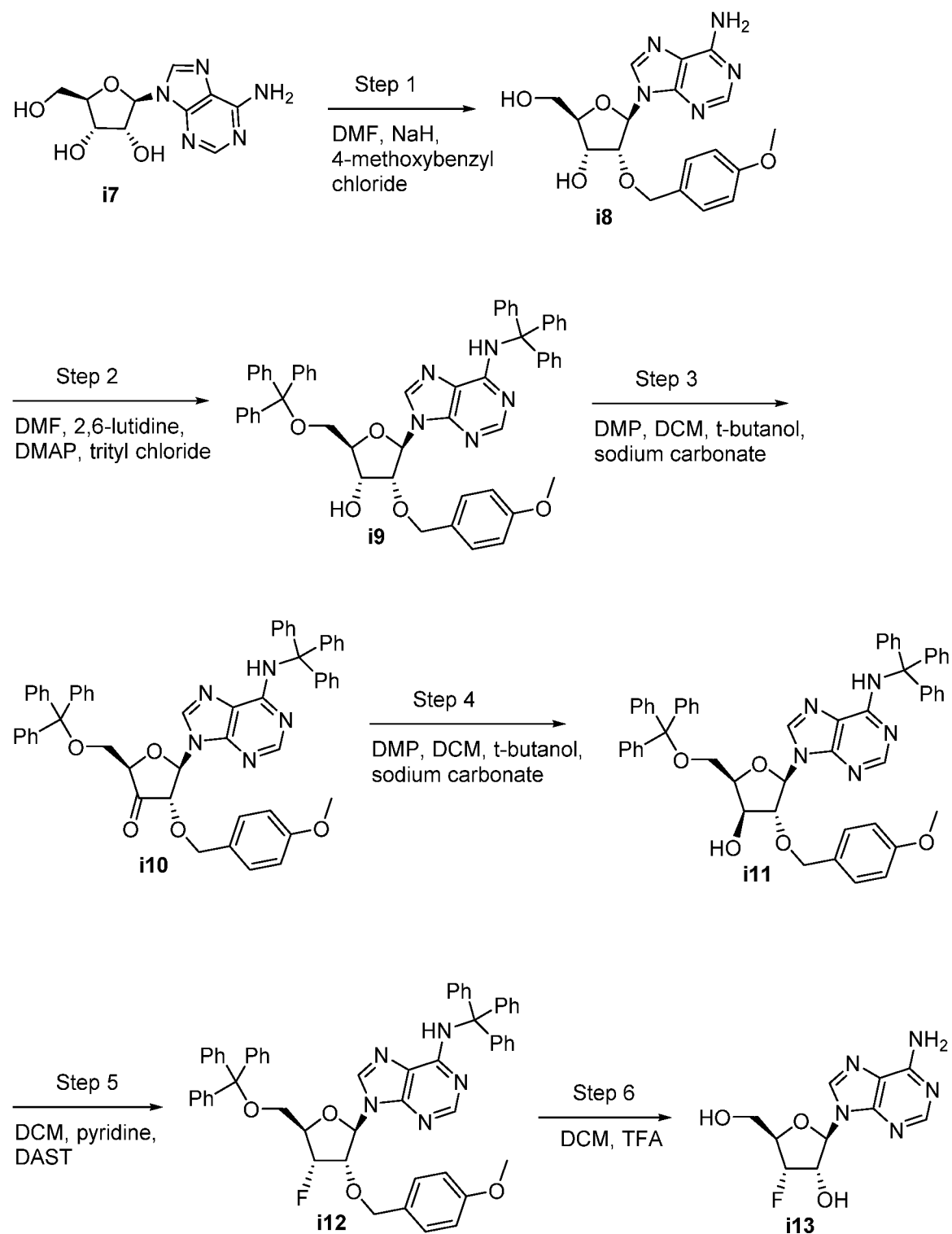
[00411] Step 3: Preparation of *N*-(9-((2*R*,3*R*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-((tert-butyltrimethylsilyl)oxy)-4-hydroxytetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (**i4**): Compound **i3** (6.78 g, 8.17 mmol) was dissolved in MeOH (120 mL) and a 2.0 M dimethylamine solution in MeOH (20.4 mL, 40.8 mmol) was added. The reaction mixture was stirred for 17 h at rt. Silica gel (12g) was added and the solvent was removed *in vacuo*. The crude material was purified by chromatography on silica gel (gradient elution 25-75% EtOAc/heptane) to give the desired compound **i4** as a tan solid: 3.9 g; ¹H NMR (400 MHz, CDCl₃) δ 8.94 (s, 1H), 8.65 (s, 1H), 8.16 (s, 1H), 7.97 – 7.90 (m, 2H), 7.58 – 7.38 (m, 3H), 7.38 – 7.32 (m, 2H), 7.32 – 7.00 (m, 7H), 6.80 – 6.65 (m, 4H), 5.83 (d, *J* = 1.2 Hz, 1H), 5.38 (d, *J* = 8.0 Hz, 1H), 4.42 (s, 1H), 4.29 (t, *J* = 4.6 Hz, 1H), 4.02 – 3.95 (m, 1H), 3.75 – 3.61 (m, 6H), 3.53 (d, *J* = 5.0 Hz, 2H), 0.81 (s, 9H), 0.0 (s, 6H). LCMS (Method A) R_t 1.57 min; m/z 788.2 [M+H]⁺.

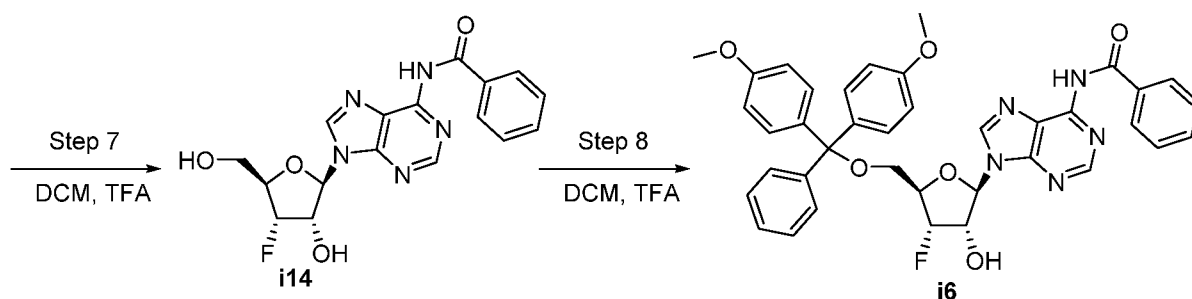
[00412] Step 4: Preparation of *N*-(9-((2*R*,3*S*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-((tert-butyltrimethylsilyl)oxy)-4-fluorotetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (**i5a**) and *N*-(9-((2*R*,3*S*,4*R*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-((tert-butyltrimethylsilyl)oxy)-4-fluorotetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (**i5b**): Compound **i4** (750 mg,

0.952 mmol) was dissolved in anhydrous DCM (7 mL) under an inert nitrogen atmosphere and the solution was cooled to 0 °C. A 1.0 M solution of DAST (1.90 mL, 1.90 mmol) was added and the reaction subsequently stirred at -5 °C for 17 h using a cryo-cool to control the reaction temperature. The vessel was warmed to 0 °C and saturated NaHCO₃ (2 mL) was added. After 30 minutes of stirring the mixture was diluted with 5% brine (20 mL) and extracted with EtOAc (2 x 20 mL). The combined organics were dried (Na₂SO₄) with the drying agent filtered off, silica gel (2g) added to the filtrate and the solvent removed *in vacuo*. The crude material was purified by chromatography on silica gel (gradient elution 10-75% EtOAc/heptane) to give a mixture of diastereoisomers **i5a** and **i5b** as a tan solid: 193 mg; Major (2R,3S,4S,5R) diastereoisomer LCMS (Method A) R_t 1.53 min; m/z 790.4 (M+H)⁺; Minor (2R,3S,4R,5R) diastereoisomer R_t 1.58 min; m/z 790.4 [M+H]⁺.

[00413] Step 5: Preparation of *N*-(9-((2R,3S,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-9H-purin-6-yl)benzamide (**i6**): The diastereomeric mixture of **i5a** and **i5b** (2.0 g, 2.53 mmol) was dissolved in anhydrous THF (100 mL) and cooled to -42 °C under an inert nitrogen atmosphere before 1.0 M TBAF (3.80 mL, 3.80 mmol) was added. The reaction was stirred for 2.5 h, then quenched with saturated NaHCO₃ (20 mL). The cold bath was removed, and the slurry was stirred for 10 minutes before the mixture was diluted with 5% brine (150 mL) and extracted with DCM (2 x 100 mL). The combined organic phases were dried (Na₂SO₄), with the drying agent filtered off, silica gel (4g) added to the filtrate and the solvent removed *in vacuo*. The crude material was purified by chromatography on silica gel (gradient elution 25-100% EtOAc/heptane) to give the desired compound **i6** as a white solid: 355 mg; ¹H NMR (400 MHz, CDCl₃) δ 9.16 (s, 1H), 8.64 (s, 1H), 8.23 (s, 1H), 7.99 (d, *J* = 7.5 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.41 – 7.31 (m, 3H), 7.31 – 7.11 (m, 7H), 6.79 (d, *J* = 8.9 Hz, 4H), 6.16 (d, *J* = 7.3 Hz, 1H), 5.77 (br s, 1H), 5.27 – 5.10 (m, 2H), 4.53 (dt, *J* = 28.0 Hz, 3.4 Hz, 1H), 3.77 (s, 6H), 3.51 (dd, *J* = 10.7, 3.7 Hz, 1H), 3.34 (dd, *J* = 10.7, 3.3 Hz, 1H); ¹⁹F NMR (376.4 MHz, CDCl₃) δ -197.5; ¹³C NMR (101 MHz, CDCl₃) δ 164.66, 158.64, 158.62, 152.60, 151.43, 149.34, 144.22, 141.66, 135.29, 135.13, 133.40, 132.93, 129.96, 128.87, 127.99, 127.93, 127.86, 127.07, 122.65, 113.26, 93.85, 92.02, 87.56 (d, *J* = 144 Hz), 83.56 (d, *J* = 23 Hz), 77.30, 74.63 (d, *J* = 16 Hz), 62.82 (d, *J* = 11 Hz), 55.26; LCMS (Method A) R_t 0.89 min; m/z 676.3 [M+H]⁺.

[00414] Alternatively, Intermediate **i6** was also prepared according to the following Scheme 1A':





[00415] Step 1: Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-2-(hydroxymethyl)-4-((4-methoxybenzyl)oxy)tetrahydrofuran-3-ol (**i8**): To a suspension of adenosine (**i7**, 100g, 374 mmol) in DMF (2.64 L) at 4 °C under nitrogen was added 60% sodium hydride (19.46 g, 486 mmol) in one portion and the reaction mixture stirred under nitrogen for 60 min. 4-Methoxybenzyl chloride (60.9 ml, 449 mmol) was added dropwise over a 10 min period and the suspension stirred and warmed to rt for 16 h. The reaction was quenched with water (50 mL), a short path condenser then fitted and the pale yellow mixture was heated (115°C) *in vacuo* to remove the DMF (60-90°C). The reaction volume was reduced to ~300 mL and then partitioned between water (2.5 L) and EtOAc (2 x 500 mL) with the pH of the aqueous phase ~8. The aqueous phase was separated and then extracted with 4:1 DCM-IPA (8x 500 mL). The combined DCM-IPA phase was dried (Na₂SO₄), the drying agent filtered off and the filtrate concentrated *in vacuo* to yield a semi-solid residue. The crude residue was stirred in EtOH (130 mL) at 55 °C for 1 h, filtered off, the solid washed with EtOH and dried *in vacuo* to afford a white solid (55.7 g, 38%, regioisomer ratio 86:14). This material was re-subjected to a hot slurry in EtOH (100 mL at 55 °C), hot filtered, the solid washed with cold EtOH to give the desired compound **i8** as a white crystalline solid (47.22 g): ¹H NMR (400 MHz, DMSO-d₆) δ 8.30 (s, 1H), 8.08 (s, 1H), 7.33 (br s, 2H), 7.06 (d, *J* = 8.6 Hz, 2H), 6.73 (d, *J* = 8.6 Hz, 2H), 6.03 (d, *J* = 6.3 Hz, 1H), 5.46 (dd, *J* = 7.3, 4.4 Hz, 1H), 5.28 (d, *J* = 5.1 Hz, 1H), 4.57 (d, *J* = 11.6 Hz, 1H), 4.53 (dd, *J* = 6.4, 5.0 Hz, 1H), 4.37 (d, *J* = 11.6 Hz, 1H), 4.33 (dd, *J* = 5.0, 2.9 Hz, 1H), 4.02 (q, *J* = 3.3 Hz, 1H), 3.69 (s, 3H), 3.67 (m, 1H), 3.56 (m, 1H); LCMS (Method B) Rt 1.86 mins; *m/z* 388.0 (*M*+*H*⁺).

[00416] Step 2: Preparation of (2*R*,3*R*,4*R*,5*R*)-4-((4-methoxybenzyl)oxy)-5-(6-(tritylamino)-9*H*-purin-9-yl)-2-((trityloxy)methyl)tetrahydrofuran-3-ol (**i9**): To compound **i8** (45.5g, 117 mmol) in DMF (310 mL) was added 2,6-lutidine (68.4 mL, 587 mmol), DMAP (3.59 g, 29.4 mmol) and trityl chloride (82 g, 294 mmol). The reaction

mixture was slowly heated to 80 °C. The reaction mixture was stirred for 15 h at 80°C and then cooled to rt. The reaction was poured into aq. sat. NH₄Cl (1500 mL) and extracted with EtOAc (3x 1L). The combined organic phases were dried (Na₂SO₄), the drying agent filtered off and the filtrate concentrated *in vacuo*. The crude product was purified by chromatography on silica gel (gradient elution EtOAc-Heptane 0-100%) to yield the desired compound **i9** as an off white solid (85.79g): ¹H NMR (400 MHz, CDCl₃) δ 8.01 (s, 1H), 7.87 (s, 1H), 7.41 (m, 12H), 7.28 (m, 18H), 7.18 (d, *J* = 8.6 Hz, 2H), 6.95 (s, 1H), 6.80 (d, *J* = 8.6 Hz, 2H), 6.11 (d, *J* = 4.4 Hz, 1H), 4.77-4.67 (m, 2H), 4.62 (d, *J* = 11.6 Hz, 1H), 4.32 (q, *J* = 5.3 Hz, 1H), 4.21 (m, 1H), 3.79 (s, 3H), 3.49 (dd, *J* = 10.5, 3.3 Hz, 1H), 3.36 (dd, *J* = 10.5, 4.5 Hz, 1H), 2.66 (d, *J* = 5.7 Hz, 1H); LCMS (Method G) Rt 1.53 mins; *m/z* 872.0 (M+H⁺).

[00417] Step 3: Preparation of (2*R*,4*S*,5*R*)-4-((4-methoxybenzyl)oxy)-5-(6-(tritylamino)-9*H*-purin-9-yl)-2-((trityloxy) methyl)dihydrofuran-3(2*H*)-one (**i10**): To a solution of Dess-Martin Periodinane (DMP, 3.04 g, 7.17 mmol) in DCM (72 mL) at rt was added *tert*-butanol (0.713 mL, 7.45 mmol) and sodium carbonate (0.134 g, 1.261 mmol), followed by a dropwise addition over 1 h of a solution of compound **i9** (5.00 g, 5.73 mmol) in DCM (72 mL). The resulting reaction mixture was stirred at rt for 4 hours before additional DCM (110 mL) was added. After a further 3 h additional DMP (0.63 g) and DCM (50 mL) were added. The reaction stirred for 13 h and then quenched by addition of sat. Na₂S₂O₅ (40 mL), sat. NaHCO₃ (150 mL) and brine (50 mL). The organic phase was separated and the aqueous phase then re-extracted with DCM (2 x 150 mL). The combined DCM was dried (Na₂SO₄), the drying agent filtered off and the filtrate concentrated *in vacuo*. The crude material was purified by chromatography on silica gel (gradient elution EtOAc / heptane (0-80%)) to afford compound **i10** as a white foam (4.36 g): ¹H NMR (400 MHz, CDCl₃) δ 7.95 (s, 1H), 7.78 (s, 1H), 7.46-7.15 (m, 30H), 7.05 (d, *J* = 8.6 Hz, 2H), 6.98 (s, 1H), 6.73 (d, *J* = 8.6 Hz, 2H), 6.13 (d, *J* = 7.8 Hz, 1H), 5.23 (dd, *J* = 7.9, 0.8 Hz, 1H), 4.80 (d, *J* = 11.8 Hz, 1H), 4.72 (d, *J* = 11.8 Hz, 1H), 4.35 (ddd, *J* = 4.0, 2.4, 0.8 Hz, 1H), 3.76 (s, 3H), 3.52 (dd, *J* = 10.5, 4.0 Hz, 1H), 3.43 (dd, *J* = 10.5, 2.4 Hz, 1H); LCMS (Method C) Rt 1.53 mins; *m/z* 870.0 (M+H⁺).

[00418] Step 4: Preparation of (2*R*,3*S*,4*R*,5*R*)-4-((4-methoxybenzyl)oxy)-5-(6-(tritylamino)-9*H*-purin-9-yl)-2-((trityloxy)methyl)tetrahydrofuran-3-ol (**i11**): To a solution of compound **i10** (98 mg, 0.113 mmol) in DCM (3 mL) at -20°C was added

glacial AcOH (0.15 mL) followed by NaBH₄ (13 mg, 0.34 mmol). After 1 h the reaction mixture was quenched with 5% brine (20 mL) and extracted with EtOAc (25 mL). The organic phase was separated and dried (Na₂SO₄), the drying agent filtered off and the filtrate concentrated *in vacuo* to a white solid. The crude solid (3*S*:3*R* ratio 7:1) was slurried in hot MeOH (3 mL, warmed to 50 °C) with DCM (~0.5 mL) added dropwise and the suspension cooled. The mother liquor was decanted off and the solid was dried *in vacuo* (63 mg, 3*S*:3*R* ratio 13:1). Recrystallization from MeOH:DCM (4 mL, v/v 5:1) gave compound **i11** as a single diastereomer (ratio 50:1): ¹H NMR (400 MHz, CDCl₃) δ 7.90 (s, 1H), 7.74 (s, 1H), 7.48 – 7.13 (m, 32H), 6.95 – 6.84 (m, 2H), 5.80 (s, 1H), 4.68 (d, *J* 11.3 Hz, 1H), 4.49 (d, *J* 11.3 Hz, 1H), 4.36 (s, 1H), 4.33 – 4.27 (m, 1H), 4.23 (d, *J* 3 Hz, 1H), 3.83 (s, 3H), 3.59 – 3.52 (m, 2H); LCMS (Method H) Rt 1.76 mins; *m/z* 872.2 (M+H)⁺.

[00419] Step 5: Preparation of 9-((2*R*,3*S*,4*R*,5*R*)-4-fluoro-3-((4-methoxybenzyl)oxy)-5-((trityloxy)methyl)tetrahydro-furan-2-yl)-N-trityl-9*H*-purin-6-amine (**i12**): To a solution of compound **i11** (240 mg, 0.275 mmol) in anhydrous DCM (15 mL) at 0 °C was added anhydrous pyridine (0.223 mL, 2.75 mmol). After 5 min, diethylaminosulfur trifluoride (DAST, 0.182 mL, 1.38 mmol) was added dropwise. After 5 min, the cooling bath was removed and the reaction stirred for 4.5 h. The reaction mixture was diluted with chloroform (20 mL), dry silica gel was added, and the mixture concentrated *in vacuo* before adding toluene (20 mL) and concentrating to dryness *in vacuo*. The crude material was purified by chromatography on silica gel (gradient elution 10-50% EtOAc / heptane) to give the desired compound **i12** as a white solid (121 mg): ¹H NMR (400 MHz, CDCl₃) δ 7.93 (s, 1H), 7.82 (s, 1H), 7.42 – 7.20 (m, 30H), 7.13-7.05 (m, 3H), 6.74 (d, *J* 8.3 Hz, 2H), 6.09 – 6.05 (m, 1H), 5.15- 5.06 (m, 1H), 5.00 (dd, *J* 54.4, and 4.4 Hz, 1H), 4.60-4.50 (m, 2H), 4.49-4.39 (m, 1H), 3.77 (s, 3H), 3.51-3.38 (m, 1H), 3.32 (dd, *J* = 10.6, 4.0 Hz, 1H); ¹⁹F NMR (376.4 MHz, CDCl₃) δ -198.09; LCMS (Method I) Rt 1.27 mins; *m/z* 874.5 (M+H)⁺.

[00420] Step 6: Preparation of (2*R*,3*S*,4*S*,5*R*)-2-(6-amino-9*H*-purin-9-yl)-4-fluoro-5-(hydroxymethyl)tetrahydrofuran-3-ol (**i13**): To a solution of compound **i12** (70 mg, 0.080 mmol) in DCM (1 mL) was added TFA (0.5 mL, 6.49 mmol). After 45 min the reaction mixture was diluted with MeOH (10 mL) and concentrated *in vacuo*. The crude material was dissolved in MeOH (10 mL) and TEA (0.1 mL) was added before silica gel

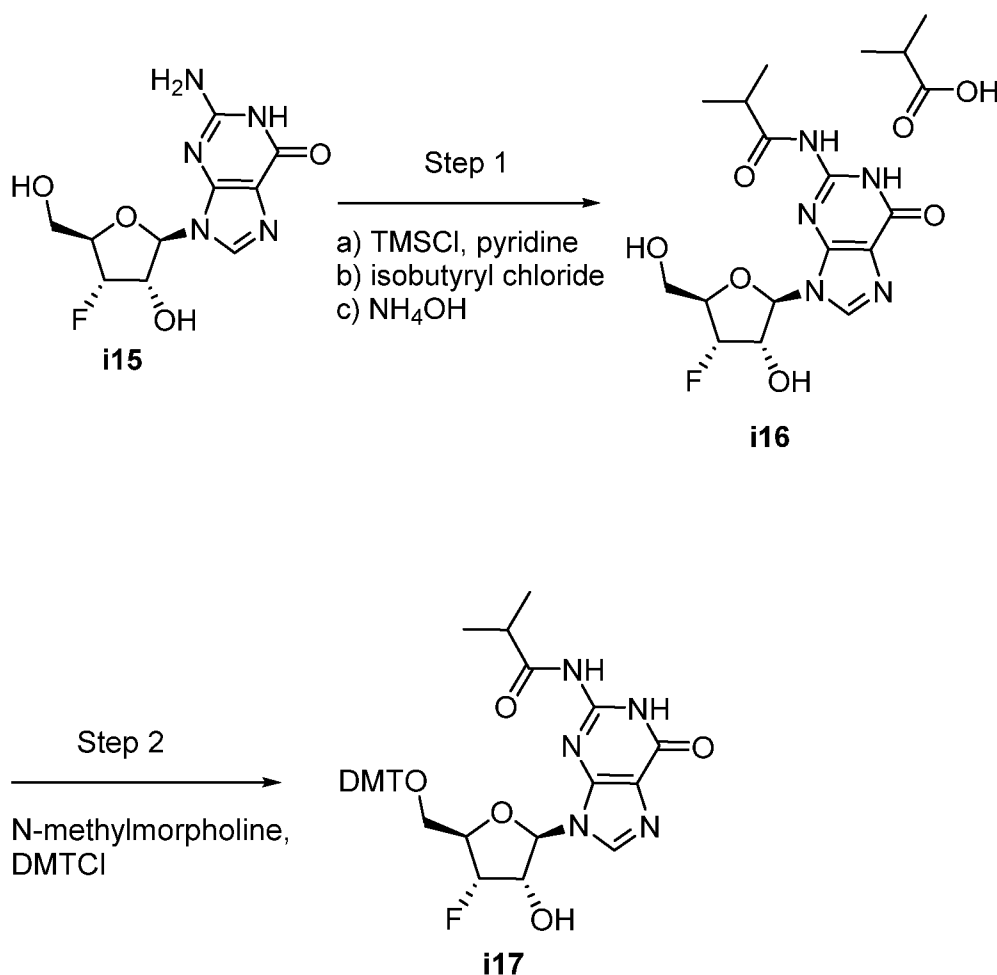
was added and the suspension concentrated *in vacuo*. The crude material was purified by chromatography on silica gel (gradient elution 0-10% MeOH / DCM) to give the desired compound **i13** as a white solid (21 mg) containing TEA.TFA salt and used as is: ^1H NMR (400 MHz, Methanol- d_4) δ 8.33 (s, 1H), 8.21 (s, 1H), 6.02 (d, J 7.9 Hz, 1H), 5.12 (dd, J 54.5, 4.3 Hz, 1H), 4.96 (ddd, J 25.1, 8.0, 4.3 Hz, 1H), 4.44 (dt, J 27.6, 2.5 Hz, 1H), 3.94 – 3.69 (m, 2H); ^{19}F NMR (376.4 MHz, Methanol- d_4) δ -200.02; LCMS (Method G) Rt 0.51 mins; m/z 270.1 ($\text{M}+\text{H}$) $^+$.

[00421] Step 7: Preparation of *N*-(9-((2*R*,3*S*,4*S*,5*R*)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (**i14**): To compound **i13** (3.88 g, 14.41 mmol) in pyridine (65 mL) at 0 °C was added benzoyl chloride (8.36 mL, 72.1 mmol) slowly followed by TMSCl (9.21 mL, 72.1 mmol). The reaction mixture was stirred while warming to rt for 4 h. After another 1 h the solution was quenched with water (35 mL), followed by conc. NH_4OH (17 mL) after 5 min resulting in a pale tan solid. The mixture was diluted with water (100 mL) and extracted with MeTHF (3 x 75 mL). The combined organic phases were dried (Na_2SO_4), the drying agent filtered off and the filtrate concentrated *in vacuo* to a tan semi-solid crude material, which was purified by chromatography on silica gel (gradient elution 0-20% MeOH / DCM) to give the desired compound **i14** (2.75g): ^1H NMR (400 MHz, CDCl_3) δ 8.78 (s, 1H), 8.09 (s, 1H), 8.08 – 8.01 (m, 2H), 7.66 (t, J = 7.4 Hz, 1H), 7.57 (t, J = 7.5 Hz, 2H), 6.13 (br s, 1H), 5.92 (d, J = 7.9 Hz, 1H), 5.41 – 5.11 (m, 2H), 4.60 (d, J = 28.4 Hz, 1H), 4.13 – 3.98 (m, 2H), 3.86 (d, J = 13.0 Hz, 1H). ^{19}F NMR (376.4 MHz, CDCl_3) δ -199.36; LCMS (Method G) Rt 0.72 mins; m/z 374.2 ($\text{M}+\text{H}$) $^+$.

[00422] Step 8: Preparation of *N*-(9-((2*R*,3*S*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (**i6**): To compound **i14** (2.73 g, 10.14 mmol) in pyridine (55 mL) was added DMTCl (4.12 g, 12.17 mmol) in one portion. The reaction was stirred at rt for 72 h before the yellowish solution was quenched by addition of MeOH (20 mL) and then concentrated *in vacuo* to a semi-solid following addition of toluene (2 x 50 mL) to azeotrope residual pyridine. The resulting material was dissolved in DCM (100 mL), washed with sat. NaHCO_3 (100 mL), brine then dried (Na_2SO_4). The drying agent was filtered off and the filtrate evaporated *in vacuo*. The resulting residue was purified by chromatography on silica gel (gradient elution 0-10% MeOH / DCM with 0.04% TEA) to

give compound **i6** as a white solid (3.70g): ^1H NMR (400 MHz, CDCl_3) δ 9.16 (s, 1H), 8.64 (s, 1H), 8.23 (s, 1H), 7.99 (d, J 7.5 Hz, 2H), 7.59 (t, J 7.4 Hz, 1H), 7.48 (t, J 7.6 Hz, 2H), 7.41 – 7.31 (m, 3H), 7.31 – 7.11 (m, 7H), 6.79 (d, J 8.9 Hz, 4H), 6.16 (d, J 7.3 Hz, 1H), 5.77 (br s, 1H), 5.27 – 5.10 (m, 2H), 4.53 (dt, J 28.0 Hz, 3.4 Hz, 1H), 3.77 (s, 6H), 3.51 (dd, J 10.7, 3.7 Hz, 1H), 3.34 (dd, J 10.7, 3.3 Hz, 1H); ^{19}F NMR (376.4 MHz, CDCl_3) δ -197.5; ^{13}C NMR (101 MHz, CDCl_3) δ 164.66, 158.64, 158.62, 152.60, 151.43, 149.34, 144.22, 141.66, 135.29, 135.13, 133.40, 132.93, 129.96, 128.87, 127.99, 127.93, 127.86, 127.07, 122.65, 113.26, 93.85, 92.02, 87.56 (d, J 144 Hz), 83.56 (d, J 23 Hz), 77.30, 74.63 (d, J 16 Hz), 62.82 (d, J 11 Hz), 55.26; LCMS (Method C) Rt 2.72 mins; m/z 676.3 ($\text{M}+\text{H}$) $^+$.

[00423] Intermediate **i17** *N*-(9-((2*R*,3*S*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)isobutyramide (used in Example 4) was prepared according to the following Scheme 1B:

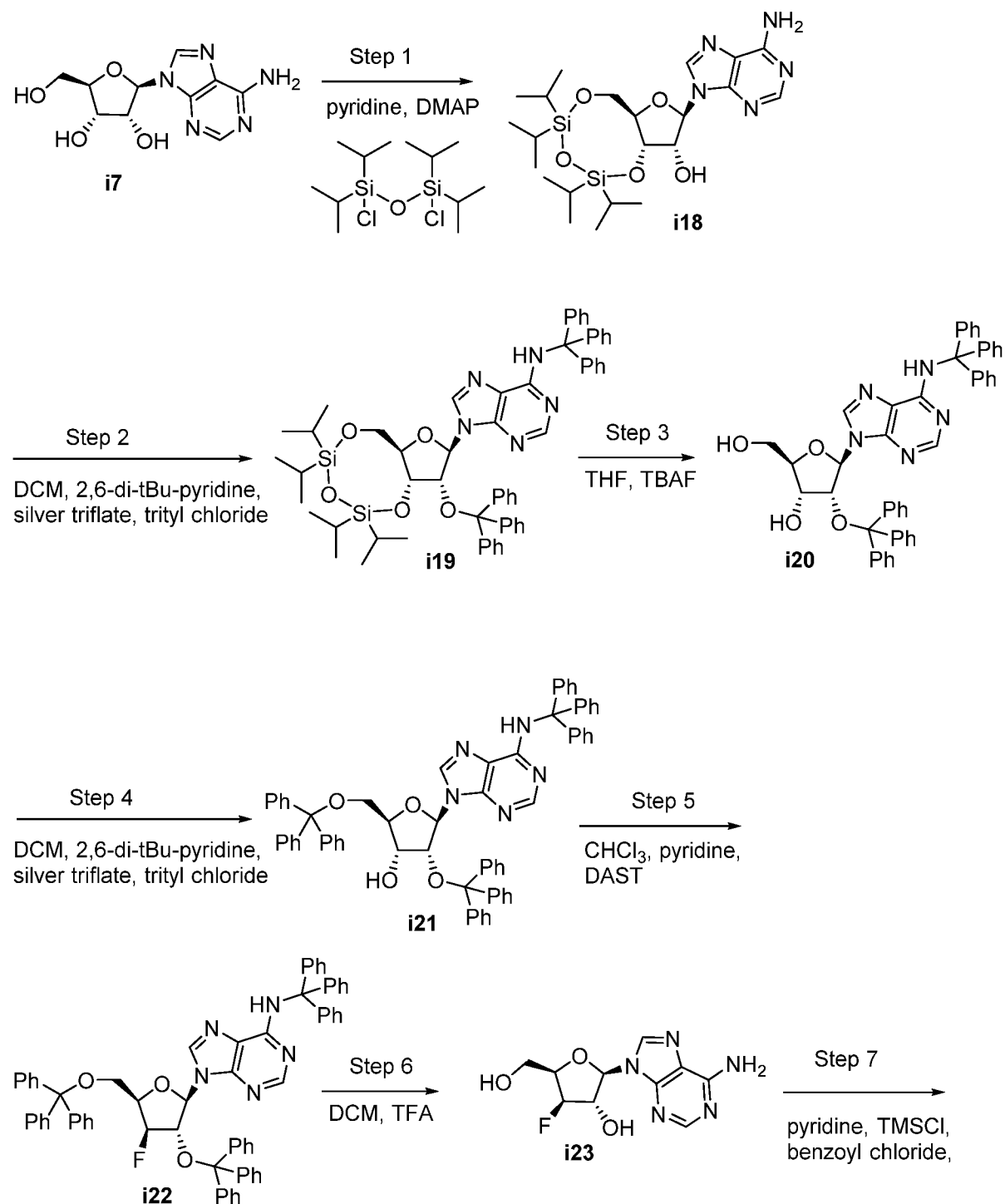


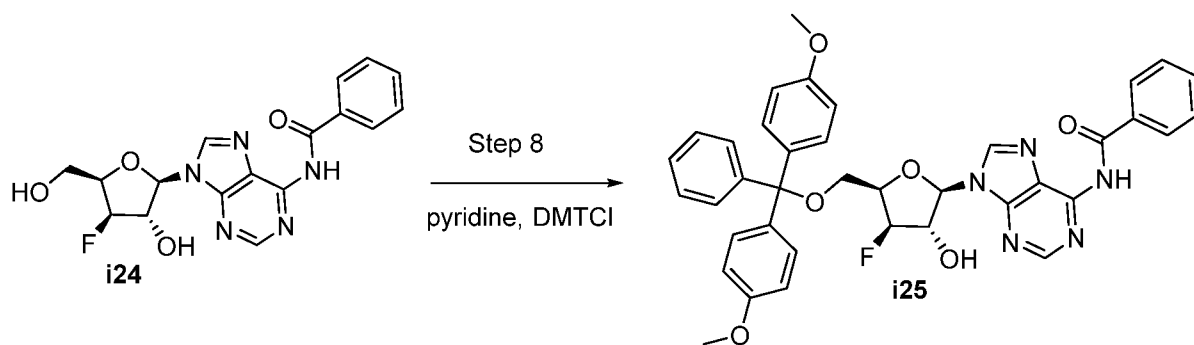
[00424] Step 1: Preparation of *N*-(9-((2*R*,3*S*,4*S*,5*R*)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)isobutyramide isobutyric acid salt (**i16**): To a solution of 2-amino-9-((2*R*,3*S*,4*S*,5*R*)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,9-dihydro-6*H*-purin-6-one (**i15**, 1.69 g, 5.91 mmol, Carbosynth, San Diego, CA) in pyridine (27.0, 334 mmol) at rt was added chlorotrimethylsilane (5.67 mL, 44.4 mmol) dropwise over 5 min. The reaction was stirred at rt for 2 h after which time the solution was cooled to 0 °C and isobutyryl chloride (1.86 mL, 17.74 mmol) was added dropwise over 10 min. The mixture was allowed to stir at 0 °C for 5 min and then warmed to rt for 3.5 h. The reaction was then cooled to 0 °C and quenched by addition of water (9.0 mL), stirred for 10 min at 0 °C and then warmed to rt. After stirring for 5 min, concentrated ammonia (28% aq. NH₄OH solution, 18 mL) was added and the mixture was stirred for 35 min, the mixture was then diluted with water (80 mL) and washed with DCM (40 mL). The aqueous layer was separated and concentrated *in vacuo* with the resulting crude material then purified by chromatography on silica gel (gradient elution 0-50% MeOH / DCM) to afford the compound **i16** (1.32 g): ¹H NMR (400 MHz, CD₃OD) δ 8.27 (s, 1H), 6.01 (d, *J* 7.6 Hz, 1H), 5.02–5.20 (m, 1H), 4.76–4.84 (m, 1H), 4.29–4.41 (m, 1H), 3.79 (d, *J* = 3.3 Hz, 2H), 2.72 (hept, *J* 6.8 Hz, 1H), 2.47 (hept, *J* 6.8 Hz, 1H), 1.23 (d, *J* 6.8 Hz, 6H), 1.12 (d, *J* 6.8 Hz, 6H); ¹⁹F NMR (376 MHz, CD₃OD) δ -200.84; LCMS (Method B) Rt 0.66 min; *m/z* 356.2 (M+H⁺).

[00425] Step 2: Preparation of *N*-(9-((2*R*,3*S*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)isobutyramide (**i17**): To a suspension of compound **i16** (2.18 g, 6.14 mmol) in 1:1 THF:CHCl₃ (400 mL) at rt was added *N*-methylmorpholine (2.70 mL, 24.54 mmol) followed by 4,4'-dimethoxytrityl chloride (8.32 g, 24.54 mmol) in a single portion. The mixture was stirred at rt for 35 min after which time the reaction was quenched by addition of sat. aq. NaHCO₃ solution (250 mL). The organic layer was separated layers and the aqueous layer further extracted with DCM (2 × 150 mL). The combined organic layers were dried (MgSO₄) the drying agent then filtered off and the filtrate concentrated *in vacuo* to give an oil. The resulting crude material was purified by chromatography on silica gel (gradient 0-60% EtOAc to remove *N*-methylmorpholine, followed by 0-60% MeOH/DCM) to afford to the compound **i17** (3.57 g): ¹H NMR (400 MHz, CD₃OD) δ 8.04 (s, 1H), 7.36–7.42 (m, 2H), 7.17–7.31 (m, 7H), 6.78–6.85 (m, 4H),

6.00 (d, J 7.1 Hz, 1H), 5.00–5.25 (m, 2H), 4.41 (m, 1H), 3.76 (s, 6H), 3.46 (dd, J 10.6, 4.3 Hz, 1H), 3.37 (dd, J 10.6, 3.0 Hz, 1H), 2.57 (hept, J 6.8 Hz, 1H), 1.19 (d, J 6.8 Hz, 3H), 1.16 (d, J 6.8 Hz, 3H); ^{19}F NMR (376 MHz, CD_3OD) δ -200.35; LCMS (Method C) Rt 2.16 min; m/z 658.3 ($\text{M}+\text{H}^+$).

[00426] Intermediate **i25** (used in Example 8) was prepared according to the following Scheme 1C:





[00427] Step 1 Preparation of (6*aR*,8*R*,9*R*,9*aS*)-8-(6-amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-9-ol (**i18**): To a suspension of adenosine (**i7**, 5.80 g, 21.70 mmol) in pyridine (40 mL) at 0 °C was added DMAP (1.33 g, 10.85 mmol) followed by 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (7.64 mL, 23.87 mmol). The resulting mixture was warmed to rt and allowed to stir 5 h after which time the mixture was concentrated *in vacuo* to give a yellow oil. A mixture of MeCN and diethyl ether were added to the oil resulting in the formation of a white precipitate. The resulting precipitate was filtered off and collected to give compound **i18** as a white solid (8.61 g): ¹H (400 MHz, CD₃OD) δ 8.28 (s, 1H), 8.16 (s, 1H), 5.97 (d, *J* 0.8 Hz, 1H), 4.83 (dd, *J* 8.4, 4.9 Hz, 1H), 4.57 (d, *J* = 4.8 Hz, 1H), 4.22–4.13 (m, 2H), 4.06 (dd, *J* 12.8, 2.5 Hz, 1H), 1.15–1.05 (m, 28H); LCMS (Method C) Rt 3.20 mins; *m/z* 510.1 (M+H⁺).

[00428] Step 2: Preparation of 9-((6*aR*,8*R*,9*R*,9*aR*)-2,2,4,4-tetraisopropyl-9-(trityloxy)tetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl)-*N*-trityl-9*H*-purin-6-amine (**i19**): To a suspension of compound **i18** (15.52 g, 30.4 mmol) in DCM (200 mL) at rt was added 2,6-di-*tert*-butylpyridine (23.94 mL, 107 mmol) followed by silver triflate (22.69, 88 mmol), the resulting mixture was sonicated to dissolve most of the silver triflate and then cooled to 0 °C. Triphenylmethyl chloride (25.4 g, 88 mmol) was then added portion wise and the solution turned dark yellow in color and a precipitate was observed, after 5 min the reaction was allowed to warm to rt. The reaction was allowed to stir for 18 h after which time the mixture was filtered through Celite™ and washed with DCM. The filtrate was washed with sat. aq. NaHCO₃ solution, brine, and the combined organic phases were dried (MgSO₄), the drying agent filtered off and the filtrate concentrated *in vacuo*. The resulting crude material was triturated from MeCN and filtered to afford compound **i19** as a white solid (24.68 g): ¹H NMR (400 MHz,

CDCl₃) δ 7.79 (s, 1H), 7.58 (d, *J* 6.0 Hz, 6H), 7.40–7.15 (m, 24H), 6.79 (s, 1H), 6.72 (s, 1H), 5.59 (dd, *J* 9.5, 5.2 Hz, 1H), 5.19 (d, *J* = 5.2 Hz, 1H), 4.70 (s, 1H), 4.30 (d, *J* 9.5 Hz, 1H), 4.06 (s, 2H), 1.30–1.03 (m, 21H), 0.94 (s, 3H), 0.80 (d, *J* = 7.0 Hz, 3H); LCMS (Method C) Rt 4.83 mins; *m/z* 995.3 (M+H⁺).

[00429] Step 3: Preparation of (2*R*,3*R*,4*R*,5*R*)-2-(hydroxymethyl)-5-(6-(tritylamino)-9*H*-purin-9-yl)-4-(trityloxy)tetra-hydrofuran-3-ol (**i20**): To a solution of compound **i19** (24.7 g, 24.84 mmol) in THF (100 mL) at rt was added TBAF (1.0 M solution in THF, 49.7 mL, 49.7 mmol) dropwise. The resulting mixture was stirred at rt for 4 h after which time the reaction was concentrated *in vacuo* to give an oil. The crude material was then triturated from MeOH and filtered to afford compound **i20** as a white solid (18.47 g): ¹H NMR (400 MHz, DMSO-d₆) δ 8.30 (s, 1H), 7.69 (s, 1H), 7.56 (s, 1H), 7.44–7.36 (m, 6H), 7.32 (t, *J* 7.6 Hz, 6H), 7.27–7.16 (m, 9H), 7.12–6.96 (m, 9H), 6.05 (d, *J* 7.3 Hz, 1H), 5.47 (s, 1H), 5.01 (s, 1H), 4.79 (d, *J* 11.9 Hz, 1H), 4.02–3.78 (m, 1H), 3.43 (d, *J* 14.7 Hz, 1H), 3.28 (s, 1H), 3.15 (d, *J* 4.7 Hz, 1H); HRMS (Method E) calculated for C₄₈H₄₂N₅O₄ requires *m/z* 752.3237 found 752.3240 (M+H⁺).

[00430] Step 4: Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(6-(tritylamino)-9*H*-purin-9-yl)-4-(trityloxy)-2-((trityloxy)methyl) tetrahydrofuran-3-ol (**i21**): To a solution of compound **i20** (18.05 g, 24.01 mmol) in DCM (200 mL) at rt was added 2,6-di-tert-butylpyridine (6.47 mL, 28.8 mmol) followed by silver triflate (7.40 g, 28.8 mmol) and the resulting mixture was sonicated to dissolve most of the silver triflate and then the mixture cooled to 0 °C. Triphenylmethyl chloride (8.03 g, 28.8 mmol) was added portion wise and the solution turned a dark brown color and a precipitate was observed; after 5 min the reaction was allowed to warm to rt. The reaction was allowed to stir for 4 h after which time the mixture was filtered through Celite™ and washed with DCM. The filtrate was washed with sat. aq. NaHCO₃ solution, brine, dried (Na₂SO₄), the drying agent then filtered off and the filtrate concentrated *in vacuo* to give a white solid. The resulting crude material was purified by chromatography on silica gel (isocratic elution 10% EtOAc / DCM) to afford compound **i21** as a white solid (16 g): ¹H NMR (400 MHz, CDCl₃) δ 7.95 (s, 1H), 7.91 (s, 1H), 7.48–7.38 (m, 6H), 7.38–7.14 (m, 35H), 7.09 (t, *J* 7.7 Hz, 6H), 7.03 (s, 1H), 6.35 (d, *J* 7.4 Hz, 1H), 5.17 (s, 1H), 4.07 (s, 1H), 3.28 (d, *J* 14.0 Hz, 1H), 3.02 (d, *J* 13.8 Hz, 1H), 2.86 (d, *J* 4.5 Hz, 1H); LCMS (Method C) Rt 4.20 mins; *m/z* 995.2 (M+H⁺).

[00431] Step 5: Preparation of 9-((2*R*,3*S*,4*S*,5*R*)-4-fluoro-3-(trityloxy)-5-((trityloxy)methyl)tetrahydrofuran-2-yl)-*N*-trityl-9*H*-purin-6-amine (**i22**): To a solution of compound **i21** (11.51 g, 11.58 mmol) in CHCl₃ (75 mL) at 0 °C was added pyridine (14.05 mL, 174 mmol) followed by dropwise addition of DAST (8.05 mL, 57.9 mmol). After 5 min the mixture was allowed to warm to rt and stirred at that temperature for 15 h after which time the reaction was quenched by addition of sat. aq. NaHCO₃ solution and diluted with DCM. The phases were separated and the organic phase was further washed with sat. aq. NaHCO₃ followed by brine. The combined organic layers were dried (Na₂SO₄) the drying agent then filtered off and the filtrate concentrated *in vacuo* to give a yellow oil. The resulting crude material was purified by chromatography on silica gel (gradient elution 0-10% EtOAc / CH₂Cl₂) to afford compound **i22** (5.02 g): ¹H NMR (400 MHz, DMSO-d₆) δ 7.92 (d, *J* 15.4 Hz, 2H), 7.47–7.00 (m, 47H), 6.16 (s, 1H), 4.69 (d, *J* 20.4 Hz, 1H), 4.32 (dt, *J* 30.3, 7.3 Hz, 1H), 4.15 (d, *J* 50.7 Hz, 1H), 3.28 - 3.18 (m, 1H), 3.17–3.08 (m, 1H); ¹⁹F NMR (376 MHz, DMSO-d₆) δ -197.98; LCMS (Method E) Rt 3.21 mins; *m/z* 996.4 (M+H⁺).

[00432] Step 6: Preparation of (2*R*,3*S*,4*R*,5*R*)-2-(6-amino-9*H*-purin-9-yl)-4-fluoro-5-(hydroxymethyl)tetrahydrofuran-3-ol (**i23**): To a solution of compound **i22** (10.3 g, 10.34 mmol) in DCM (20 mL) at 0 °C was added trifluoroacetic acid (11.95 mL, 155 mmol) dropwise. After 10 min the reaction was allowed to warm to rt and allowed to stir for 16 h after which time water was added (15 mL) and the aqueous layer was washed with DCM (3 × 50 mL). The aqueous layer was separated and then lyophilized to give a clear oil which was purified by chromatography on silica gel (gradient elution 0-10% MeOH / DCM) to afford compound **i23** (2.57 g): ¹H NMR (400 MHz, DMSO-d₆) δ 8.19 (s, 1H), 8.12 (s, 1H), 7.46 (s, 2H), 6.29 (s, 1H), 5.95 (s, 1H), 5.09 (d, *J* 52.0 Hz, 1H), 5.05 (br s, 1H), 4.75 (d, *J* 15.7 Hz, 1H), 4.41 - 4.23 (m, 1H), 3.87–3.61 (m, 2H); ¹⁹F NMR (376 MHz, DMSO-d₆) δ -200.82; LCMS (Method C) Rt 0.49 mins; *m/z* 270.1 (M+H⁺).

[00433] Step 7: Preparation of *N*-(9-((2*R*,3*S*,4*R*,5*R*)-4-fluoro-3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (**i24**): To a solution of compound **i23** (2.62, 6.84 mmol) in pyridine (25 mL) at 0 °C was added chlorotrimethylsilane (4.37 mL, 34.2 mmol) dropwise. After 5 min the reaction was allowed to warm to rt and stirred for 10 min. Benzoyl chloride (3.97 mL, 34.2 mmol) was then added and the reaction was allowed to stir at rt for 4 h, upon completion the

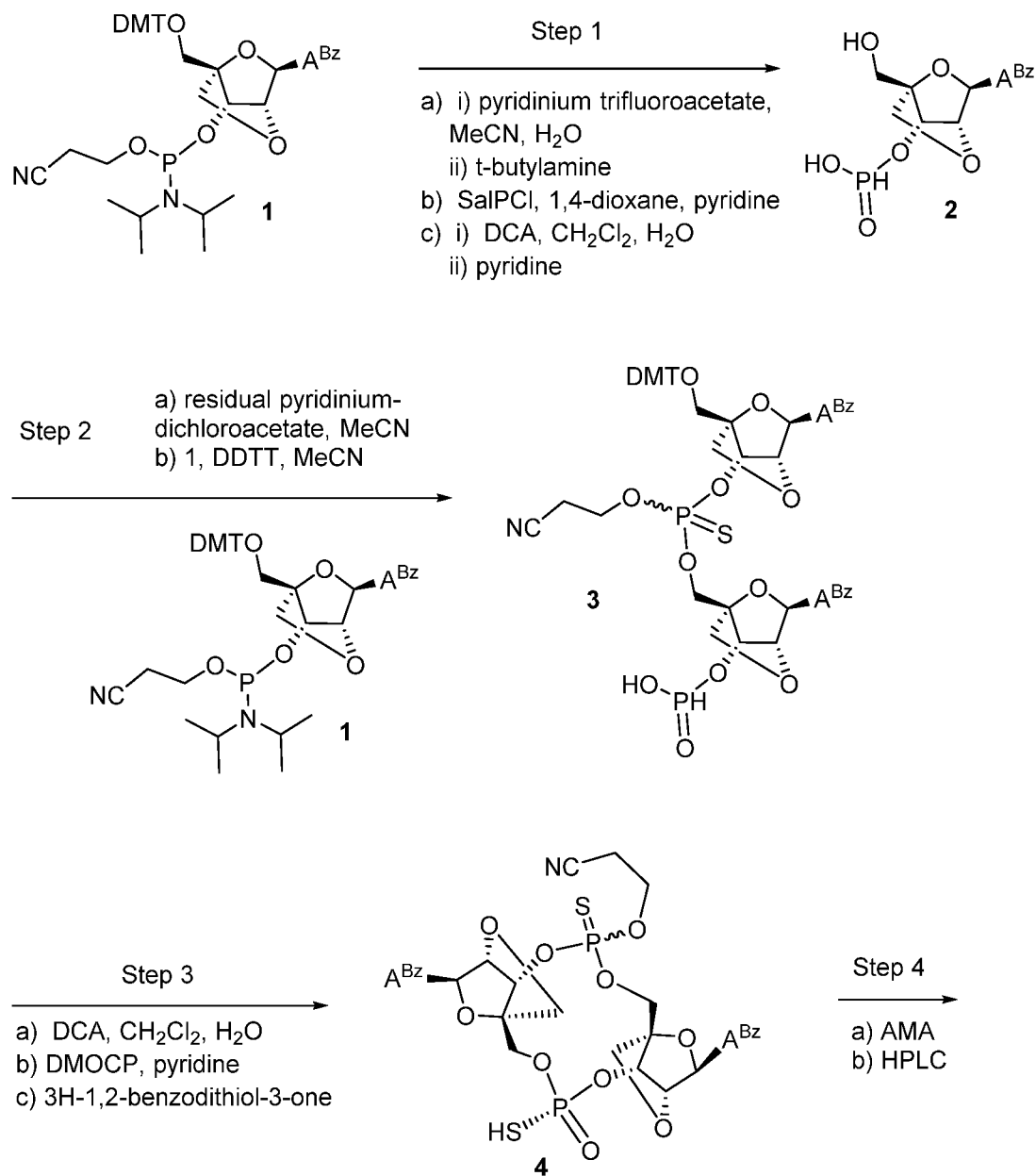
mixture was cooled to 0 °C and quenched by addition of water (3.0 mL). Concentrated ammonia (28% aq. NH₄OH solution, 6.0 mL) was then added and the mixture was allowed to warm to rt and stirred for 30 min after which time the mixture was concentrated *in vacuo*. The resulting material was diluted further with water and extracted with EtOAc with the organic layer then being dried (Na₂SO₄) and the drying agent filtered off; the filtrate was concentrated *in vacuo* to give an oil. The resulting crude material was purified by chromatography on silica gel (gradient elution 0-10% MeOH / DCM) to afford compound **i24** (2.68 g): ¹H NMR (400 MHz, DMSO-d₆) δ 11.22 (s, 1H), 8.78 (s, 1H), 8.43 (s, 1H), 8.02–8.08 (m, 2H), 7.61–7.69 (m, 1H), 7.52–7.59 (m, 2H), 6.38 (d, *J* 4.6 Hz, 1H), 6.11 (d, *J* 2.3 Hz, 1H), 5.03–5.23 (m, 2H), 4.81–4.88 (m, 1H), 4.30–4.46 (m, 1H), 3.69–3.87 (m, 2H); ¹⁹F NMR (376 MHz, DMSO-d₆) δ -200.62; LCMS (Method G) Rt 1.49 mins; *m/z* 374.1 (M+H⁺).

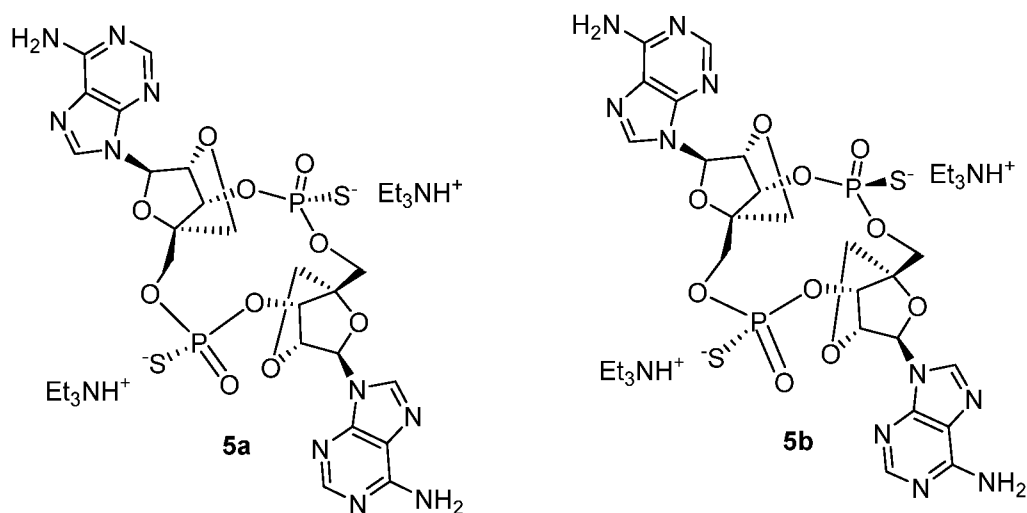
[00434] Step 8: Preparation of *N*-(9-((2*R*,3*S*,4*R*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (**i25**): To a solution compound **i24** (812 mg, 2.18 mmol) in pyridine (3.0 mL) at rt was added DMTCl (1.35 g, 3.91 mmol). The mixture was stirred for 20 h after which time the reaction was quenched by addition of MeOH and concentrated *in vacuo* to give an oil. The resulting crude material was purified by chromatography on silica gel (gradient elution 0-10% MeOH / DCM) to afford compound **i25** as a white solid (1.07 g): ¹H NMR (400 MHz, DMSO-d₆) δ 11.23 (s, 1H), 8.78 (s, 1H), 8.24 (s, 1H), 8.00–8.08 (m, 2H), 7.61–7.69 (m, 1H), 7.52–7.59 (m, 2H), 7.39–7.44 (m, 2H), 7.13–7.33 (m, 8H), 6.86 (dd, *J* 8.8, 8.1 Hz, 4H), 6.43 (d, *J* 4.8 Hz, 1H), 6.14 (d, *J* = 2.3 Hz, 1H), 5.11–5.30 (m, 1H), 4.84–4.94 (m, 1H), 4.52–4.67 (m, 1H), 3.73 (d, *J*=2.3 Hz, 6H); ¹⁹F NMR (376 MHz, DMSO-d₆) δ -199.41; LCMS (Method C) Rt 1.86 mins; *m/z* 676.2 (M+H⁺).

Example 2: Synthesis of 3'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) (5a) and 3'3'-RS-(2'O4'C-LNA-A)(2'O,4'C-LNA-A) (5b)

[00435] (2*R*,3*R*,3*aS*,5*R*,7*aR*,9*S*,10*R*,10*aS*,12*R*,14*aR*)-2,9-bis(6-amino-9*H*-purin-9-yl)-5,12-dimercaptotetrahydro-2*H*,7*H*,9*H*,14*H*-3,14*a*:10,7*a*-bis(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecine 5,12-dioxide (**5a**), also referred to as 3'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A), or dithio-(*Rp,Rp*)-cyclic-[2'O,4'C-LNA-A(3',5')p-2'O,4'C-LNA-A(3',5')p]; and

(2*R*,3*R*,3*aS*,5*R*,7*aR*,9*S*,10*R*,10*aS*,12*S*,14*aR*)-2,9-bis(6-amino-9*H*-purin-9-yl)-5,12-dimercaptotetrahydro-2*H*,7*H*,9*H*,14*H*-3,14a:10,7a-bis(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecine 5,12-dioxide (**5b**), also referred to as 3'3'-RS-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A), or dithio-(*Rp,Sp*)-cyclic-[2'O,4'C-LNA-A(3',5')p-2'O,4'C-LNA-A(3',5')p], were prepared according to the following Scheme 2:





[00436] Step 1: Preparation of (1*S*,3*R*,4*R*,7*S*)-3-(6-benzamido-9*H*-purin-9-yl)-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl hydrogen phosphonate (**2**): To a solution of (1*R*,3*R*,4*R*,7*S*)-3-(6-benzamido-9*H*-purin-9-yl)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl (2-cyanoethyl) diisopropylphosphoramidite (**1**, 1.0 g, 1.2 mmol, Exiqon, Woburn, MA) in MeCN (10 mL) and H₂O (0.05 mL) was added pyridinium trifluoroacetate (270 g, 1.5 mmol). After 25 min, to the stirring reaction mixture at rt was added *tert*-butyl amine (5.0 mL). After 15 min, the reaction solution was concentrated *in vacuo* and water was removed as an azeotrope after concentration with MeCN (3 x 15 mL) to obtain a white foam. To a solution of the white foam in 1,4-dioxane (13 mL) was added a solution of SalPCl (226 mg, 1.0 mmol), in 1,4-dioxane (5 mL). After 7 min, to the cloudy white mixture was added pyridine (3 mL). After 1 h, to the cloudy reaction mixture was introduced water (2 mL). After 5 min, the mixture was poured into a 1N NaHCO₃ solution (100 mL). The solution was extracted with EtOAc (3 x 100 mL) and the organic layer was condensed to dryness *in vacuo*. The residue was dissolved in DCM (10 mL) to give a white mixture. To this solution was added water (150 μ L) and 9 % (v/v) solution of DCA in DCM (10 mL). After 10 min of stirring at rt, to the orange solution was charged pyridine (1.5 mL). The resulting clear solution was concentrated *in vacuo* and water was removed as an azeotrope after concentration with MeCN (3 x 20 mL). On the last evaporation, the resulting cloudy slurry of compound **2** was left in MeCN (20 mL).

[00437] Step 2: Preparation of (1*R*,3*R*,4*R*,7*S*)-3-(6-benzamido-9*H*-purin-9-yl)-1-((((((1*R*,3*R*,4*R*,7*S*)-3-(6-benzamido-9*H*-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)-2,5-dioxabicyclo[2.2.1]heptan-7-yl hydrogen phosphonate (**3**): A solution of compound **1** (1.0 g, 1.2 mmol, Exiqon) in MeCN (10 mL) was dried through concentration *in vacuo*. This process was repeated two more times to remove water as an azeotrope. On the last azeotrope, to the solution of compound **1** in MeCN (10 mL) was introduced ten 3Å molecular sieves and the solution was stored under an atmosphere of nitrogen. To a stirred mixture of compound **2** with residual pyridinium dichloroacetate in MeCN (20 mL) was added the solution of compound **1** in MeCN (10 mL). After 40 min, to the stirred mixture was added DDTT (263 mg, 1.3 mmol). After 70 min, the yellow solution was concentrated *in vacuo* to give compound **3** as a yellow paste.

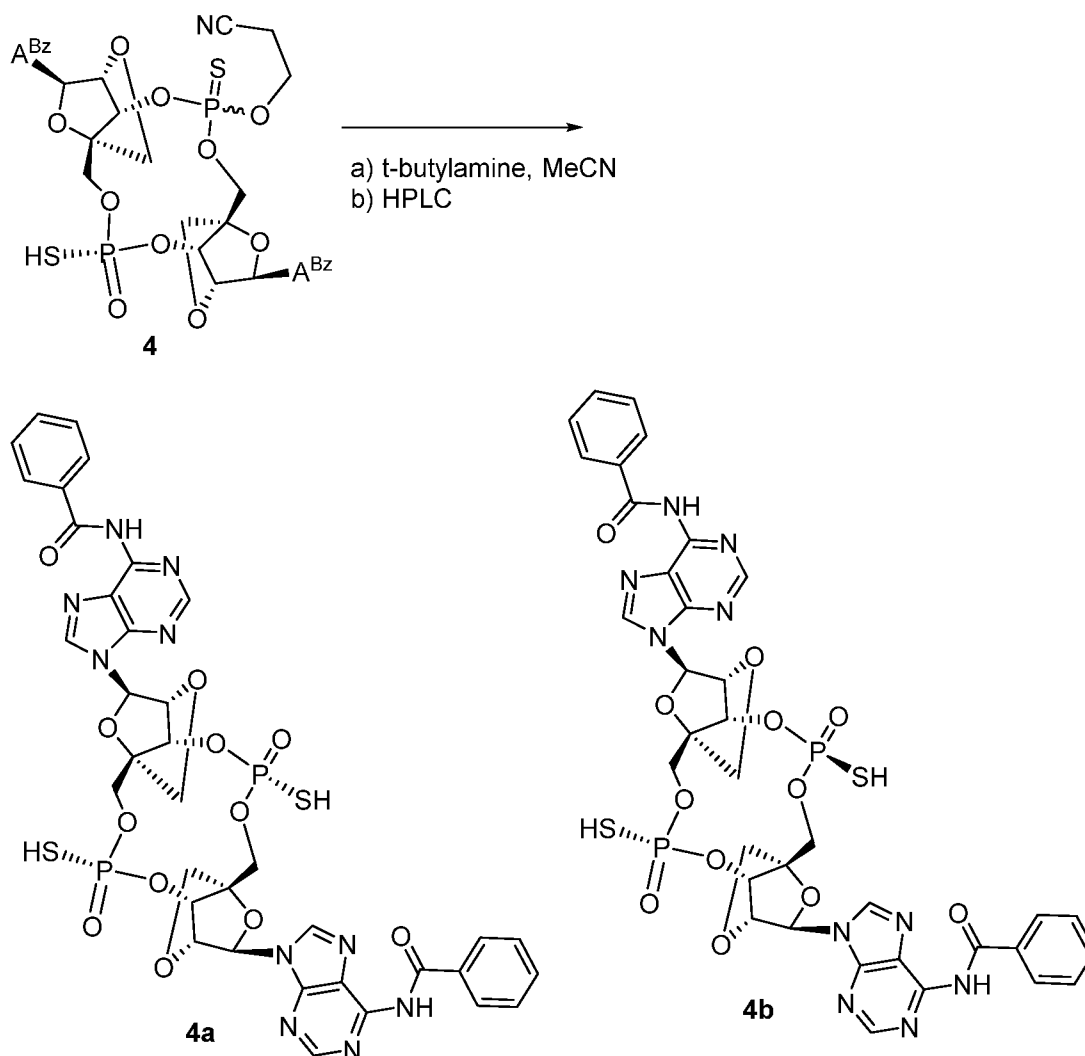
[00438] Step 3: Preparation of N,N'-(((2*S*,3*R*,3*aS*,7*aR*,9*R*,10*R*,10*aS*,12*R*,14*aR*)-5-(2-cyanoethoxy)-12-mercapto-12-oxido-5-sulfidotetrahydro-2*H*,7*H*,9*H*,14*H*-3,14*a*:10,7*a*-bis(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecine-2,9-diyl)bis(9*H*-purine-9,6-diyl))dibenzamide (**4**): To a solution of compound **3** in DCM (30 mL) was added water (180 µL) and 8.5 % (v/v) solution of DCA in DCM (20 mL). After stirring for 15 min at rt, to the red-orange solution was introduced pyridine (10 mL). The resulting yellow solution was concentrated *in vacuo* until approximately 10 mL of the yellow mixture remained. To the yellow mixture was introduced pyridine (30 mL) and the mixture was concentrated *in vacuo* until approximately 10 mL of the yellow mixture remained. To the yellow mixture was added pyridine (30 mL) and the mixture was concentrated *in vacuo* until approximately 10 mL of the yellow mixture remained. To the stirred yellow mixture in pyridine (50 mL) was added DMOCP (631 mg, 3.4 mmol). After 15 min, to the brownish yellow solution was added water (750 µL), followed immediately by the introduction of 3*H*-1,2-benzodithiol-3-one (304 mg, 1.8 mmol). After 30 min, the brownish yellow solution was poured into a 1N aqueous NaHCO₃ solution (250 mL). After 15 min, the biphasic mixture was extracted with EtOAc (200 mL). After separation, the aqueous layer was back extracted with EtOAc (2 x 150 mL). The organic extracts were combined and concentrated *in vacuo*. To the concentrated yellow oil was added toluene (20 mL) and the mixture was evaporated *in vacuo* to remove residual pyridine. This procedure was repeated again with toluene (30 mL). The resulting oil was purified by silica gel chromatography (0% to 50% MeOH in DCM) to provide a mixture of compound **4** (604 mg, 52% yield) as beige solid.

[00439] Step 4: Preparation of 3'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) (**5a**) and 3'3'-RS-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) (**5b**): To a stirred solution of compound **4** (472 mg, 0.5 mmol) in EtOH (5.0 mL) was added AMA (6.5 mL) and the yellow solution was heated at 50 °C. After 2 h, the yellow solution was allowed to cool and concentrated *in vacuo*. The yellow residue in 10mM TEAA (3 mL) was purified by reverse phase silica gel chromatography (0% to 25% MeCN in 10 mM aqueous TEAA) to obtain compound **5a** (92 mg, 27% yield) as a white TEAH⁺ salt after lyophilization. LCMS-ESI: 712.95 [M-H]⁻ (calculated for C₂₂H₂₄N₁₀O₁₀P₂S₂: 714.56); R_t: 1.06 min by UPLC (20 mM NH₄OAc, 2% to 80% MeCN). ¹H NMR (400 MHz, 45 °C, D₂O) δ 8.45 (d, *J* = 4.4 Hz, 2H), 8.30 (d, *J* = 5.6 Hz, 2H), 6.36 (d, *J* = 4.4 Hz, 2H), 5.12 (s, 4H), 4.63 (d, *J* = 12.4 Hz, 2H), 4.34-4.24 (m, 6H), 3.33 (q, *J* = 7.2 Hz, 12H), 2.09 (m, 1H), 1.40 (t, *J* = 5.2 Hz, 18H). ³¹P NMR (45 °C, D₂O) δ 54.57.

[00440] The Rp,Sp isomer was also isolated after purification in the reverse phase chromatography step, to provide compound **5b** (35 mg, 10% yield) as the TEAH⁺ salt after lyophilization. LCMS-ESI: 712.95 [M-H]⁻ (calculated for C₂₂H₂₄N₁₀O₁₀P₂S₂: 714.56); R_t: 1.01 min by UPLC (20 mM NH₄OAc, 2% to 80% MeCN). ¹H NMR (400 MHz, 45 °C, D₂O) δ 8.58 (s, 1H), 8.46 (s, 1H), 8.31 (s, 1H), 8.27 (s, 1H), 6.38 (s, 2H), 5.32 (s, 1H), 5.11 (s, 1H), 5.07 (d, *J* = 10.4 Hz, 2H), 4.62 (d, *J* = 11.2 Hz, 1H), 4.53 (d, *J* = 11.2 Hz, 1H), 4.41 – 4.31 (m, 4H), 4.24 (t, *J* = 16.4 Hz, 1H), 3.33 (q, *J* = 7.2 Hz, 10H), 1.41 (t, *J* = 7.2 Hz, 15H). ³¹P NMR (45 °C, D₂O) δ 55.33, 54.48.

[00441] The substantially pure compound **5a** (2'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A)) was co-crystalized with wild type STING protein, and the x-ray structure of this compound bound to STING confirmed the stereochemistry as depicted above in Scheme 2, compound **5a**. The corresponding stereoisomer having RS configuration on the phosphorus linkers was separated as compound **5b**, with a shorter retention time (1.01 min vs 1.06 min for the RR isomer **5a**). While additional compounds below have not yet been co-crystalized, experience with these compounds indicates that the Rp, Rp isomer consistently has a longer retention time than the corresponding Rp, Sp isomer, Sp, Rp isomer or Sp, Sp isomer. The identity of the Rp, Rp isomers in the following examples is further verified as having improved biological activity (e.g. binding to STING, induction of IFNβ) compared to the Rp, Sp isomer, Sp, Rp isomer or Sp, Sp isomer.

[00442] The compound *N,N'*-(((2*R*,3*R*,3*aS*,5*R*,7*aR*,9*R*,10*R*,10*aS*,12*R*,14*aR*)-5,12-dimercapto-5,12-dioxidotetrahydro-2*H*,7*H*,9*H*,14*H*-3,14*a*:10,7*a*-bis(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecine-2,9-diyl)bis(9*H*-purine-9,6-diyl))dibenzamide (**4a**), also referred to as 3'3'-RR-(2'O,4'C-LNA-BzA)(2'O,4'C-LNA-BzA), was prepared from compound **4** as follows:



To a stirred solution of compound **4** (57.39 mg, 0.06 mmol) in MeCN (1.5 mL) was added *tert*-butylamine (0.3 mL). After 2 h, the white, cloudy mixture was concentrated *in vacuo*. The beige residue in 10mM TEAA (3 mL) was purified by reverse phase silica gel chromatography (0% to 20% MeCN in 10 mM aqueous TEAA) to obtain compound **4a** (27.8 mg, 51% yield) as a white bis-TEAH⁺ salt after lyophilization. LCMS-ESI: 922.78 [M-H]⁻ (calculated for C₃₆H₃₂N₁₀O₁₂P₂S₂: 921.00); R_t: 1.40 min by UPLC condition (2% to 80% MeCN in 20 mM NH₄OAc). ¹H NMR (400 MHz, 45 °C, D₂O) δ 8.84 (s, 2H), 8.65 (s, 2H), 8.024 (d, *J* = 8.0 Hz, 4H), 7.74 (t, *J* = 7.6 Hz, 2H), 7.57 (t, *J* = 7.6 Hz, 2H), 6.47 (s, 2H), 5.18 (d, *J* = 9.2 Hz, 2H), 5.12 (s, 2H), 4.62 (d, *J* = 11.6 Hz, 2H), 4.35-4.31

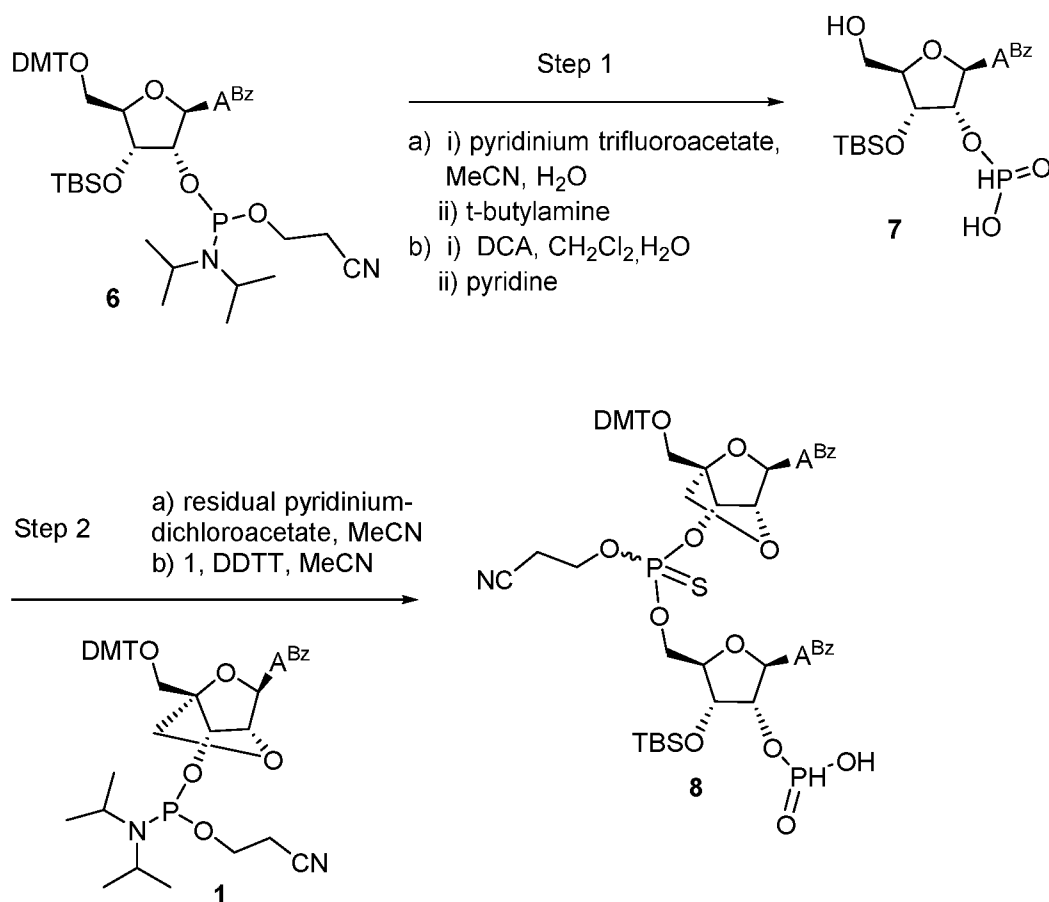
(m, 4H), 4.26 (d, $J = 8.4$ Hz, 2H), 3.32 (q, $J = 14.8, 7.2$ Hz, 14H), 2.09 (m, 1H), 1.41 (t, $J = 7.2$ Hz, 20H). ^{31}P NMR (45 °C, D_2O) δ 54.87. The RS isomer 3'3'-RS-(2'O,4'C-LNA-BzA)(2'O,4'C-LNA-BzA) **4b** was also isolated in the chromatography step.

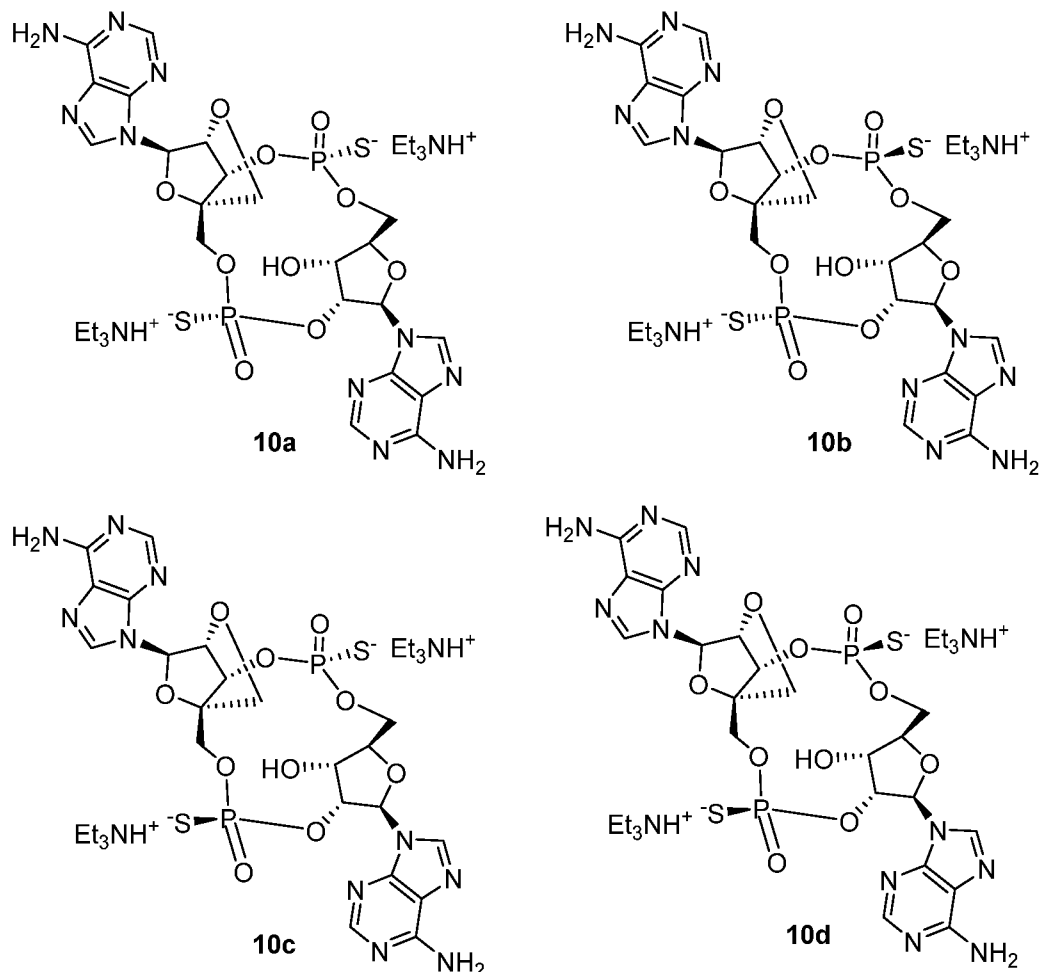
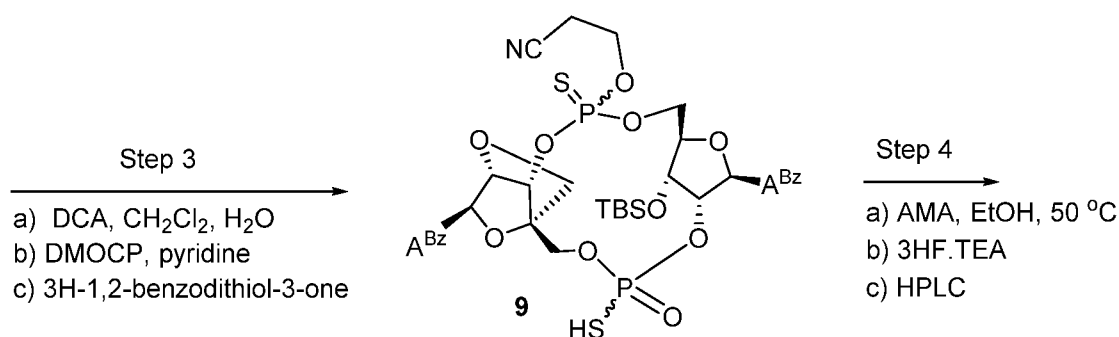
Example 3: Synthesis of 2'3'-RR-(A)(2'O,4'C-LNA-A) (10a),

2'3'-RS-(A)(2'O,4'C-LNA-A) (10b), 2'3'-SR-(A)(2'O,4'C-LNA-A) (10c) and

2'3'-SS-(A)(2'O,4'C-LNA-A) (10d)

[00443] 2'3'-RR-(A)(2'O,4'C-LNA-A) (**10a**), also referred to as dithio-(*Rp,Rp*)-cyclic-[A(2',5')p-2'O,4'C-LNA-A(3',5')p]; 2'3'-RS-(A)(2'O,4'C-LNA-A) (**10b**), also referred to as dithio-(*Rp,Sp*)-cyclic-[A(2',5')p-2'O,4'C-LNA-A(3',5')p]; 2'3'-SR-(A)(2'O,4'C-LNA-A) (**10c**), also referred to as dithio-(*Sp,Rp*)-cyclic-[A(2',5')p-2'O,4'C-LNA-A(3',5')p]; and 2'3'-SS-(A)(2'O,4'C-LNA-A) (**10d**), also referred to as dithio-(*Sp,Sp*)-cyclic-[A(2',5')p-2'O,4'C-LNA-A(3',5')p] were prepared according to the following Scheme 3:





[00444] Step 1: Preparation of (2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-3-yl hydrogen phosphonate (7): To a solution of (2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (**6**, 1.0 g, 1.0 mmol, ChemGenes, Wilmington, MA) in MeCN (8 mL) and H₂O (50 μ L) was added pyridinium trifluoroacetate (0.27 g, 1.4 mmol). After 15 min, to the stirring reaction mixture at rt was added *tert*-butyl amine (5.0 mL, 47.6 mmol). After 15 min, the

reaction solution was concentrated *in vacuo* and water was removed as an azeotrope after concentration *in vacuo* with MeCN (3 x 15 mL) to obtain a colorless foam. The colorless foam was dissolved in DCM (13 mL) to give a colorless solution. To this solution was added water (150 μ L) and 8 % (v/v) solution of DCA in DCM (10 mL). After 10 min of stirring at rt, the red solution was charged with pyridine (1.5 mL). The resulting clear solution was concentrated *in vacuo* and water was removed as an azeotrope after concentration *in vacuo* with MeCN (3 x 20 mL). On the last evaporation, the resulting peach slurry of compound **7** was left in MeCN (25 mL).

[00445] Step 2: Preparation of (2*R*,3*R*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((((((1*R*,3*R*,4*R*,7*S*)-3-(6-benzamido-9*H*-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-((tert-butyl)dimethylsilyl)oxy)tetrahydrofuran-3-yl hydrogen phosphonate (**8**): A solution of compound **1** (1.0 g, 1.14 mmol, Exiqon) in MeCN (25 mL) was dried through concentration *in vacuo*. This process was repeated two more times to remove water as an azeotrope. On the last azeotrope, to the solution of compound **1** in MeCN (10 mL) was introduced ten 3Å molecular sieves and the solution was stored under an atmosphere of nitrogen. To a stirred mixture of compound **7** with residual pyridinium dichloroacetate in MeCN (25 mL) was added the solution of compound **1** in MeCN (10 mL). After 1 h, to the stirred mixture was added DDTT (280 mg, 1.4 mmol). After 2 h, the yellow mixture was concentrated *in vacuo* to give compound **8** as a yellow paste.

[00446] Step 3: Preparation of Protected-dithio-2'3'-(A)(2'O,4'C-LNA-A) (**9**): To a solution of compound **8** in DCM (30 mL) was added water (180 μ L) and 15 % (v/v) solution of DCA in DCM (20 mL). After 10 min at rt, to the red solution was introduced pyridine (10 mL). The resulting yellow solution was concentrated *in vacuo* until approximately 20 mL of the yellow mixture remained. Addition of pyridine (30 mL) and concentration *in vacuo* to 10 mL was done twice, leaving a yellow mixture of approximately 10 mL. The stirred yellow mixture was brought to 50 mL with pyridine and DMOCP (629 mg, 3.4 mmol) was added. After 20 min, to the brownish yellow solution was added water (750 μ L), followed immediately by the introduction of 3*H*-1,2-benzodithiol-3-one (299 mg, 1.8 mmol). After 45 min, the brownish yellow solution was poured into a 1N aqueous NaHCO₃ solution (250 mL). After 30 min, the biphasic

mixture was extracted with EtOAc (200 mL). After separation, the aqueous layer was back extracted with EtOAc (1 x 200 mL, 1 x 150 mL). The organic extracts were combined and concentrated *in vacuo*. To the concentrated yellow oil was added toluene (75 mL) and the mixture was evaporated *in vacuo* to remove residual pyridine. This procedure was repeated twice with toluene (75 mL). The resulting oil was purified by silica gel chromatography (0% to 60% MeOH in DCM) to provide a mixture of compound **9** (733 mg, 68% yield) as a yellow paste.

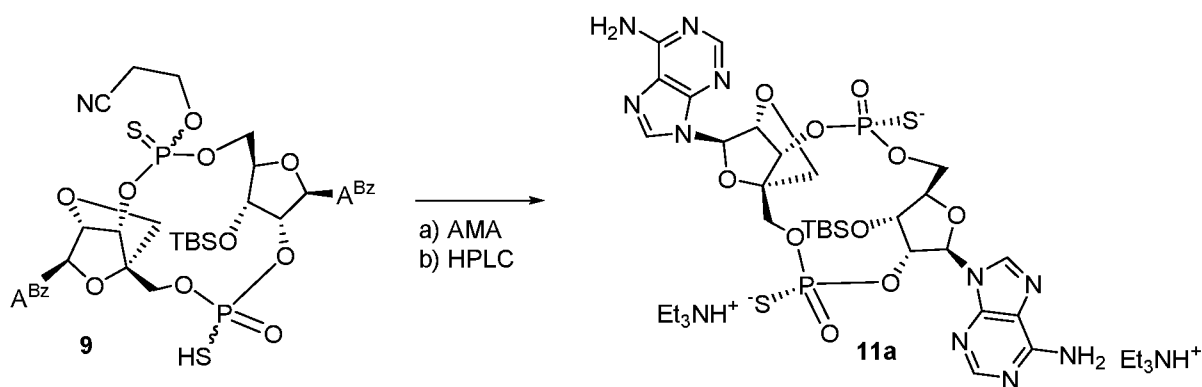
[00447] Step 4: Preparation of 2'3'-RR-(A)(2'O,4'C-LNA-A) (**10a**), 2'3'-RS-(A)(2'O,4'C-LNA-A) (**10b**), 2'3'-SR-(A)(2'O,4'C-LNA-A) (**10c**) and 2'3'-SS-(A)(2'O,4'C-LNA-A) (**10d**): To a stirred solution of compound **9** (600 mg, 0.56 mmol) in EtOH (19 mL) was added AMA (13.5 mL) and the yellow solution was heated at 50 °C. After 2 h, the clear solution was allowed to cool and concentrated *in vacuo*. To the residual solid (363 mg, 0.44 mmol) was introduced triethylamine trihydrofluoride (4.3 mL) and the yellow solution was heated to 40 °C. After 3 h, the yellow solution was allowed to cool to rt. This yellow solution was slowly added to a cooled solution of 1M TEAB (24 mL) and TEA (4.3 mL). The yellow mixture was allowed to stir for 1 h. The clear paste residue was purified by reverse phase silica gel chromatography (0% to 50% MeCN in 10 mM aqueous TEAA) to obtain compound **10a** (14 mg, 5% yield) as a white bis-TEAH⁺ salt after lyophilization. LCMS-ESI: 701.75 [M-H]⁻ (calculated for C₂₁H₂₄N₁₀O₁₀P₂S₂: 702.53); R_t: 6.602 min by LCMS conditions (20 mM NH₄OAc, 2% to 50%). ¹H NMR (400 MHz, 45 °C, D₂O) δ 8.61 (s, 1H), 8.42 (m, 3H), 6.46 (d, *J* = 5.2 Hz, 1H), 6.42 (s, 1H), 5.60 (br s, 1H), 5.43 (s, 1H), 5.4 (s, 1H), 5.00 (s, 1H), 4.63-4.60 (m, 1H), 4.87-4.47 (m, 2H), 4.41-4.38 (m, 2H), 3.41 (d, *J* = 5.2 Hz, 10H), 1.50 (s, 15H). ³¹P NMR (45 °C, D₂O) δ 55.46, 53.69.

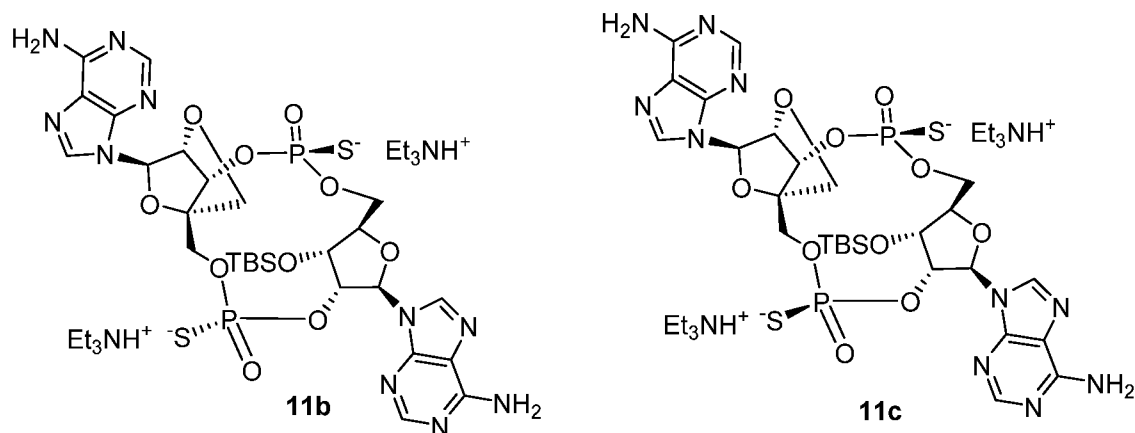
[00448] The isomer 2'3'-RS-(A)(2'O,4'C-LNA-A) **10b** was also isolated after purification in the reverse phase chromatography step as the TEAH⁺ salt after lyophilization (20 mg, 6 %). LCMS-ESI: 701.75 [M-H]⁻ (calculated for C₂₁H₂₄N₁₀O₁₀P₂S₂: 702.53); R_t: 6.198 min by LCMS conditions (20 mM NH₄OAc, 2% to 50%). ¹H NMR. (400 MHz, 45 °C, D₂O) δ 8.75 (s, 1H), 8.50-8.46 (m, 3H), 6.53-6.51 (m, 1H), 6.43 (d, *J* = 6 Hz, 1H), 5.53-5.50 (m, 2H), 5.28 (s, 1H), 4.40-4.36 (m, 4H), 3.41 (q, *J* = 6 Hz, 24H), 1.49 (t, *J* = 6 Hz, 36H). ³¹P NMR (45 °C, D₂O) δ 57.52, 55.43.

[00449] The isomer 2'3'-SR-(A)(2'O,4'C-LNA-A) **10c** was also isolated after purification in the reverse phase chromatography step as the bis-TEAH⁺ salt after lyophilization (27 mg, 9%). LCMS-ESI: 701.75 [M-H]⁻ (calculated for C₂₁H₂₄N₁₀O₁₀P₂S₂: 702.53); R_t: 6.064 min by LCMS conditions (20 mM NH₄OAc, 2% to 50. ¹H NMR. (400 MHz, 45 °C, D₂O) δ 8.77 (s, 1H), 8.59 (s, 1H), 8.48 (s, 1H), 8.45 (s, 1H), 6.49 (d, *J* = 8 Hz, 1H), 6.42 (s, 1H), 5.67 (br s, 1H), 5.47 (s, 1H), 5.36 (d, *J* = 6 Hz, 1H), 5.01 (s, 1H), 4.72-4.59 (m, 7H), 3.41 (q, *J* = 7.2 Hz, 13H), 1.49 (t, *J* = 6.4 Hz, 20H). ³¹P NMR (45 °C, D₂O) δ 54.79, 53.33.

[00450] The isomer 2'3'-SS-(A)(2'O,4'C-LNA-A) **10d** was also isolated after purification in the reverse phase chromatography step as the bis-TEAH⁺ salt after lyophilization (6.0 mg, 2%). LCMS-ESI: 701.75 [M-H]⁻ (calculated for C₂₁H₂₄N₁₀O₁₀P₂S₂: 702.53); R_t: 5.757 min by LCMS conditions (20 mM NH₄OAc, 2% to 50%). ¹H NMR. (400 MHz, 45 °C, D₂O) δ 8.84 (s, 1H), 8.66 (s, 1H), 8.46 (s, 1H), 8.42 (s, 1H), 6.49 (d, *J* = 8 Hz, 1H), 6.41 (s, 1H), 5.68-5.64 (m, 1H), 5.37 (s, 2H), 4.90 (br s, 1H), 4.55 (d, *J* = 22 Hz, 1H), 4.49-4.47 (m, 2H), 4.37-4.34 (m, 2H), 3.39 (q, *J* = 6.8 Hz, 10H), 1.47 (t, *J* = 7.2 Hz, 15H). ³¹P NMR (45 °C, D₂O) δ 57.34, 55.20.

[00451] The compounds 2'3'-RR-(3'-OTBS-A)(2'O,4'C-LNA-A) (**11a**); 2'3'-RS-(3'-OTBS-A)(2'O,4'C-LNA-A) (**11b**); and 2'3'-SS-(3'-OTBS-A)(2'O,4'C-LNA-A) (**11c**), were prepared from the compound **9** in Step 4 a) of Scheme 3 as follows:





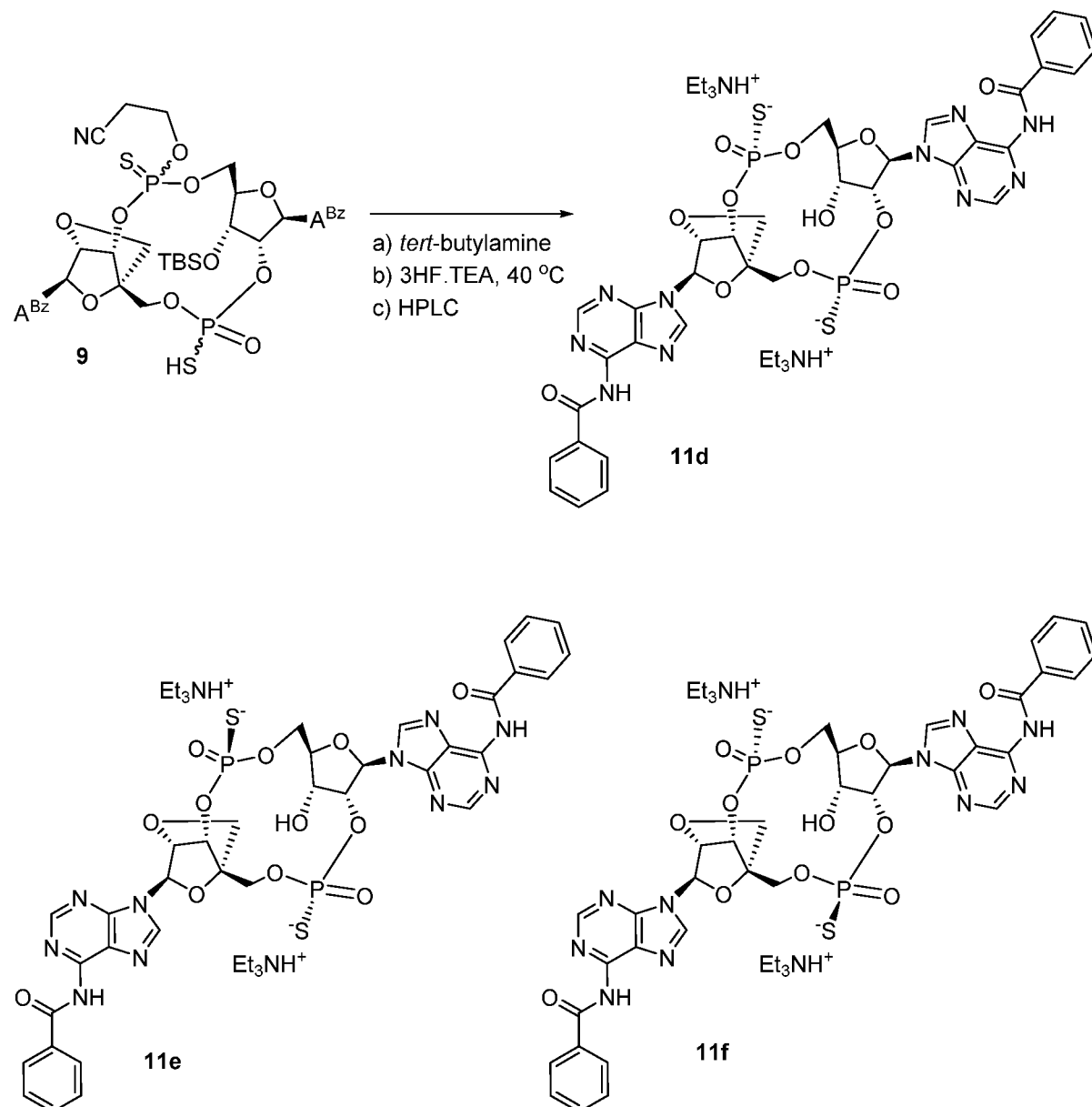
[00452] To a stirred solution of compound **9** (600 mg, 0.56 mmol) in EtOH (19 mL) was added AMA (13.5 mL) and the yellow solution was heated at 50 °C. After 2 h, the clear solution was allowed to cool and concentrated *in vacuo*. A portion of the residual intermediate was purified by reverse phase silica gel chromatography (0% to 50% MeCN in 10 mM aqueous TEAA) to obtain compound **11a** (6.4 mg, 6% yield) as a white bis-TEAH⁺ salt after lyophilization. LCMS-ESI: 815.75 [M-H]⁻ (calculated for C₂₇H₃₆N₁₀O₁₀P₂S₂Si : 816.80); R_t: 8.66 min by LCMS conditions (20 mM NH₄OAc, 2% to 50%). ¹H NMR (400 MHz, 45 °C, D₂O) δ 8.61 (s, 1H), 8.45-8.43- (m, 3H), 6.51 (d, *J* = 8 Hz, 1H), 6.43 (s, 1H), 5.60 (br s, 1H), 5.43 (s, 1H), 5.4 (s, 1H), 5.39-5.38 (m, 1H), 4.93-4.92 (m, 1H), 4.63-4.60 (m, 1H), 4.70-4.46 (m, 2H), 4.48-4.41 (m, 4H), 3.42 (q, *J* = 7.0 Hz, 11H), 2.1 (s, 0.5H), 1.49 (t, *J* = 7.0 Hz, 17H), 1.20 (s, 9H), 0.49 (s, 6H). ³¹P NMR (45 °C, D₂O) δ 55.94, 54.37.

[00453] 2'3'-RS-(3'-OTBS-A)(2'O,4'C-LNA-A) **11b** was also isolated by HPLC to provide 11.4 mg (11 % yield) as the bis-TEAH⁺ salt after lyophilization. LCMS-ESI: 815.75 [M-H]⁻ (calculated for C₂₇H₃₆N₁₀O₁₀P₂S₂Si : 816.80); R_t: 8.05 min by LCMS conditions (20 mM NH₄OAc, 2% to 50%). ¹H NMR. (400 MHz, 45 °C, D₂O) δ 8.73 (s, 1H), 8.50-8.46 (m, 3H), 6.56-6.54 (d, *J* = 8.0 Hz, 1H), 6.43 (s, 1H), 5.55 (s, 2H), 5.27 (s, 1H), 4.54 (d, *J* = 8.0 Hz, 1H), 4.39-4.33 (m, 3H), 3.41 (q, *J* = 7.6 Hz, 12H), 2.15 (s, 0.5), 1.51 (t, *J* = 7 Hz, 18H), 1.20 (s, 9H), 0.47 (s, 6H). ³¹P NMR (45 °C, D₂O) δ 57.31, 55.66.

[00454] 2'3'-SS-(3'-OTBS-A)(2'O,4'C-LNA-A) **11c** was also isolated by HPLC to provide 2.38 mg (2 % yield) as the bis-TEAH⁺ salt after lyophilization. LCMS-ESI: 815.75 [M-H]⁻ (calculated for C₂₇H₃₆N₁₀O₁₀P₂S₂Si : 816.80); R_t: 7.46 min by LCMS conditions (20 mM NH₄OAc, 2% to 50%). ¹H NMR. (400 MHz, 45 °C, D₂O) δ 8.82 (s,

1H), 8.70 (s, 1H), 8.48 (s, 1H), 8.44 (s, 1H), 6.53 (d, $J = 8.8$ Hz, 1H), 6.43 (s, 1H), 5.40 - 5.37 (m, 2H), 4.57 - 4.48 (m, 6H), 4.39 - 4.31 (m, 4H), 4.39-4.33 (m, 3H), 3.41 (q, $J = 7.2$ Hz, 17H), 1.50 (t, $J = 7.2$ Hz, 25H), 1.20 (s, 9H), 1.51-1.23 (m, 5H) 0.47 (s, 6H). ^{31}P NMR (45 °C, D_2O) δ 57.30, 55.01.

[00455] The compounds 2'3'-RR-(BzA)(2'O,4'C-LNA-BzA) (**11d**); 2'3'-RS-(BzA)(2'O,4'C-LNA-BzA) (**11e**); and 2'3'-SR-(BzA)(2'O,4'C-LNA-BzA) (**11f**) were prepared from the compound **9** from Step 3 of Scheme 3 as follows:



[00456] To a stirred solution of compound **9** (114.59 mg, 0.11 mmol) in MeCN (5 mL) was added *tert*-butylamine (1 mL). After 3 h, the yellow solution was condensed *in vacuo* to dryness. To the residual solid was introduced triethylamine trihydrofluoride

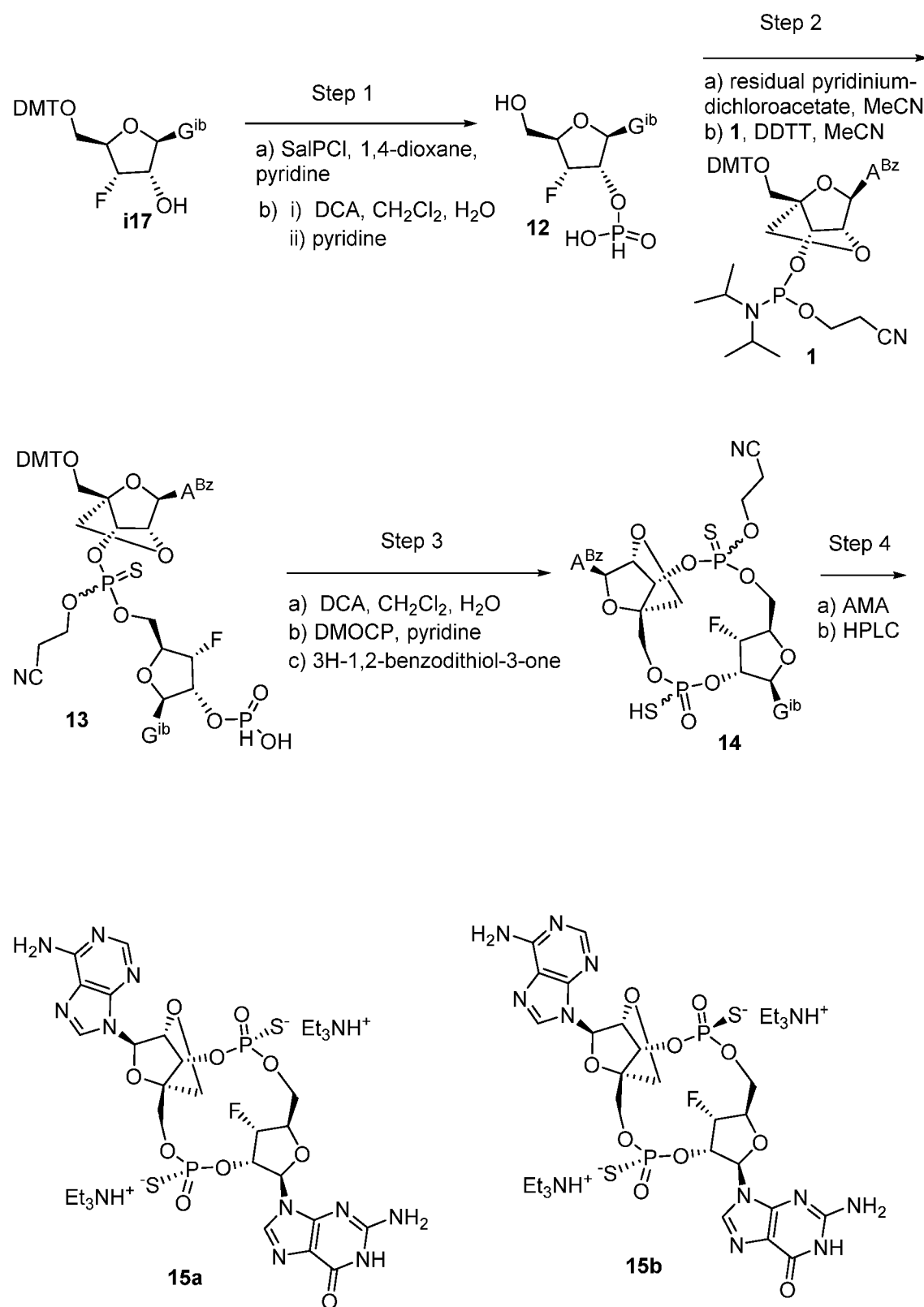
(1 mL) and the yellow solution was heated to 40 °C. After 2.5 h, the yellow solution was allowed to cool to rt. This yellow solution was slowly added to a cooled solution of 1M TEAB (8 mL) and TEA (1 mL). The yellow mixture was allowed to stir for 1 h. The solution was purified by reverse phase silica gel chromatography (0% to 50% MeCN in 10 mM aqueous TEAA) to obtain compounds **11d**, **11e** and **11f**. The compound **11d** (5 mg, 5% yield) was isolated as a white bis-TEAH⁺ salt after lyophilization. LCMS-ESI: 909.70 [M-H]⁻ (calculated for C₃₅H₃₂N₁₀O₁₂P₂S₂: 910.77); R_t: 9.70 min by LCMS conditions (MeCN in 20 mM NH₄OAc, 2% to 50%). ¹H NMR (400 MHz, 45 °C, D₂O) δ 8.86 (s, 1H), 8.84 (s, 1H), 8.73 (s, 1H), 8.73 (s, 1H), 8.05 (pseudo triplet, *J* = 8 Hz, 4H), 7.75 (m, 2H), 7.59 (dd, *J* = 8 Hz, 4H), 6.51 (d, *J* = 8 Hz, 1H), 6.48 (s, 1H), 5.79-5.74 (m, 1H), 5.57 (s, 1H), 5.34 (d, *J* = 6.8 Hz, 1H), 4.97 (s, 1H), 4.56-4.31 (m, 6H), 3.34 (q, *J* = 8.0 Hz, 11H), 1.43 (t, *J* = 8.0 Hz, 17H). ³¹P NMR (45 °C, D₂O) δ 54.77, 53.02.

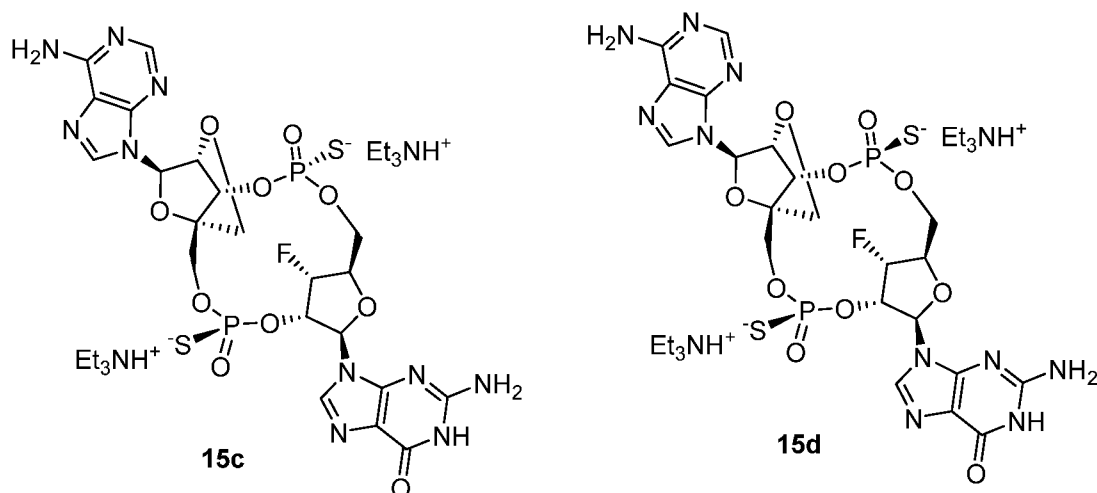
[00457] The Rp,Sp isomer was also isolated after purification in the reverse phase chromatography step, to provide compound **11e** (6.46, 6 % yield) as the bis-TEAH⁺ salt after lyophilization. LCMS-ESI: 909.70 [M-H]⁻ (calculated for C₃₅H₃₂N₁₀O₁₂P₂S₂: 910.77); R_t: 9.87 min by LCMS conditions (MeCN in 20 mM NH₄OAc, 2% to 50%). ¹H NMR (400 MHz, 45 °C, D₂O) δ 8.95 (s, 1H), 8.90 (s, 1H), 8.81 (s, 1H), 8.75 (s, 1H), 8.07 (dd, *J* = 20, 8 Hz, 4H), 7.77 (m, 3H), 7.69 – 7.60 (m, 5H), 6.57 (d, *J* = 8 Hz, 1H), 6.49 (s, 1H), 5.76-5.73 (m, 2H), 5.50 (s, 1H), 5.30 (d, *J* = 5.6 Hz, 1H), 4.88 (d, *J* = 3.2 Hz, 1H), 4.63-4.30 (m, 9H), 3.34 (q, *J* = 8.0 Hz, 10H), 1.43 (t, *J* = 8.0 Hz, 16H). ³¹P NMR (45 °C, D₂O) δ 56.73, 55.21.

Example 4: Synthesis of Synthesis of 2'3'-RR-(3'F-G)(2'O,4'C-LNA-A) (15a), 2'3'-RS-(3'F-G)(2'O,4'C-LNA-A) (15b), 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A) (15c) and 2'3'-SS-(3'F-G)(2'O,4'C-LNA-A) (15d)

[00458] 2'3'-RR-(3'F-G)(2'O,4'C-LNA-A) (**15a**), also referred to as dithio-(Rp,Rp)-cyclic-[3'F-G(2',5')p-2'O,4'C-LNA-A(3',5')p]; 2'3'-RS-(3'F-G)(2'O,4'C-LNA-A) (**15b**), also referred to as dithio-(Rp,Sp)-cyclic-[3'F-G(2',5')p-2'O,4'C-LNA-A(3',5')p]; 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A) (**15c**), also referred to as dithio-(Sp,Rp)-cyclic-[3'F-G(2',5')p-2'O,4'C-LNA-A(3',5')p]; and 2'3'-SS-(3'F-G)(2'O,4'C-LNA-A) (**15d**), also referred to as dithio-(Sp,Sp)-cyclic-[3'F-G(2',5')p-2'O,4'C-LNA-A(3',5')p] were prepared

according to the following Scheme 4:





[00459] Step 1: Preparation of (2*R*,3*S*,4*R*,5*R*)-4-fluoro-5-(hydroxymethyl)-2-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate (**12**): To a solution of N-(9-((2*R*,3*S*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)isobutyramide (**i17**, 806 mg, 1.2 mmol, see Example 1) in 1,4-dioxane (12 mL) and pyridine (5 mL) was added a solution of SalPCl (327 mg, 1.6 mmol) in 1,4-dioxane (5 mL). After 100 min, to the stirred reaction mixture at rt was introduced water (1.9 mL), and after 25 min, the resulting mixture was poured into a 1*N* aqueous NaHCO₃ solution (80 mL). This aqueous mixture was extracted with EtOAc (2 x 50 mL), followed by DCM (2 x 50 mL), and the layers were partitioned. The EtOAc and DCM extracts were combined and concentrated to dryness *in vacuo*. The residue was purified by normal phase silica gel chromatography (1% to 50%, 0.5% pyridine in DCM) to obtain an intermediate compound (500 mg, 0.69 mmol, 56% yield) as a clear oil. The colorless oil was dissolved in DCM (15 mL) to give a colorless solution. To this solution was added water (150 μ L) and an 11 % (v/v) solution of DCA in DCM (15 mL). After 10 min of stirring at rt, to the red solution was charged pyridine (3.5 mL). The resulting white mixture was concentrated *in vacuo* and water was removed as an azeotrope after concentration with MeCN (30 mL). This azeotrope process was repeated two more times with MeCN (30 mL). On the last evaporation, the resulting white slurry of compound **12** was left in MeCN (20 mL).

[00460] Step 2: Preparation of (2*S*,3*R*,4*S*,5*S*)-5-((((((1*R*,3*R*,4*R*,7*S*)-3-(6-benzamido-9*H*-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 hydrogen phosphonate (**13**): A solution of compound **1** (1.0 g, 1.14 mmol, Exiqon) in MeCN (20 mL) was dried through concentration *in vacuo*. This process was repeated two more times to remove water as an azeotrope. On the last azeotrope, to the solution of compound **1** in MeCN (10 mL) was introduced ten 3Å molecular sieves and the solution was stored under an atmosphere of nitrogen. To a stirred mixture of compound **12** with residual pyridinium dichloroacetate in MeCN (20 mL) was added the solution of compound **1** in MeCN (10 mL). After 1 h, to the stirred mixture was added DDTT (228 mg, 1.1 mmol). After 1 h, the yellow mixture was concentrated *in vacuo* to give compound **13** as a yellow paste.

[00461] Step 3: Preparation of Protected-dithio-2'3'-(3'F-G)(2'O,4'C-LNA-A) (14**):** To a solution of compound **13** in DCM (30 mL) was added water (180 µL) and a 15% (v/v) solution of DCA in DCM (20 mL). After 10 min at rt, to the red solution was introduced pyridine (10 mL). The resulting yellow solution was concentrated *in vacuo* until approximately 20 mL of the yellow mixture remained. To the yellow mixture was introduced pyridine (20 mL) and the mixture was concentrated *in vacuo* until approximately 10 mL of the yellow mixture remained. Addition of pyridine (first 20mL, then 30 mL) and concentration *in vacuo* to 10 mL was done twice, leaving a yellow mixture of approximately 10 mL. The stirred yellow mixture was brought to 40 mL with pyridine and DMOCP (383 mg, 2.1 mmol) was added. After 10 min, to the brownish yellow solution was added water (0.7 mL), followed immediately by the introduction of 3H-1,2-benzodithiol-3-one (175 mg, 1.0 mmol). After 1 h, the brownish yellow solution was poured into a 1N aqueous NaHCO₃ solution (200 mL). After 10 min, the biphasic mixture was extracted with EtOAc (150 mL). After separation, the aqueous layer was back extracted with EtOAc (150 mL) and DCM (150 mL). The organic extracts were combined and concentrated *in vacuo*. To the concentrated yellow oil was added toluene (75 mL) and the mixture was evaporated *in vacuo* to remove residual pyridine. This procedure was repeated twice with toluene (75 mL). The resulting oil was purified by silica gel chromatography (0% to 65% MeOH in DCM) to provide compound **14** (604 mg, 92.5% yield) as a yellow paste.

[00462] Step 4: Preparation of 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A) (**15c**) and 2'3'-SS-(3'F-G)(2'O,4'C-LNA-A) (**15d**): To a stirred solution of a mixture of compound **14** (550 mg, 0.58 mmol) in EtOH (19 mL) was added AMA (13.5 mL) and the yellow solution was heated at 50 °C. After 2 h, the clear solution was allowed to cool and concentrated *in vacuo*. The clear paste residue was purified by reverse phase silica gel chromatography (0% to 50% MeCN in 10 mM aqueous TEAA) to obtain compound **15c** (26.7 mg, 22% yield) as a white TEAH⁺ salt after lyophilization. The isomer 2'3'-SS-(3'F-G)(2'O,4'C-LNA-A) **15d** was also isolated after purification in the reverse phase chromatography step as a white TEAH⁺ salt after lyophilization (24.4 mg, 8%).

[00463] The overall reaction was repeated on a larger scale, and all four isomers, including compounds **15a** and **15b** were similarly isolated by HPLC from the last step. The isomer 2'3'-RR-(3'F-G)(2'O,4'C-LNA-A) **15a** was isolated as a white TEAH⁺ salt after lyophilization (60.8 mg, 2.9%). LCMS-ESI: 719.50 [M-H]⁻ (calculated for C₂₁H₂₃FN₁₀O₁₀P₂S₂: 720.54); R_t: 5.946 min by LCMS conditions (20 mM NH₄OAc, 2% to 80%). ¹H NMR (400 MHz, 45 °C, D₂O) δ 8.18 (s, 1H), 7.90 (s, 1H), 7.81 (s, 1H), 6.07 (s, 1H), 5.98 (d, *J* = 8.0 Hz, 1H), 5.86 – 5.79 (m, 2H), 5.61 (d, *J* = 56 Hz, 1H), 5.10 (s, 1H), 4.92 (s, 1H), 4.59 (s, 1H), 4.42–4.13 (m, 6H), 3.99 (d, *J* = 8.0 Hz, 1H), 3.11 (q, *J* = 8 Hz, 8H), 1.83 (s, 0.6H), 1.19 (t, *J* = 8 Hz, 12H). ¹⁹F NMR (400 MHz, 45 °C, D₂O) δ – 198.07 (s). ³¹P NMR (45 °C, D₂O) δ 55.25, 52.31.

[00464] The isomer 2'3'-RS-(3'F-G)(2'O,4'C-LNA-A) **15b** was isolated as a white TEAH⁺ salt after lyophilization (61 mg, 2.9%). LCMS-ESI: 719.55 [M-H]⁻ (calculated for C₂₁H₂₃FN₁₀O₁₀P₂S₂: 720.54); R_t: 5.606 min by LCMS conditions (20 mM NH₄OAc, 2% to 80%). ¹H NMR (400 MHz, 45 °C, D₂O) δ 8.17 (s, 1H), 8.09 (s, 1H), 7.86 (s, 1H), 6.08 (s, 1H), 6.00 (d, *J* = 12.0 Hz, 1H), 5.74 – 5.67 (m, 2H), 5.54 (d, *J* = 52 Hz, 1H), 5.27 (s, 1H), 4.88 (s, 1H), 4.61 (s, 1H), 4.36 – 4.30 (m, 2H), 4.14 – 4.12 (m, 3H), 4.01 (d, *J* = 8.0 Hz, 1H), 3.10 (q, *J* = 8 Hz, 10H), 1.52 - 1.46 (t, *J* = 8 Hz, 15). ¹⁹F NMR (400 MHz, 45 °C, D₂O) δ - 199.63 (s). ³¹P NMR (45 °C, D₂O) δ 53.50, 53.15.

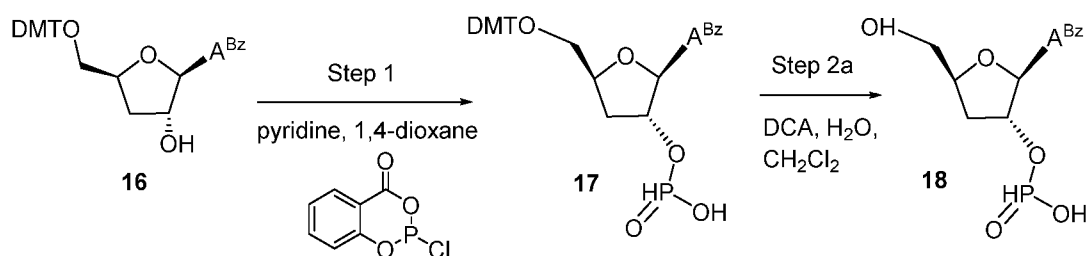
[00465] The isomer 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A) **15c** was isolated as a white TEAH⁺ salt after lyophilization (63.4 mg, 3.1%). LCMS-ESI: 719.70 [M-H]⁻ (calculated for C₂₁H₂₃FN₁₀O₁₀P₂S₂: 720.54); R_t: 5.573 min by LCMS conditions (20 mM NH₄OAc, 2% to 80%). ¹H NMR (400 MHz, 45 °C, D₂O) δ 8.18 (s, 1H), 8.05 (s, 1H), 7.82 (s, 1H), 6.08 (s, 1H), 6.01 (d, *J* = 8.0 Hz, 1H), 5.92 – 5.86 (m, 1H), 5.54 (d, *J* = 52 Hz, 1H), 5.06

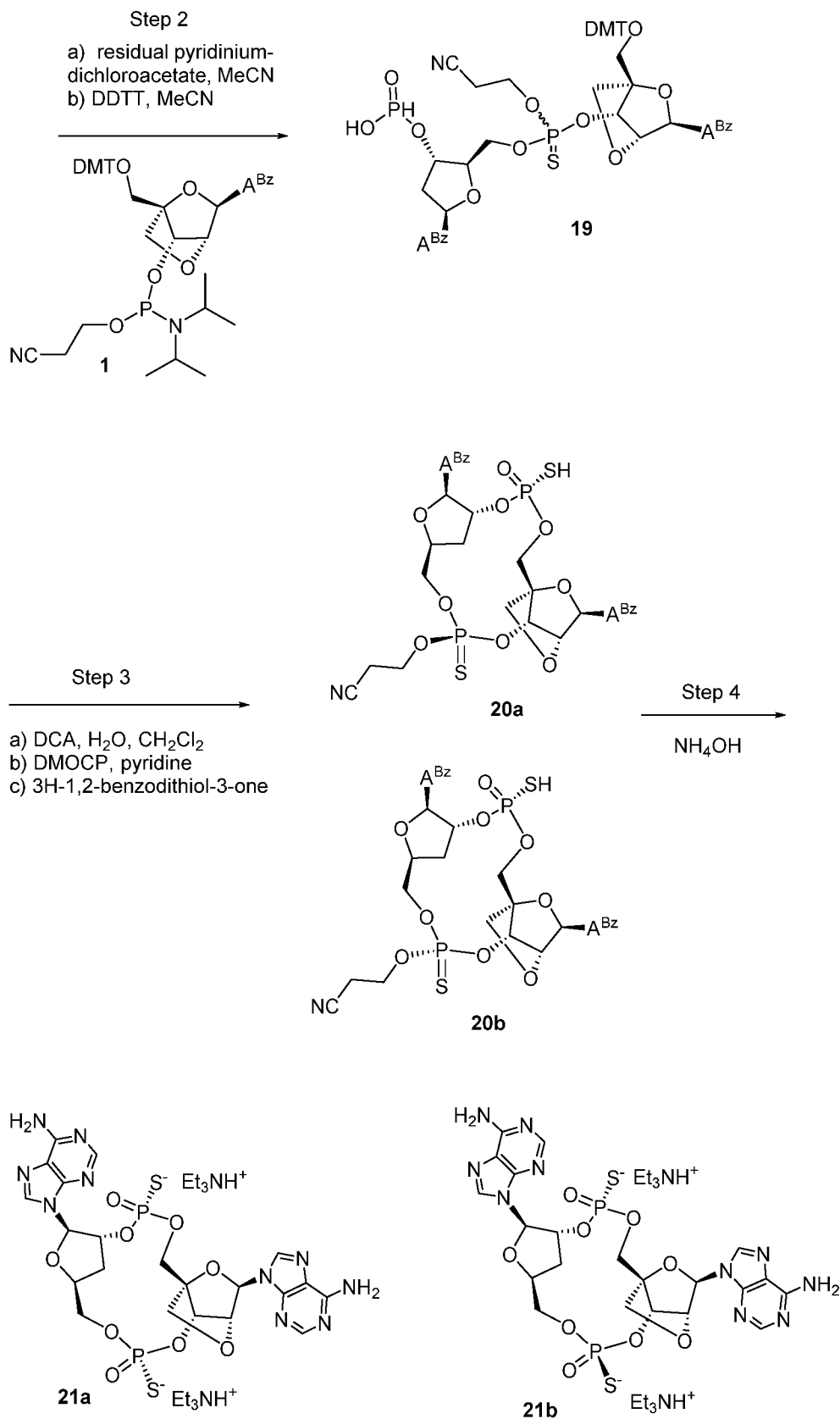
(d, $J = 4$ Hz, 1H), 4.95 (s, 1H), 4.65 (s, 1H), 4.59 (s, 1H), 4.40 – 4.13 (m, 6H), 4.01 (d, $J = 8.0$ Hz, 1H), 3.10 (q, $J = 8$ Hz, 17H), 1.52 – 1.46 (t, $J = 8$ Hz, 26H). ^{19}F NMR (400 MHz, 45 °C, D_2O) δ - 198.60 (s). ^{31}P NMR (45 °C, D_2O) δ 55.38, 54.62.

[00466] The isomer 2'3'-SS-(3'H-G)(2'O,4'C-LNA-A) **15d** was isolated as a white TEAH^+ salt after lyophilization (28.3 mg, 1.4%). LCMS-ESI: 719.50 $[\text{M-H}]^-$ (calculated for $\text{C}_{21}\text{H}_{23}\text{FN}_{10}\text{O}_{10}\text{P}_2\text{S}_2$: 720.54); R_t : 5.393 min by LCMS conditions (20 mM NH_4OAc , 2% to 80%). ^1H NMR (400 MHz, D_2O) δ 8.23 (s, 1H), 8.18 (s, 1H), 7.90 (s, 1H), 6.10 (s, 1H), 6.03 (d, $J = 8.0$ Hz, 1H), 5.78 – 5.71 (m, 1H), 5.43 (d, $J = 52$ Hz, 2H), 5.27 (s, 1H), 4.89 (s, 1H), 4.61 (s, 1H), 4.46 (m, 2H), 4.33 (d, $J = 12.0$ Hz, 2H), 4.15 (d, $J = 8.0$ Hz, 3H), 4.01 (d, $J = 8.0$ Hz, 1H), 3.11 (q, $J = 8$ Hz, 12H), 1.19 (t, $J = 8$ Hz, 18H). ^{19}F NMR (400 MHz, 45 °C, D_2O) δ - 199.83 (s). ^{31}P NMR (45 °C, D_2O) δ 56.21, 53.88.

**Example 5: Synthesis of 2'3'-RR-(3'H-A)(2'O,4'C-LNA-A) (21a) and
2'3'-RS-(3'H-A)(2'O,4'C-LNA-A) (21b)**

[00467] 2'3'-RR-(3'H-A)(2'O,4'C-LNA-A) (**21a**), also referred to as dithio-(R_P, R_P)-cyclic-[3'H-A(2',5')p-2'O,4'C-LNA-A(3',5')p]; and 2'3'-RS-(3'H-A)(2'O,4'C-LNA-A) (**21b**), also referred to as dithio-(R_P, S_P)-cyclic-[3'H-A(2',5')p-2'O,4'C-LNA-A(3',5')p], were prepared according to the following Scheme 5:





[00468] Step 1: Preparation of (2*R*,3*R*,5*S*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl hydrogen phosphonate

(**17**): To a solution of N-(9-((2*R*,3*R*,5*S*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-hydroxytetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (**16**, 1.0 g, 1.5 mmole, 1 eq, Berry and Associates, Dexter, MI) in dioxane (12 mL) was added pyridine (3.2 mL, 40 mmole, 26 eq) followed by SalPCl (370 mg, 1.8 mmole, 1.2 eq). The reaction mixture is stirred for 1 h, then quenched with 10 mL of water followed by saturated NaHCO₃ solution (50 mL). The reaction mixture was extracted with EtOAc (3 x 50 mL), the combined organic layers were dried with Na₂SO₄, filtered and concentrated *in vacuo*. Purification by SiO₂ MPLC (DCM, MeOH and pyridine) gave 1.1 g of compound **17**.

[00469] **Step 2:** Preparation of (2*R*,3*R*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-2-((((((1*R*,3*R*,4*R*,7*R*)-3-(6-benzamido-9*H*-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)tetrahydrofuran-3-yl hydrogen phosphonate (**19**): Compound **1** (1.5 g, 1.6 mmole, 1.1 eq, Exiqon) was co-evaporated with anhydrous MeCN (3 x 20 mL) leaving 12 mL of MeCN. (Step 2a) To a solution of compound **17** (1.5 mmole, 1 eq) in DCM (20 mL) was added water (0.27 mL, 15 mmole, 10 eq) followed by 40 mL of 6% DCA in DCM solution. The reaction mixture was stirred for 20 min, then quenched with pyridine (4 mL, 50 mmole, 34 eq) and concentrated *in vacuo* to provide (2*R*,3*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3-yl hydrogen phosphonate (**18**). The crude mixture of compound **18** was co-evaporated with anhydrous MeCN (3 x 15 mL) leaving 4 mL of MeCN. The solution of compound **1** (1.5 g, Exiqon) in anhydrous MeCN (12 mL) was added to the solution of compound **18** in MeCN and stirred for 20 min. After the addition of DDTT (0.34 g, 1.6 mmole, 1.1 eq), the reaction mixture was stirred for 1 h then concentrated *in vacuo* to give 7.5 g of crude compound **19**.

[00470] **Step 3:** Preparation of Protected-dithio-2'3'-(3'H-A)(2'O,4'C-LNA-A) (**20a**, **20b**): To a solution of crude compound **19** (7.5 g, 1.5 mmole, 1 eq) in DCM (37 mL) was added water (0.27 mL) followed by 74 mL of a 6% DCA in DCM solution. The reaction mixture was stirred for 30 min, then quenched with pyridine (30 mL). The mixture was condensed *in vacuo* to remove the DCM, then co-evaporated with anhydrous pyridine (30 mL) leaving 15 mL. DMOCP (0.82 g, 4.5 mmole, 3 eq) was added and stirred for 15 min followed by water (0.80 mL, 45 mmole, 30 eq) and immediately after with 3*H*-1,2-

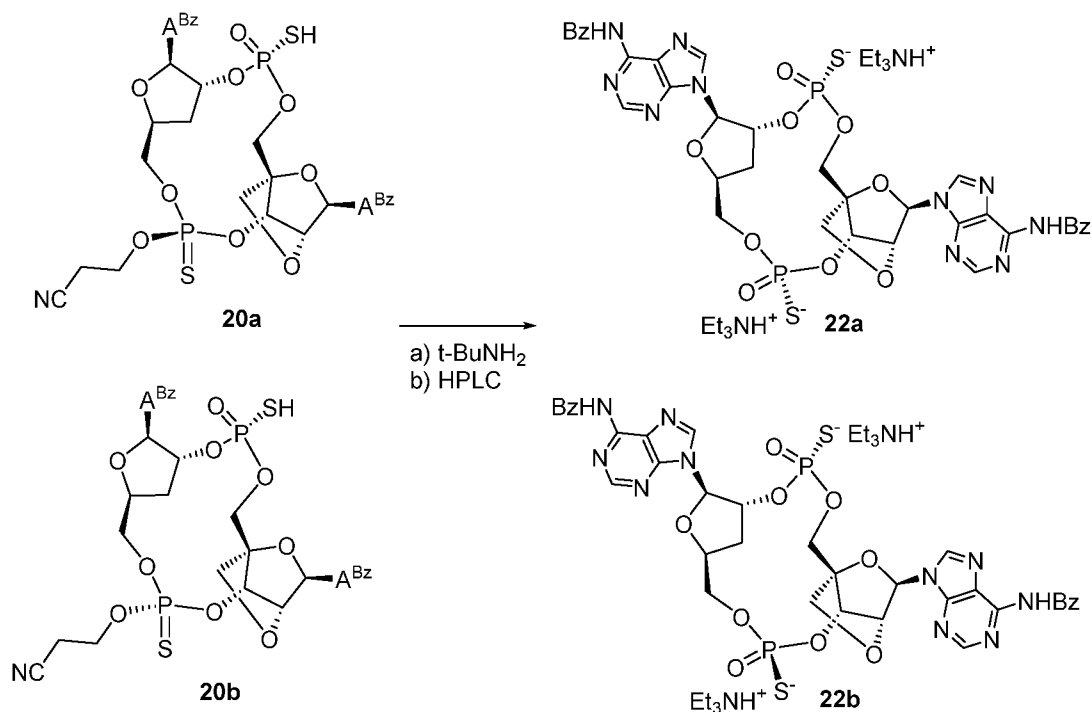
benzodithiol-3-one (0.38 g, 2.2 mmole, 1.5 eq). The reaction was allowed to proceed for 15 min and was poured into a saturated solution of NaHCO₃ (100 mL) then extracted with EtOAc (3 x 100 mL). The combined organic layers were concentrated *in vacuo* to give crude mixture of diastereomer compounds **20a** and **20b**. Prep MPLC-SiO₂ (100% DCM to 50% DCM/MeOH) gave 132 mg of compound **20a** enriched in the RR diastereomer (LCMS) and 100 mg of **20b** enriched in the RS diastereomers (LCMS).

[00471] Step 4: Preparation of 2'3'-RR-(3'H-A)(2'O,4'C-LNA-A) (**21a**) and 2'3'-RS-(3'H-A)(2'O,4'C-LNA-A) (**21b**): To a solution of compound **20a** (70 mg, 0.074 mmole, 1 eq) in MeOH (1.5 mL) was added concentrated NH₄OH (1.5 mL). The mixture was capped and stirred for 3 h at 50 °C. The mixture was cooled to rt and concentrated *in vacuo* to give 60 mg of crude compound **21a**. The reaction was purified using a prep-MPLC-C18 (100% 10 mM TEAA to 50% MeCN/ 10 mM TEAA) to give 19 mg of compound **21a** (>95% pure) as the bis-TEAH⁺ salt. LCMS-ESI: 685.80 [M-H]⁻ (calculated for C₂₁H₂₄N₁₀O₉P₂S₂: 686.06); R_t: 7.2 min (2% to 50% MeCN in 20 mM NH₄OAc aqueous solution). ¹H NMR. (400 MHz, 25 °C, D₂O) δ 8.21 (s, 1H), 8.17 (s, 1H), 8.14 (s, 2H), 6.13 (s, 1H), 6.10 (d, *J* = 4.0 Hz, 1H), 5.24-5.22 (m, 1H), 5.01 (s, 1H), 4.84 (d, *J* = 4.4 Hz, 1H), 4.37-4.33 (m, 1H), 4.13-4.09 (m, 2H), 4.02-3.92 (m, 2H), 3.10 (q, *J* = 7.2 Hz, 12H), 2.74-2.71 (m, 1H), 2.42-2.37 (m, 1H), 1.18 (t, *J* = 7.6 Hz, 18H). ³¹P NMR (25 °C, D₂O) δ 56.34, 53.89.

[00472] To a solution of compound **20b** (100 mg, 0.11 mmole, 1 eq) in MeOH (1.5 mL) was added concentrated NH₄OH (1.5 mL). The mixture was capped and stirred for 3 h at 50 °C. The mixture was cooled to rt and concentrated *in vacuo* to give 60 mg of crude compound **21b**. The reaction was purified using a prep-MPLC-C18 (100% 10 mM TEAA to 50% MeCN/ 10 mM TEAA) to give 7 mg of compound **21b** (>95% pure) as the bis-TEAH⁺ salt. LCMS-ESI: 685.80 [M-H]⁻ (calculated for C₂₁H₂₄N₁₀O₉P₂S₂: 686.06); R_t: 6.4 min (2% to 50% MeCN in 20 mM NH₄OAc aqueous solution). ¹H NMR. (400 MHz, 25 °C, D₂O) δ 8.44 (s, 1H), 8.42 (s, 1H), 8.24 (s, 1H), 8.22 (s, 1H), 6.21 (s, 1H), 6.15 (d, *J* = 5.2 Hz, 1H), 5.39-5.36 (m, 1H), 5.32 (s, 1H), 5.03 (d, *J* = 6.4 Hz, 1H), 4.73 – 4.69 (m, 1H), 4.43-4.33 (m, 2H), 4.24-4.16 (m, 4H), 3.19 (q, *J* = 7.2 Hz, 12H), 1.27 (t, *J* = 7.6 Hz, 18H). ³¹P NMR (25 °C, D₂O) δ 54.68, 53.43.

[00473] The compounds 2'3'-RR-(3'H-BzA)(2'O,4'C-LNA-BzA) (**22a**) and 2'3'-RS-(3'H-BzA)(2'O,4'C-LNA-BzA) (**22b**) were prepared from the compounds **20a**

and **20b**, respectively, from Step 3 of Scheme 4 as follows:



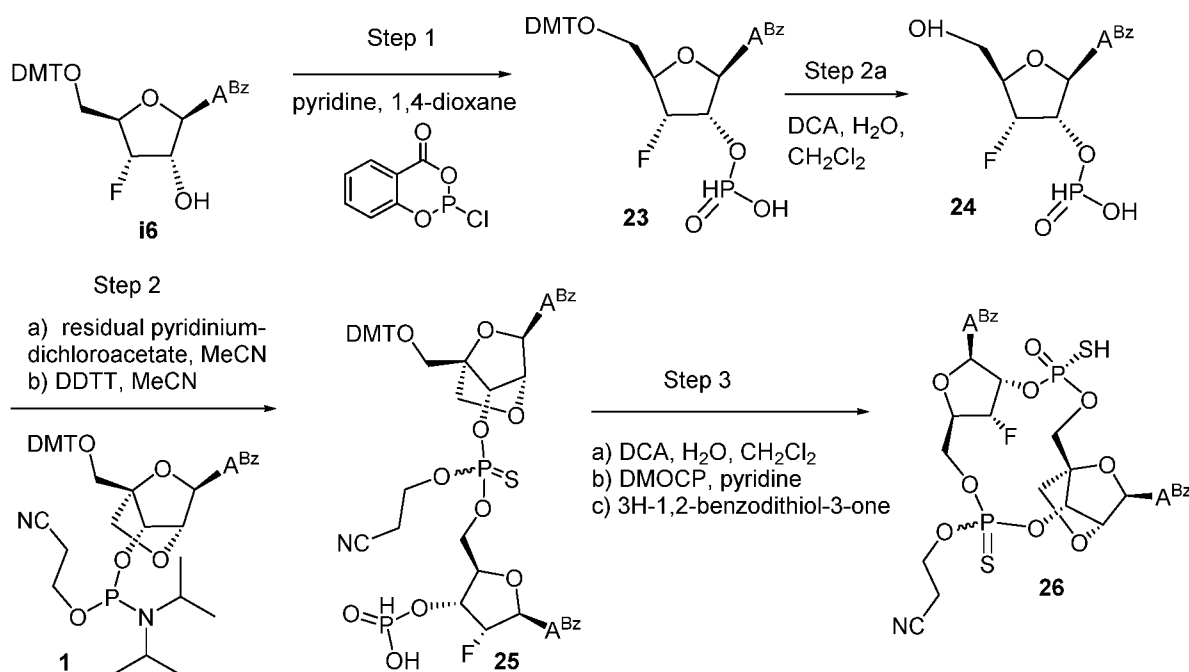
[00474] To a solution of **20a** (25 mg, 0.026 mmol, 1 eq) in MeCN (1 mL) was added *tert*-BuNH₂ (140 μ L, 1.2 mmol, 48 eq). The reaction mixture was stirred for 15 min then concentrated *in vacuo*. The residue was taken up in anhydrous MeCN and co-evaporated to dryness twice. The reaction was purified using a prep-MPLC-C18 (10% to 50% MeCN/ 10 mM TEAA) to give 8 mg of compound **22a** (>95% pure) as the bis-TEAH⁺ salt. LCMS-ESI: 895.8 [M+H]⁺ (calculated for C₃₅H₃₂N₁₀O₁₁P₂S₂: 894.12); R_t: 7.5 min (2% to 80% MeCN in 20 mM NH₄OAc aqueous solution). ¹H NMR. (400 MHz, 25 °C, MeOD) δ 8.75 (s, 1H), 8.73 (s, 1H) 8.72 (s, 1H) 8.57 (s, 1H), 8.10 (d, *J* = 7.2 Hz, 4H), 7.67 (t, *J* = 8.0 Hz, 2H), 7.56 (t, *J* = 7.2 Hz, 4H) 6.34 (d, *J* = 2 Hz, 1H), 6.26 (s, 1H), 5.42-5.38 (m, 1H), 5.14 (s, 1H), 4.78 (d, *J* = 4.0 Hz, 1H), 4.55-4.49 (m, 1H), 4.23 (d, *J* = 7.6 Hz, 1H), 4.19-4.13 (m, 1H), 3.95-3.93 (m, 2H), 3.15 (q, *J* = 7.6 Hz, 12H), 1.27 (t, *J* = 7.6 Hz, 18H). ³¹P NMR (25 °C, MeOD) δ 58.9, 55.7.

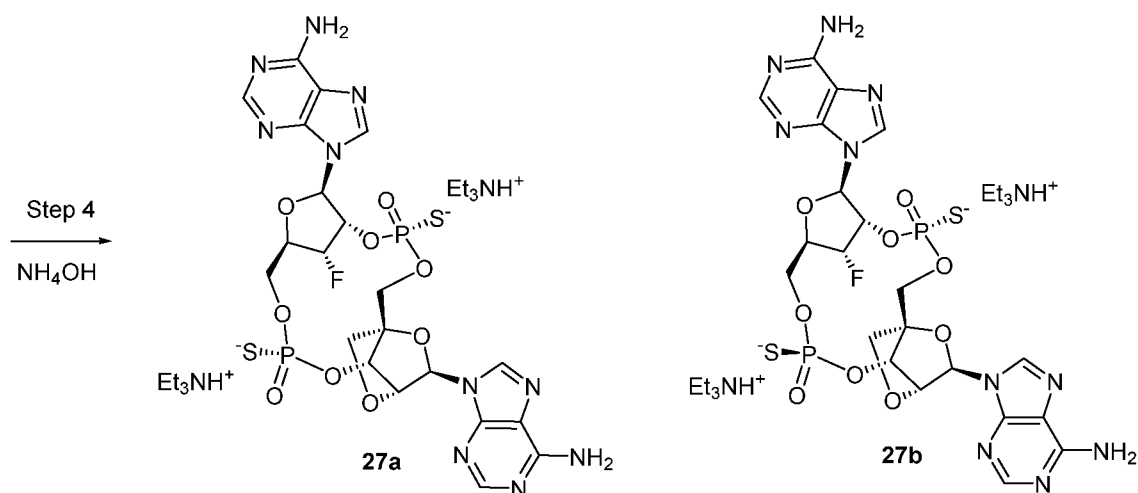
[00475] To a solution of **20b** (26 mg, 0.027 mmol, 1 eq) in MeCN (1 mL) was added *tert*-BuNH₂ (140 μ L, 1.2 mmol, 48 eq). The reaction mixture was stirred for 15 min then concentrated *in vacuo*. The residue was taken up in anhydrous MeCN and co-evaporated to dryness twice. The reaction was purified using a prep-MPLC-C18 (10% to 50% MeCN/ 10 mM TEAA) to give 8.1 mg of compound **22b** (>95% pure) as the bis-TEAH⁺ salt. LCMS-ESI: 895.7 [M+H]⁺ (calculated for C₃₅H₃₂N₁₀O₁₁P₂S₂: 894.12); R_t: 7.43 min

(2% to 80% MeCN in 20 mM NH₄OAc aqueous solution). ¹H NMR. (400 MHz, 25 °C, MeOD) δ 8.92 (s, 1H), 8.75 (s, 1H), 8.73 (s, 1H), 8.71 (s, 1H), 8.10 (d, J = 7.6 Hz, 4H), 7.65 (t, J = 7.6 Hz, 2H), 7.55 (t, J = 7.6 Hz, 4H), 6.34-6.26 (m, 2H), 5.5 (m, 1H), 5.37 (s, 1H), 5.05 (d, J = 5.2 Hz, 1H), 4.64 (m, 1H), 4.44-4.3 (m, 2H), 4.30-4.10 (m, 4H), 4.02-3.98 (d, J = 8, 1H), 3.08-3.07 (m, 12H), 1.27 (t, J = 6.8 Hz, 18H).

Example 6: Synthesis of 2'3'-RR-(3'F-A)(2'O,4'C-LNA-A) (27a) and 2'3'-RS-(3'F-A)(2'O,4'C-LNA-A) (27b)

[00476] 2'3'-RR-(3'F-A)(2'O,4'C-LNA-A) (27a), also referred to as dithio-(R_P,R_P)-cyclic-[3'F-A(2',5')p-2'O,4'C-LNA-A(3',5')p]; and 2'3'-RS-(3'F-A)(2'O,4'C-LNA-A) (27b), also referred to as dithio-(R_P,S_P)-cyclic-[3'F-A(2',5')p-2'O,4'C-LNA-A(3',5')p] were prepared according to the following Scheme 6:





[00477] Step 1: Preparation of (2*R*,3*S*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl hydrogen phosphonate (**23**): To a solution of N-(9-((2*R*,3*S*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (**i6**, 1.1 g, 1.6 mmole, 1 eq, see Example 1) in dioxane (12.1 mL) was added pyridine (3.6 mL) followed by a solution of SalPCl (0.39 g, 1.9 mmole, 1.2 eq) in dioxane (6.6 mL). The reaction mixture was stirred for 2 h then quenched with 2.9 mL of water followed by 85 mL of a saturated NaHCO₃ solution. After extraction with EtOAc (55 mL) and DCM (55 mL), the organic layers were combined and concentrated *in vacuo*. The above conditions were repeated twice more giving 1.74 g of crude material, which was purified on Prep MPLC-SiO₂ (100% DCM to 50% 99.5:0.5 MeOH:pyridine in DCM) to give 1.04 g of compound **23**.

[00478] Step 2: Preparation of (2*R*,3*S*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((((((1*R*,3*R*,4*R*,7*S*)-3-(6-benzamido-9*H*-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-fluorotetrahydrofuran-3-yl phosphonate (**25**): Compound **1** (1.0 g, 1.13 mmole, 1 eq, Exiqon) was co-evaporated with anhydrous MeCN (3 x 5 mL) leaving 3 mL of MeCN. (Step 2a) To a solution of compound **23** in DCM (1.04g, 13.8 mL) was added water (0.207 mL) followed by 13.8 mL of a 6% DCA in DCM solution. The reaction was stirred for 10 minutes then quenched with pyridine (1.9 mL) and concentrated *in vacuo* to give (2*R*,3*S*,4*R*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-4-fluoro-5-(hydroxymethyl)tetrahydrofuran-3-yl hydrogen phosphonate (**24**). The

crude mixture of **24** was concentrated with anhydrous pyridine (2 x 2 mL) to remove the DCA, then co-evaporated with anhydrous MeCN (3 x 10 mL) leaving 5 mL of MeCN. The solution of compound **1** in 3 mL of anhydrous MeCN was added and the reaction stirred for 45 min. After the addition of DDTT (0.31 g), the reaction mixture was stirred for 90 min and concentrated *in vacuo* to give compound **25**.

[00479] Step 3: Preparation of Protected-dithio-2'3'-(3'F-A)(2'O,4'C-LNA-A) (**26**): To a solution of compound **25** in DCM (from step 2, 18 mL) was added water (0.203 mL) followed by 18 mL of a 6% DCA in DCM solution. The reaction mixture was stirred for 10 min and quenched with pyridine (12 mL). The mixture was concentrated *in vacuo* to remove the DCM, then co-evaporated with anhydrous pyridine (2 x 30 mL) leaving 20 mL. Anhydrous pyridine (50 mL) was added followed by DMOCP (0.77 g) and stirred for 15 min. Water (0.723 mL), immediately followed by 3*H*-1,2-benzodithiol-3-one (0.29 g), was added to the reaction. The reaction was allowed to proceed for 10 min, then was diluted with a 3% solution of NaHCO₃ (170 mL) and extracted with EtOAc (2 x 200 mL). The combined organic layers were concentrated to give 8.02 g of material, which was purified on Prep MPLC-SiO₂ (100% DCM to 35% DCM/MeOH) to give 140 mg of compound **26** as a mixture of diastereomers.

[00480] Step 4: Preparation of 2'3'-RR-(3'F-A)(2'O,4'C-LNA-A) (**27a**) and 2'3'-RS-(3'F-A)(2'O,4'C-LNA-A) (**27b**): To a solution of compound **26** (140 mg, 0.15 mmole, 1 eq) in MeOH (2.3 mL) was added concentrated NH₄OH (2.3 mL). The mixture was capped and heated to 50 °C for 3 h. The mixture was then cooled to rt, concentrated *in vacuo* to remove MeOH, and lyophilized to give 36 mg of the desired compounds as a mixture of diastereomers, which was purified using Prep MPLC-C18 (100% 10 mM TEAA to 25% MeCN/10 mM TEAA) to give 6.4 mg of compound **27a** (>95% pure by LCMS) and 8.4 mg compound **27b** (>95% pure) as the TEAH⁺ salt.

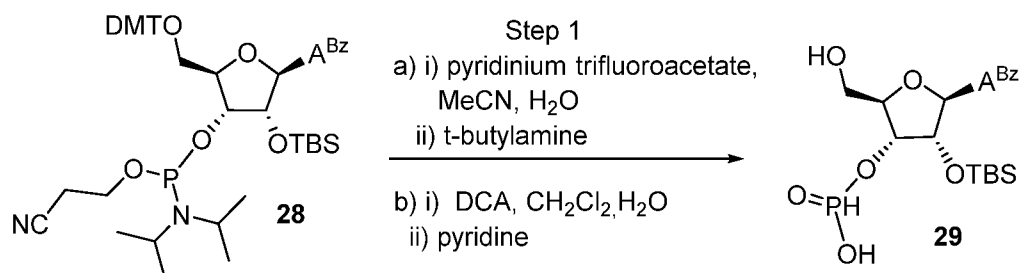
Compound **27a**: LCMS-ESI: 703.7. [M-H]⁻ (Calculated for C₂₁H₂₃FN₁₀O₉P₂S₂: 704.54); ¹H NMR (400 MHz, 25 °C, D₂O) δ 8.22 (s, 1H), 8.06 (d, *J* = 4.0, 1H), 8.03 (s, 1H), 6.15 (d, *J* = 8.8, 1H), 6.08 (s, 1H), 5.6-5.46 (m, 1H), 5.37-5.30 (m, 1H), 5.23 (d, *J* = 5.6, 1H), 5.04 (s, 1H), 4.56-4.50 (m, 1H), 4.30-4.26 (m, 2H), 4.15-4.10 (m, 4H). ¹⁹F NMR (400 MHz, 25 °C, D₂O) δ -197.9 – -198.2, ³¹P NMR (25 °C, D₂O) δ 55.2; 54.3.

Compound **27b**: LCMS-ESI: 703.7. [M-H]⁻ (Calculated for C₂₁H₂₃FN₁₀O₉P₂S₂: 704.54); ¹H NMR (400 MHz, 25 °C, D₂O) δ 8.39 (s, 1H), 8.29 (s, 1H), 8.17 (s, 1H), 6.25 (d, *J* =

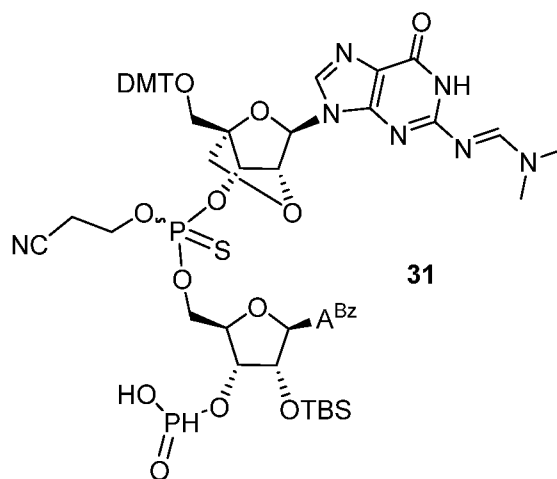
8.8, 1H), 6.19 (d, $J = 6.8$, 1H), 5.74 (d, $J = 3.2$, 1H), 5.61-5.47 (m, 1H), 5.27 (s, 1H), 5.20 (d, $J = 7.2$, 1H), 4.29-4.22 (m, 4H), 4.14 (d, $J = 8.8$, 2H). ^{19}F NMR (400 MHz, 25 °C, D_2O) δ -198.3 – -198.6, ^{31}P NMR (25 °C, D_2O) δ 54.5; 53.9.

Example 7: Synthesis of 3'3'-RR-(A)(2'O,4'C-LNA-G) (33a), 3'3'-RS-(A)(2'O,4'C-LNA-G) (33b), 3'3'-SR-(A)(2'O,4'C-LNA-G) (33c), and 3'3'-SS-(A)(2'O,4'C-LNA-G) (33d)

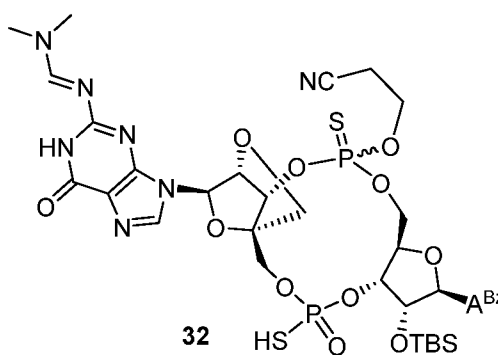
[00481] 2-amino-9-((2*R*,3*R*,3*aS*,5*R*,7*aR*,9*R*,10*R*,10*aS*,12*R*,14*aR*)-9-(6-amino-9*H*-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2*H*,7*H*,14*H*-3,14*a*-(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-2-yl)-1,9-dihydro-6*H*-purin-6-one (**33a**), also referred to as 3'3'-RR-(A)(2'O,4'C-LNA-G), or dithio-(*R_P*,*R_P*)-cyclic-[A(3',5')p-2'O,4'C-LNA-G(3',5')p]; 2-amino-9-((2*R*,3*R*,3*aS*,5*S*,7*aR*,9*R*,10*R*,10*aS*,12*R*,14*aR*)-9-(6-amino-9*H*-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2*H*,7*H*,14*H*-3,14*a*-(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-2-yl)-1,9-dihydro-6*H*-purin-6-one (**33b**), also referred to as 3'3'-RS-(A)(2'O,4'C-LNA-G), or dithio-(*R_P*,*S_P*)-cyclic-[A(3',5')p-2'O,4'C-LNA-G(3',5')p]; 2-amino-9-((2*R*,3*R*,3*aS*,5*R*,7*aR*,9*R*,10*R*,10*aS*,12*S*,14*aR*)-9-(6-amino-9*H*-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2*H*,7*H*,14*H*-3,14*a*-(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-2-yl)-1,9-dihydro-6*H*-purin-6-one (**33c**), also referred to as 3'3'-SR-(A)(2'O,4'C-LNA-G), or dithio-(*S_P*,*R_P*)-cyclic-[A(3',5')p-2'O,4'C-LNA-G(3',5')p]; and 2-amino-9-((2*R*,3*R*,3*aS*,5*S*,7*aR*,9*R*,10*R*,10*aS*,12*S*,14*aR*)-9-(6-amino-9*H*-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2*H*,7*H*,14*H*-3,14*a*-(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-2-yl)-1,9-dihydro-6*H*-purin-6-one (**33d**), also referred to as 3'3'-SS-(A)(2'O,4'C-LNA-G), or dithio-(*S_P*,*S_P*)-cyclic-[A(3',5')p-2'O,4'C-LNA-G(3',5')p], were prepared according to the following Scheme 7:



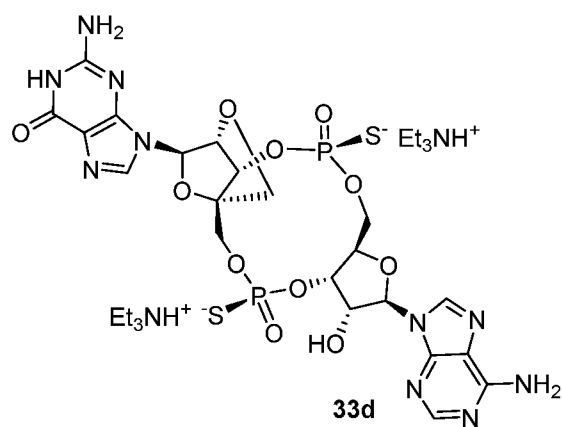
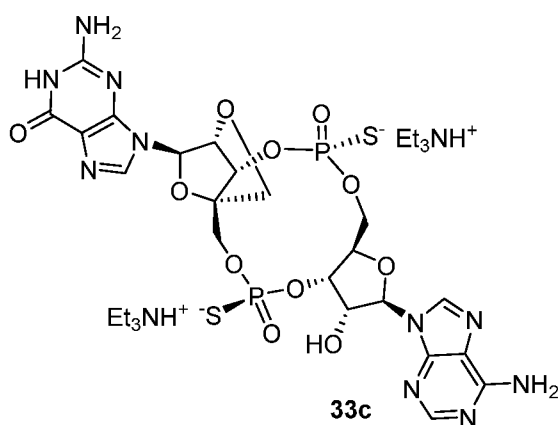
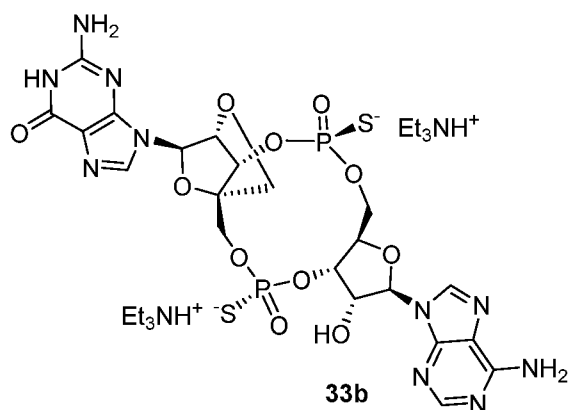
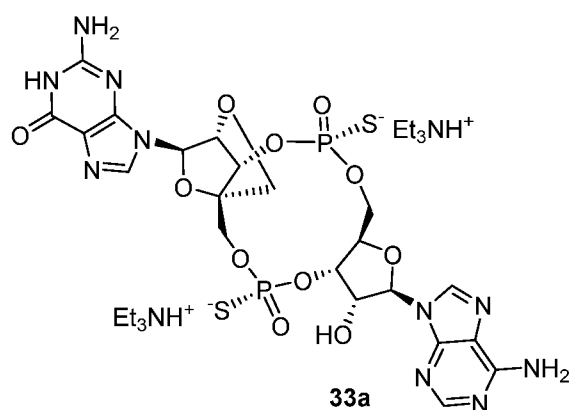
a) residual pyridinium-dichloroacetate, MeCN
b) DDTT, MeCN



a) DCA, CH₂Cl₂, H₂O
b) DMOCP, pyridine
c) 3H-1,2-benzodithiol-3-one



a) AMA, EtOH, 50 °C
b) 3HF.TEA, 40 °C
c) HPLC



[00482] Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)-2-(hydroxymethyl)tetrahydrofuran-3-yl hydrogen phosphonate (**29**): To a solution of (2*R*,3*R*,4*R*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (**28**, 0.99 g, 1.0 mmol, ChemGenes) in MeCN (14 mL) and H₂O (100 μ L) was added pyridinium trifluoroacetate (0.29 g, 1.5 mmol). After fifteen min, *tert*-butyl amine (5.0 mL, 47.6 mmol) was added with stirring at rt. After fifteen min, the reaction solution was concentrated *in vacuo* and water was removed as an azeotrope after concentration with MeCN (3 x 15 mL) to obtain a colorless foam. The colorless foam was dissolved in DCM (15 mL) to give a colorless solution. To this solution was added water (165 μ L) and 9 % (v/v) solution of DCA in DCM (10 mL). After ten min of stirring at rt, to the red solution was charged pyridine (1.5 mL). The resulting clear solution was concentrated *in vacuo* and water was removed as an azeotrope after concentration with MeCN (3 x 20 mL) to

obtain a mixture of oil and solid. On the last evaporation, the resulting peach slurry of compound **29** was left in MeCN (25 mL).

[00483] Step 2: Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(6-benzamido-9*H*-purin-9-yl)-2-((((((1*R*,3*R*,4*R*,7*S*)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-(((*E*)-(dimethylamino)methylene)amino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-(((tert-butyl)dimethylsilyl)oxy)tetrahydrofuran-3-yl hydrogen phosphonate (**31**): To a solution of (1*R*,3*R*,4*R*,7*S*)-1-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-(2-(((*E*)-(dimethylamino)methylene)amino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl (2-cyanoethyl) diisopropylphosphoramidite (**30**, 1.0 g, 1.17 mmol, Exiqon, Woburn, MA) in MeCN (20 mL) was dried through concentration *in vacuo*. This process was repeated two more times to remove water as an azeotrope. On the last azeotrope, to the solution of compound **30** in MeCN (10 mL) was introduced ten 3 Å molecular sieves and the solution was stored under an atmosphere of nitrogen. To a stirred mixture of compound **29** with residual pyridin-1-ium dichloroacetate in MeCN (25 mL) was added the solution of compound **30** in MeCN (10 mL). After one h, to the stirred mixture was added DDTT (249 mg, 1.2 mmol). After one h, the yellow mixture was concentrated *in vacuo* to give compound **31** as a yellow paste.

[00484] Step 3: Preparation of *N*-(9-((2*S*,3*R*,3*aS*,7*aR*,9*R*,10*R*,10*aR*,14*aR*)-10-(((tert-butyl)dimethylsilyl)oxy)-5-(2-cyanoethoxy)-2-(2-(((*E*)-(dimethylamino)methylene)amino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-12-mercapto-12-oxido-5-sulfidohexahydro-2*H*,7*H*,14*H*-3,14*a*-(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-9-yl)-9*H*-purin-6-yl)benzamide (**32**): To a solution of compound **31** in DCM (20 mL) was added water (180 µL) and 10 % (v/v) solution of DCA in DCM (20 mL). After 10 min at rt, to the red solution was introduced pyridine (71 mL). The resulting yellow solution was concentrated *in vacuo* until approximately 20 mL of the yellow mixture remained. To the yellow mixture was introduced pyridine (17 mL) and the mixture was concentrated *in vacuo* until approximately 5 mL of the yellow mixture remained. To the yellow mixture was added pyridine (20 mL) and the mixture was concentrated *in vacuo* until approximately 5 mL of the yellow mixture remained. To the remaining mixture was added 35 mL of pyridine. To the stirred yellow mixture in pyridine (40 mL) was added DMOCP (598 mg, 3.2 mmol). After 20 min, to the brownish yellow solution was added water (700 µL),

followed immediately by the introduction of 3*H*-1,2-benzodithiol-3-one (271 mg, 1.6 mmol). After 45 min, the brownish yellow solution was poured into a 1N aqueous NaHCO₃ solution (200 mL). After 30 min, the biphasic mixture was extracted with EtOAc (200 mL). After separation, the aqueous layer was back extracted with EtOAc (2 x 150 mL), and DCM (1 x 150 mL). The organic extracts were combined and concentrated *in vacuo*. The resulting oil was purified by silica gel chromatography (0% to 60% MeOH in DCM) to provide different fractions of compound **32** and impurities (921 mg, 88% yield) as a yellow paste.

[00485] Step 4: Preparation of 3'3'-RR-(A)(2'O,4'C-LNA-G) (33a): To stirred solutions of each fraction of compound **32** (921 mg, 0.88 mmol) in EtOH (7.5 mL) were added aqueous NH₄OH and methyl amine (15 mL) and each yellow solution was heated at 50 °C. After 2 h, the clear solution was allowed to cool and concentrated *in vacuo*. To the residual was added water and lyophilized. To the residual solid (551 mg, 0.6 mmol) was introduced triethylamine trihydrofluoride (6.5 mL) and the yellow solution was heated to 40 °C. After 3 h, the yellow solution was allowed to cool to rt. This yellow solution was slowly added to a cooled solution of 1M TEAB (22.5 mL) and TEA (5.5 mL). The yellow mixture was allowed to stir for one h. The clear paste residue was purified by reverse phase silica gel chromatography (0% to 100% MeCN in 10 mM aqueous TEAA) to isolate compounds **33a**, **33b**, **33c** and **33d**. The compound **33a** (35 mg, 10% yield) was obtained as a white TEAH⁺ salt after lyophilization. LCMS-ESI: 717.80 [M-H]⁻ (calculated for C₂₁H₂₄N₁₀O₁₁P₂S₂: 718.55); R_t: 6.89 min by LCMS conditions (MeCN in 20 mM NH₄OAc, 2% to 50%). ¹H NMR. (400 MHz, 45 °C, D₂O) δ 8.33 (s, 1H), 8.13 (s, 1H), 7.81 (s, 1H), 6.10 (s, 1H), 5.93 (s, 1H), 4.99 (d, *J* = 8 Hz, 1H), 4.89 (m, 1H), 4.65 (s, 1H), 4.40-4.34 (m, 2H), 4.07-4.02 (m, 2H), 3.95 (d, *J* = 8 Hz, 1H), 3.10 (q, *J* = 8 Hz, 8H), 1.83 (s, 1H), 1.18 (t, *J* = 8 Hz, 11H). ³¹P NMR (45 °C, D₂O) δ 54.73, 54.49.

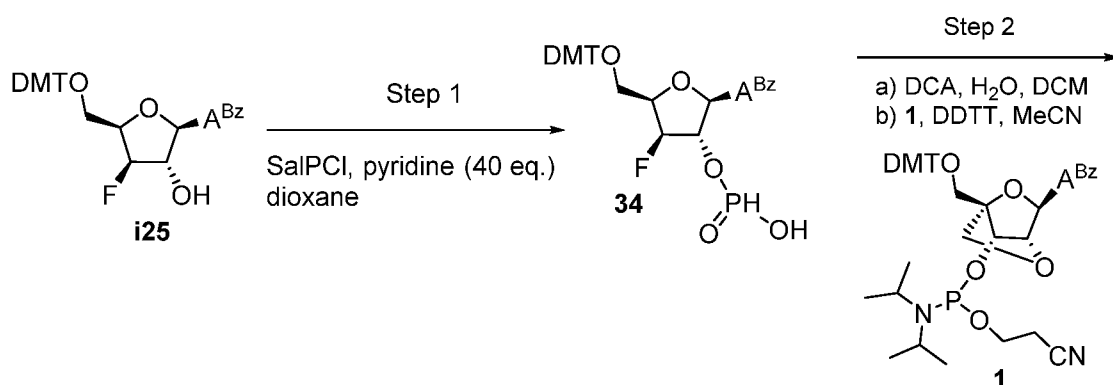
[00486] The Rp,Sp isomer was also isolated after purification in the reverse phase chromatography step, to provide compound **33b** (45 mg, 13 %) as the bis-TEAH⁺ salt after lyophilization. LCMS-ESI: 717.80 [M-H]⁻ (calculated for C₂₁H₂₄N₁₀O₁₁P₂S₂: 718.55); R_t: 6.02 min by LCMS conditions (MeCN in 20 mM NH₄OAc, 2% to 50%). ¹H NMR. (400 MHz, 45 °C, D₂O) δ 8.48 (s, 1H), 8.13 (s, 1H), 7.97 (s, 1H), 6.11 (s, 1H), 5.94 (s, 1H), 4.96 (s, 1H), 4.91-4.85 (m, 2H), 4.61 (d, *J* = 4 Hz, 1H) 4.38-4.28 (m, 3H),

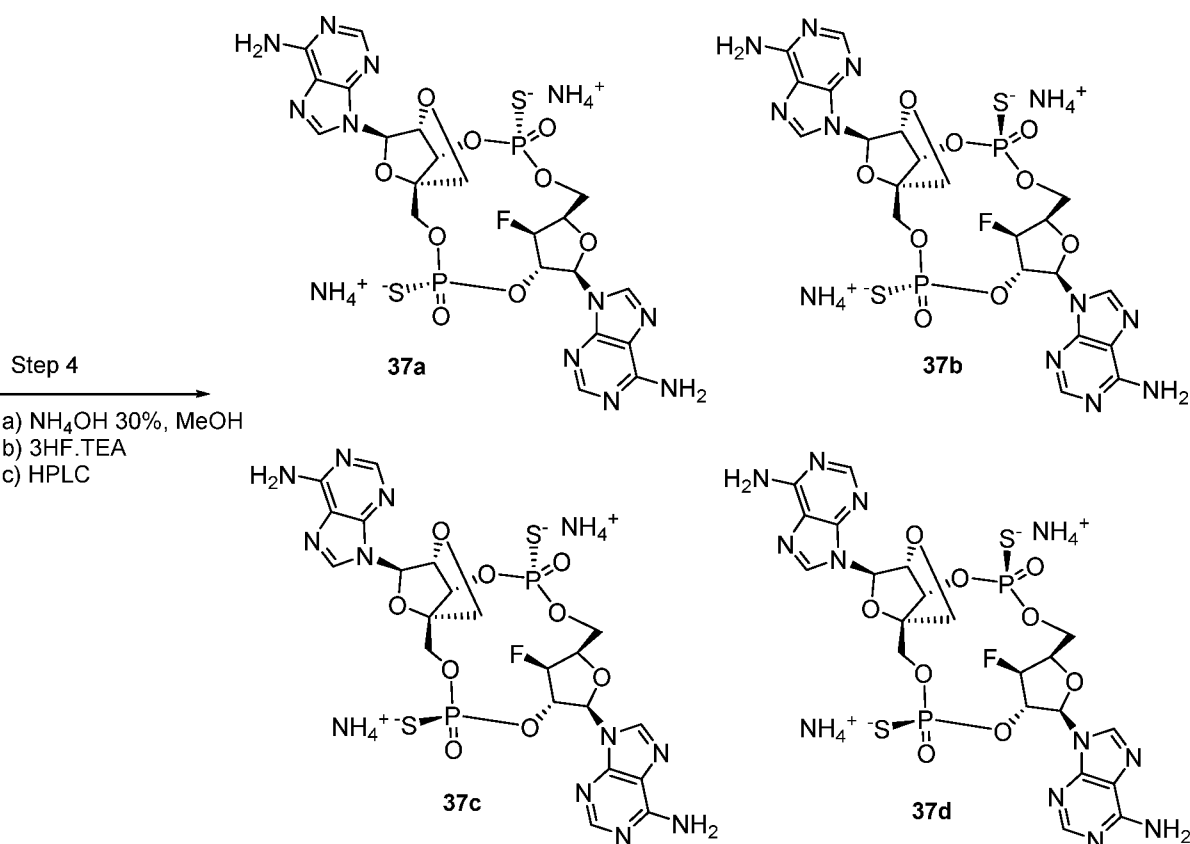
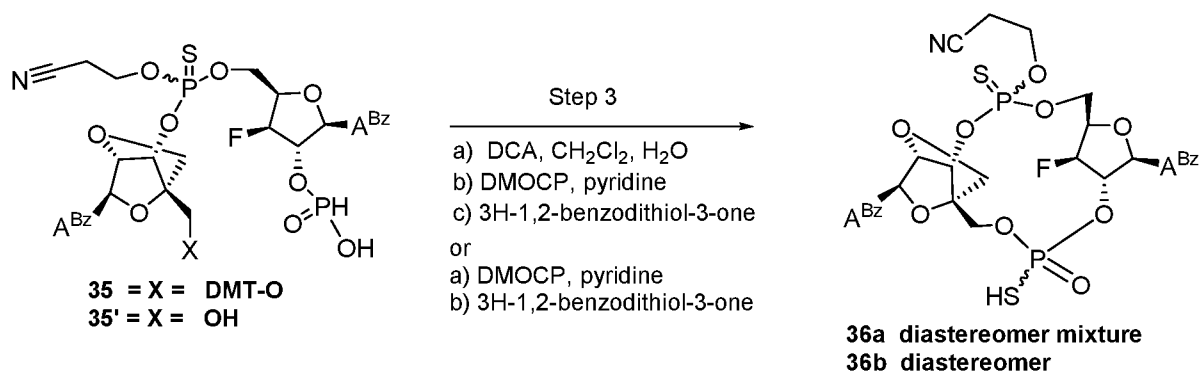
4.09-3.96 (m, 3H), 3.08 (q, $J = 8$ Hz, 14H), 1.84 (s, 1H), 1.16 (t, $J = 8$ Hz, 20H). ^{31}P NMR (45 °C, D_2O) δ 55.93, 54.61.

[00487] The Sp,Sp isomer was also isolated after purification in the reverse phase chromatography step, to provide compound **33d** (10.3 mg, 3%) as the bis- TEAH^+ salt after lyophilization. LCMS-ESI: 717.80 $[\text{M}-\text{H}]^-$ (calculated for $\text{C}_{21}\text{H}_{24}\text{N}_{10}\text{O}_{11}\text{P}_2\text{S}_2$: 718.55); R_t : 5.49 min by LCMS conditions (MeCN in 20 mM NH_4OAc , 2% to 50%). ^1H NMR. (400 MHz, 45 °C, D_2O) δ 8.41(s, 1H), 8.07 (s, 1H), 8.05 (s, 1H), 6.06 (s, 1H), 5.83 (s, 1H), 4.91 (s, 1H), 4.84-4.77 (m, 3H), 4.35-4.33 (m, 1H), 4.24 (m, 2H), 4.07-4.01 (m, 3H), 3.99-3.91(m, 1H), 3.04 (q, $J = 8$ Hz, 16H), 1.80 (s, 2H), 1.11 (t, $J = 7.2$ Hz, 24H). ^{31}P NMR (45 °C, D_2O) δ 56.0, 54.98.

Example 8: Synthesis of 2'3'-RR-(3'βF-A)(2'O,4'LNA-A) (37a), 2'3'-RS-(3'βF-A)(2'O,4'LNA-A) (37b), 2'3'-SR-(3'βF-A)(2'O,4'LNA-A) (37c), and 2'3'-SS-(3'βF-A)(2'O,4'LNA-A) (37d).

[00488] 2'3'-RR-(3'βF-A)(2'O,4'LNA-A) (**37a**) also referred to as dithio- (R_P,R_P) -cyclic-[3'βF-A(2',5')p-2'O,4'C-LNA-A(3',5')p]; 2'3'-RS-(3'βF-A)(2'O,4'LNA-A) (**37b**) also referred to as dithio- (R_P,S_P) -cyclic-[3'βF-A(2',5')p-2'O,4'C-LNA-A(3',5')p]; 2'3'-SR-(3'βF-A)(2'O,4'LNA-A) (**37c**) also referred to as dithio- (S_P,R_P) -cyclic-[3'βF-A(2',5')p-2'O,4'C-LNA-A(3',5')p], and 2'3'-SS-(3'βF-A)(2'O,4'LNA-A) (**37d**) also referred to as dithio- (S_P,S_P) -cyclic-[3'βF-A(2',5')p-2'O,4'C-LNA-A(3',5')p], were prepared according to the following Scheme 8:





[00489] Step 1: Preparation of (2*R*,3*S*,4*S*,5*R*)-2-(6-benzamido-9H-purin-9-yl)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-fluorotetrahydrofuran-3-yl hydrogen phosphonate (**34**): To a solution of compound **i25** (2.0 g, 3.0 mmol, per Example 1, Scheme 1C) in a mixture of anhydrous dioxane (10 mL) and pyridine (12 mL) was added dropwise a solution of SalPCl (780 mg, 3.90 mmol, Sigma Aldrich) in dioxane (5 mL). After 5 h of stirring, water (3.0 mL) was added and the reaction mixture was poured into a 1N aqueous NaHCO₃, then extracted with EtOAc (x3). The combined organic layers were dried (Na₂SO₄), filtered, the solvent removed *in vacuo* and the resulting oil purified by chromatography on silica gel (gradient elution 0% to 30% MeOH/DCM) to provide unreacted starting material (270 mg) and compound **34** as a white solid (1.67 g): ¹H NMR

(400 MHz, DMSO-*d*₆) δ 11.21 (s, 1H), 8.76 (s, 1H), 8.26 (s, 1H), 8.10 – 7.93 (m, 2H), 7.72 – 7.61 (m, 1H), 7.61 – 7.50 (m, 2H), 7.50 – 7.34 (m, 3H), 7.35 – 7.15 (m, 7H), 6.94 – 6.73 (m, 4H), 6.25 (d, *J* = 2.3 Hz, 1H), 5.98 (s, 1H), 5.40 (ddd, *J* = 51.2, 3.3, 1.4 Hz, 1H), 5.23 (ddd, *J* = 16.0, 9.7, 2.2 Hz, 1H), 4.65 – 4.44 (m, 1H), 3.72 (d, *J* = 2.8 Hz, 6H), 3.44 – 3.30 (m, 2H overlapped with H₂O); ¹⁹F NMR (376 MHz, DMSO) δ -199.22; ³¹P NMR (162 MHz, DMSO) δ 0.14; LCMS (Method C) *R*_t: 2.20 min; *m/z* 740.3 [M+H]⁺.

[00490] Step 2: Preparation of (2*R*,3*S*,4*S*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-5-((((((1*R*,3*R*,4*R*,7*S*)-3-(6-benzamido-9*H*-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-fluorotetrahydrofuran-3-yl hydrogen phosphonate (**35**): To a cooled 0 °C solution of compound **34** (810 mg, 1.1 mmol) in DCM (15 mL) was added water (0.17 mL) followed by dropwise addition of a solution of v/v 12% of DCA in DCM (5.0 mL). After completion of the addition the ice bath was removed and the red solution was stirred at rt. After 1 h the reaction mixture was quenched with the addition of pyridine (1.5 mL) and then the solvents were removed *in vacuo*. The resulting oil was azeotroped *in vacuo* using MeCN (3x50 mL) to give crude (2*R*,3*S*,4*S*,5*R*)-2-(6-benzamido-9*H*-purin-9-yl)-4-fluoro-5-(hydroxymethyl)tetrahydrofuran-3-yl hydrogen phosphonate (474 mg, 1.08 mmol, crude): LCMS (Method B) *R*_t: 0.58 min; *m/z* 438.1 [M+H]⁺. Compound **1** (1.15 g, 1.3 mmol, Exiqon) was dried by azeotroping *in vacuo* with MeCN (3 x 75 mL) before dissolution in anhydrous MeCN (5 mL) and addition to a stirred solution of the above formed crude phosphonate (474 mg, 1.08 mmol) in MeCN (5 mL). The resultant reaction mixture was stirred at rt for 1 h. Analysis of an aliquot indicated expected intermediates: LCMS (Method B) *R*_t: 3.63 min and 3.65 min; *m/z* 1222.5 [M+H]⁺. DDTT (289 mg, 1.4 mmol, 1.3 eq) was subsequently added and after 45 min the reaction mixture was concentrated *in vacuo* to give compound **35** as a crude mixture. The crude mixture was purified by chromatography on silica gel (gradient elution 0% to 20% MeOH/DCM with 0.15% of triethylamine) to give compound **35** (440 mg, 0.35 mmol), LCMS (Method B) *R*_t: 3.64 min and 3.71 min; *m/z* 1254.5 and 1254.4 [M+H]⁺; and compound **35'** (240 mg, 0.25 mmol), having the DMT protecting group removed, LCMS (Method B) *R*_t: 1.96 min and 2.02 min; *m/z* 952.3 and 952.4 [M+H]⁺.

[00491] Step 3: Preparation of protected-dithio-2'3'-(3'βF-A)(2'O,4'LNA-A) (**36a** / **36b**): Starting with compound **35'**, to a solution of compound **35'** (240 mg, 0.25 mmol) in 40 mL of dry pyridine was added DMOCP (93 mg, 0.5 mmol) and the reaction mixture stirred for 1 h at rt. Distilled water (70 μL) was added, followed immediately by the addition of 3*H*-1,2-benzodithiol-3-one (84 mg, 0.5 mmol) and the reaction mixture stirred overnight. The reaction mixture was quenched by addition to a solution of 1N aq. NaHCO₃, stirred for 15 min, then extracted with EtOAc (x3). The combined organic layers were dried (Na₂SO₄), filtered and the filtrate solvent removed *in vacuo*. The residue was treated with toluene (2x20 mL) then MeCN (2x 25 mL) to remove the excess pyridine until the oily mixture became a white gummy precipitate. The white precipitate was filtered off and washed with MeCN. The combined filtrates were evaporated *in vacuo* to yield an oil which was further triturated with DCM to give a precipitate of the crude desired cyclized products as a mixture of diastereoisomers: LCMS (Method B) R_t: 2.35 min and 2.58 min; m/z 966.2 and 966.4 [M+H]⁺. Alternatively starting with compound **35**, to a 0 °C cooled solution of compound **35** (500 mg, 0.4 mmol) in DCM (10 mL) was added water (50 μL) followed by v/v 12% DCA in DCM solution (2 mL). The ice bath was removed once addition was complete, and the reaction mixture stirred for 1 h before it was quenched with pyridine (1 mL). The mixture was concentrated *in vacuo* to remove the solvent, then azeotroped *in vacuo* with MeCN (2 x 10 mL), then anhydrous pyridine (2 x 30 mL). The later solution was reduced to 20 mL, then diluted with additional pyridine (20 mL) to a total volume of ~50 mL, before DMOCP (221 mg, 1.2 mmol) was added. The reaction mixture was stirred for 1 h before water (120 μL) was added, which was immediately followed by addition of 3*H*-1,2-benzodithiol-3-one (101 mg, 0.60 mmol). The reaction was stirred for 2 h before it was partitioned between 1N NaHCO₃ (100 mL) and EtOAc, and extracted with EtOAc (3x 20 mL). The combined organic layers were concentrated *in vacuo* to give a viscous amber oil. This was azeotroped *in vacuo* with MeCN (4 x 100 mL) and the resulting material was triturated with MeCN to give a light beige solid which was triturated again with DCM to yield the desired cyclized product mixture. This material was combined with previously obtained crude cyclized product and purified by chromatography on silica gel (gradient elution 0% to 20% MeOH/DCM) to give compound mixture **36a** (50 mg), LCMS (Method B) R_t: 2.31 min; m/z 966.3 [M+H]⁺; and compound **36b** (10 mg), ¹⁹F NMR (376 MHz, DMSO-d₆) δ -

200.53; ^{31}P NMR (162 MHz, DMSO- d_6) δ 64.70, 51.11; LCMS (Method B) R_t : 2.53 min; m/z 966.3 $[\text{M}+\text{H}]^+$.

[00492] Step 4: Preparation of 2'3'-RR-(3' β F-A)(2'O,4'LNA-A) (**37a**); 2'3'-RS-(3' β F-A)(2'O,4'LNA-A), (**37b**); and 2'3'-SR-(3' β F-A)(2'O,4'LNA-A) (**37c**) ammonium salts: To a solution of compound mixture **36a** (50 mg, 0.06 mmol) in EtOH (2 mL) was added AMA (ChemGenes, 1.2 mL) and the reaction mixture heated to 50 °C for 5 h. The mixture was then cooled to rt, concentrated *in vacuo* to remove the solvent, and purified using C18 reverse phase ISCO flash column with a C18Aq RediSepR_f Gold cartridge 15.5gm (eluting with a gradient 0-60% MeCN/15mM of ammonium formate in water) to give 3 diastereoisomers:

Compound **37a** (white solid, 2.4 mg) ^1H NMR (400 MHz, DMSO- d_6) δ 8.23 (d, J = 10.7 Hz, 2H), 8.14 (d, J = 9.2 Hz, 1H), 8.05 (s, 1H), 7.20 (d, J = 2.9 Hz, 2H), 7.07 (d, J = 3.0 Hz, 2H), 6.95 (d, J = 3.0 Hz, 2H), 6.12 (s, 1H), 5.93 (s, 1H), 5.62 (d, J = 50.4 Hz, 1H), 4.93 (d, J = 7.4 Hz, 2H), 4.73 (d, J = 12.7 Hz, 1H), 4.58 - 4.20 (m, 2H), 4.11 - 3.84 (m, 2H), 3.72 (dd, J = 29.5, 10.0 Hz, 2H); ^{19}F NMR (376 MHz, DMSO) δ -202.49; ^{31}P NMR (162 MHz, DMSO) δ 56.07, 52.46; HRMS calculated for $\text{C}_{21}\text{H}_{24}\text{FN}_{10}\text{O}_9\text{P}_2\text{S}_2$: 705.0623 $[\text{M}+\text{H}]^+$ found 705.0624.

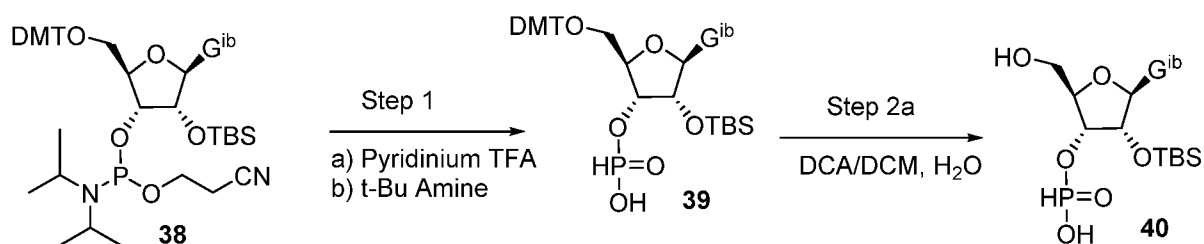
Compound **37b** (white solid, 0.9 mg) ^1H NMR (400 MHz, DMSO- d_6) δ 8.33 (d, J = 2.2 Hz, 1H), 8.26 (dd, J = 9.2, 2.3 Hz, 2H), 8.08 (d, J = 2.6 Hz, 1H), 7.99 - 7.66 (m, 3H), 7.23 (d, J = 9.1 Hz, 1H), 7.10 (d, J = 9.1 Hz, 1H), 6.98 (d, J = 9.3 Hz, 1H), 6.19 (s, 1H), 5.95 (d, J = 7.7 Hz, 1H), 5.79 - 5.56 (m, 1H), 4.94 (dd, J = 9.9, 4.2 Hz, 1H), 4.88 (s, 1H), 4.73 (t, J = 12.0 Hz, 1H), 4.55 - 4.36 (m, 1H), 4.31 (d, J = 4.3 Hz, 1H), 4.18 (dd, J = 11.8, 2.9 Hz, 1H), 4.09 - 3.85 (m, 3H), 3.85 - 3.69 (m, 3H). ^{19}F NMR (376 MHz, DMSO- d_6) δ -203.38; ^{31}P NMR (162 MHz, DMSO) δ 55.85, 51.06; HRMS calculated for $\text{C}_{21}\text{H}_{24}\text{FN}_{10}\text{O}_9\text{P}_2\text{S}_2$: 705.0623 $[\text{M}+\text{H}]^+$ found 705.0619.

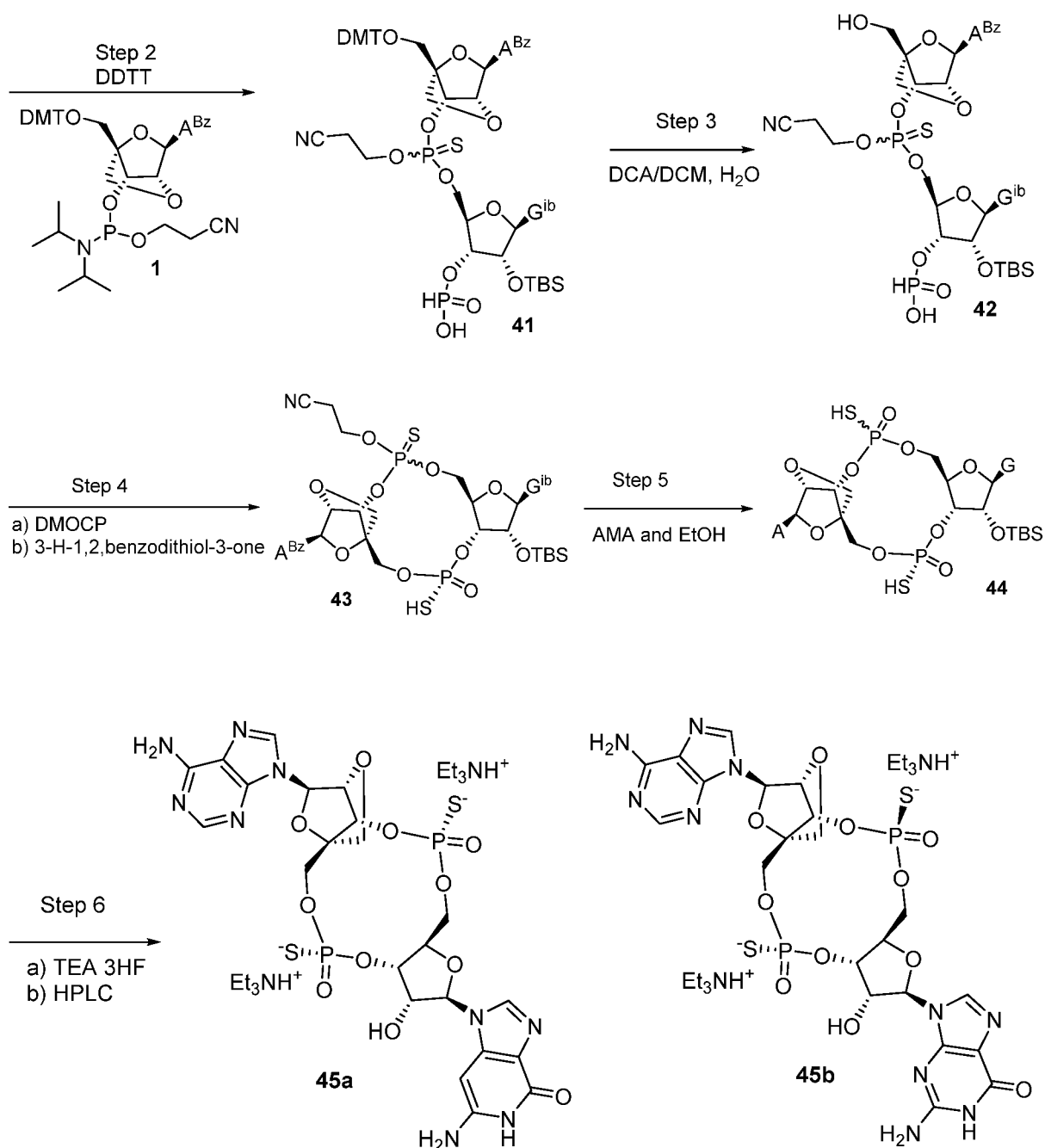
Compound **37c** (white solid, 1.6 mg) ^1H NMR (400 MHz, DMSO- d_6) δ 8.23 (d, J = 10.7 Hz, 2H), 8.14 (d, J = 9.2 Hz, 1H), 8.05 (s, 1H), 7.20 (d, J = 2.9 Hz, 2H), 7.07 (d, J = 3.0 Hz, 2H), 6.95 (d, J = 3.0 Hz, 2H), 6.12 (s, 1H), 5.93 (s, 1H), 5.62 (d, J = 50.4 Hz, 1H), 4.93 (d, J = 7.4 Hz, 2H), 4.73 (d, J = 12.7 Hz, 1H), 4.58 - 4.20 (m, 2H), 4.11 - 3.84 (m, 2H), 3.72 (dd, J = 29.5, 10.0 Hz, 2H); ^{19}F NMR (376 MHz, DMSO- d_6) δ -201.39; ^{31}P NMR (162 MHz, DMSO) δ 52.63, 51.92; HRMS calculated for $\text{C}_{21}\text{H}_{24}\text{FN}_{10}\text{O}_9\text{P}_2\text{S}_2$: 705.0623 $[\text{M}+\text{H}]^+$ found 705.0624.

Preparation of 2'3'-SS-(3'βF-A)(LNA-A) compound (**37d**) ammonium salt: To a solution of compound **36b** (10 mg, 10 mmol) in EtOH (1 mL) was added AMA (200 mL) and the reaction mixture heated to 50 °C for 5 h. The mixture was then cooled to rt, concentrated *in vacuo* to remove EtOH, and purified using C₁₈ reverse phase ISCO flash column with a C₁₈Aq RediSepRf Gold cartridge 15.5gm (eluting with a gradient 0-60% MeCN/15mM of ammonium formate in water) to give compound **37d** (1.1 mg) ¹H NMR (400 MHz, DMSO-d₆) δ 8.39 (s, 1H), 8.18 (d, J = 11.9 Hz, 2H), 8.00 (s, 1H), 7.57 - 7.35 (m, 5H), 7.26 (s, 2H), 7.13 (s, 2H), 7.01 (s, 2H), 6.17 (s, 1H), 5.93 (s, 1H), 5.70 (d, J = 49.7 Hz, 1H), 5.26 (s, 1H), 4.88 (dd, J = 10.0, 4.1 Hz, 1H), 4.54 - 4.38 (m, 3H), 4.27 - 3.98 (m, 2H), 3.97 - 3.69 (m, 3H); ¹⁹F NMR (376 MHz, DMSO) δ -202.72; ³¹P NMR (162 MHz, DMSO) δ 51.76, 51.11; HRMS calculated for C₂₁H₂₄FN₁₀O₉P₂S₂: 705.0623 [M+H]⁺ found 705.0655.

Example 9: Synthesis of 3'3'-RR-(G)(2'O,4'C-LNA-A) (45a) and 3'3'-RS-(G)(2'O,4'C-LNA-A) (45b).

[00493] 2-amino-9-((2*R*,3*R*,3*aS*,5*R*,7*aR*,9*R*,10*R*,10*aS*,12*R*,14*aR*)-2-(6-amino-9*H*-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2*H*,7*H*,14*H*-3,14*a*-(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-9-yl)-1,9-dihydro-6*H*-purin-6-one (**45a**), also referred to as 3'3'-RR-(G)(2'O,4'C-LNA-A) or dithio-(*R_P*,*R_P*)-cyclic-[G(3',5')p-2'O,4'C-LNA-A(3',5')p] and 2-amino-9-((2*R*,3*R*,3*aS*,5*S*,7*aR*,9*R*,10*R*,10*aS*,12*R*,14*aR*)-2-(6-amino-9*H*-purin-9-yl)-10-hydroxy-5,12-dimercapto-5,12-dioxidohexahydro-2*H*,7*H*,14*H*-3,14*a*-(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-9-yl)-1,9-dihydro-6*H*-purin-6-one (**45b**), also referred to as 3'3'-RS-(G)(2'O,4'C-LNA-A) or dithio-(*R_P*,*S_P*)-cyclic-[G(3',5')p-2'O,4'C-LNA-A(3',5')p], were prepared according to the following Scheme 9:





[00494] Step 1: Preparation of (2*R*,3*R*,4*R*,5*R*)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((*tert*-butyldimethylsilyl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl phosphonate (**39**): To a solution of compound **38** (1.1 g, 1.13 mmole, 1 eq, ChemGenes) in MeCN (5.8 mL) was added water (0.040 mL) followed by pyridinium trifluoroacetate (0.26 g, 1.36 mmole, 1.2 eq). The reaction was stirred for 5 min and *tert*-Butyl amine (5.75 mL, 54.7 mmole, 48 eq) was added. After 10 min of stirring the reaction was concentrated *in vacuo* to give crude compound **39** used in the next step.

[00495] Step 2: Preparation of (2*R*,3*R*,4*R*,5*R*)-2-((((((1*R*,3*R*,4*R*,7*S*)-3-(6-benzamido-9*H*-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-(((tert-butyldimethylsilyl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl phosphonate (**40**): Compound **1** (1.0 g, 1.13 mmole, 1 eq, Exiqon) was coevaporated *in vacuo* with anhydrous MeCN (3 x 5 mL) leaving 3 mL of MeCN. To a solution of compound **39** in DCM (13.8 mL) was added water (0.207 mL) followed by 13.8 mL of a 6% DCA in DCM solution. The reaction was stirred for 10 min, then quenched with pyridine (1.9 mL) and concentrated *in vacuo* to give compound **40**. The crude compound **40** was concentrated *in vacuo* with anhydrous pyridine (2 x 2 mL) to remove the DCA, then coevaporated *in vacuo* with anhydrous MeCN (3 x 10 mL) leaving 5 mL of MeCN. The solution of compound **1** (1.0 g) in anhydrous MeCN (3 mL) was added to compound **40** and stirred for 45 min. After the addition of DDTT (0.31 g), the reaction mixture was stirred for 90 min, then concentrated *in vacuo* to give compound **41**.

[00496] Step 3: Preparation of (2*R*,3*R*,4*R*,5*R*)-2-((((((1*S*,3*R*,4*R*,7*S*)-3-(6-benzamido-9*H*-purin-9-yl)-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-7-yl)oxy)(2-cyanoethoxy)phosphorothioyl)oxy)methyl)-4-(((tert-butyldimethylsilyl)oxy)-5-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)tetrahydrofuran-3-yl hydrogen phosphonate (**42**): To a solution of compound **41** in DCM (18 mL) was added water (0.203 mL) followed by 18 mL of a 6% DCA in DCM solution. The reaction mixture was stirred for 10 min, then quenched with pyridine (12 mL). The mixture was concentrated *in vacuo* to remove the DCM, then coevaporated *in vacuo* with anhydrous pyridine (2 x 30 mL) leaving 20 mL of crude compound **42** for use in the next step.

[00497] Step 4: Preparation of N-(9-((2*R*,3*R*,3*aS*,7*aR*,9*R*,10*R*,10*aR*,14*aR*)-10-(((tert-butyldimethylsilyl)oxy)-5-(2-cyanoethoxy)-9-(2-isobutyramido-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-12-mercapto-12-oxido-5-sulfidohexahydro-2*H*,7*H*,14*H*-3,14*a*-(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-2-yl)-9*H*-purin-6-yl)benzamide (**43**): Anhydrous pyridine (50 mL) was added to the solution of compound **42**, followed by DMOCP (0.77 g) and the reaction was stirred for 15 min. Water (0.723 mL) was added, immediately followed by 3*H*-1,2-benzodithiol-3-one (0.29 g). The reaction was allowed to proceed for 10 min and was diluted with a 3% solution of NaHCO₃ (170 mL) and extracted with EtOAc (2 x 200 mL). The combined organic layers

were concentrated to give 8.02 g of crude compound **43**. Prep MPLC-SiO₂ (100% DCM to 70% DCM/MeOH) gave 240 mg of **43** as a mixture of diastereomers.

[00498] Step 5: Preparation of 2-amino-9-((2*R*,3*R*,3*aS*,7*aR*,9*R*,10*R*,10*aR*,14*aR*)-2-(6-amino-9*H*-purin-9-yl)-10-((tert-butyldimethylsilyl)oxy)-5,12-dimercapto-5,12-dioxidohexahydro-2*H*,7*H*,14*H*-3,14*a*-(epoxymethano)difuro[3,2-*d*:3',2'-*j*][1,3,7,9]tetraoxa[2,8]diphosphacyclododecin-9-yl)-1,9-dihydro-6*H*-purin-6-one (**44**): To a solution of compound mixture **43** (108 mg, 0.10 mmole, 1 eq) in EtOH (1.2 mL) was added AMA (2 mL). The mixture was capped and heated to 50 °C for 90 min. The mixture was then cooled to rt and concentrated *in vacuo* to give crude compound **44** as a mixture of diastereomers used in the next step.

[00499] Step 6: Preparation of 3'3'-RR-(G)(2'O,4'C-LNA-A) (**45a**) and 3'3'-RS-(G)(2'O,4'C-LNA-A) (**45b**): To the crude mixture of compound **44** was added TEA 3HF (1.1 mL). The reaction was heated to 50 °C for 2 h. The mixture was poured into a solution of TEA and TEAB with stirring, then desalted and purified using a prep MPLC-C18 column (100% 10 mM TEAA to 30% MeCN/10 mM TEAA) to give 38 mg of a mixture of diastereomers. The crude mixture was then purified via prep HPLC (6-24% MeCN/10 mM TEAA) to give 2 mg of compound **45b** (>95% purity by LCMS) and 9 mg of compound **45a** (>95% pure). LCMS-ESI: 717.7. [M-H]⁻ (Calculated for C₂₁H₂₄FN₁₀O₁₁P₂S₂).

Compound **45a**: ¹H NMR (400 MHz, 25 °C, D₂O) δ 8.19 (s, 1H), 8.13 (s, 1H), 7.90 (s, 1H), 6.11 (s, 1H), 5.88 (d, *J* = 2.0, 1H), 4.94-4.90 (m, 1H), 4.87 (s, *J* = 8.8, 1H), 4.75-4.66 (m, 3H), 4.43-4.38 (m, 1H), 4.32-4.30 (m, 2H), 4.09-3.99 (m, 4.3H). ³¹P NMR (25 °C, D₂O) δ 54.9.

Compound **45b**: ¹H NMR (400 MHz, 25 °C, D₂O) δ 8.36 (s, 1H), 8.15 (s, 1H), 8.02 (s, 1H), 6.11 (s, 1H), 5.89 (d, *J* = 2.0, 1H), 4.98 (s, 1H), 4.93-4.91 (m, 1H), 4.85 (s, *J* = 11.2, 1H), 4.76-4.65 (m, 1H), 4.41-4.37 (m, 1H), 4.30 (d, *J* = 8.0, 1H), 4.24-4.21 (m, 1H), 4.08-4.01 (m, 4.3H). ³¹P NMR (25 °C, D₂O) δ 56.2; 54.6.

Example 10: In vitro binding analysis of LNA-CDN compounds with purified STING protein.

[00500] DNA encoding amino acids 140-379 (amino acid numbering corresponding to Swiss Prot Q86WV6) was amplified from plasmids containing the full length sequence of

human STING alleles via polymerase chain reaction with the following primers: forward TACTTCCAATCCAATGCAGCCCCAGCTGAGATCTCTG (SEQ ID NO: 9) and reverse TTATCCACTTCCAATGTTATTATTATCAAGAGAAATCCGTGCGGAG (SEQ ID NO: 10). STING variant alleles were assigned according to Yi, et al, (2013), PLoS One, 8(10), e77846 (DOI: 10.1371/journal.pone.0077846. PCR products were cloned into bacterial expression vector encoding a N-terminal hexa-histidine affinity tag (6xHIS) followed by a small ubiquitin-like modifier (SUMO) solubility sequence (Butt, et al, (2005) Protein expression and purification 43.1, 1-9) and tobacco etch virus protease cleavage site (TEV) using ligation independent cloning (Aslanidis, et al, (1990) Nucleic acids research, 18.20, 6069-6074).

[00501] Plasmids encoding 6xHIS-SUMO-TEV-STING amino acids 140-379 were transformed into Rosetta2 (DE3) *E. coli* cells (EMD Millipore) for protein expression. Cells were grown in lysogeny broth at 37°C until a 600 nM absorbance of 0.6 was reached. Cells were then transferred to 18°C and protein expression was induced overnight by the addition of isopropyl β -D-1-thiogalactopyranoside to the media at a concentration of 0.25 mM. Cells were harvested by centrifugation at 6,000 times gravity for 10 minutes. Cell pellets were re-suspended on ice in a buffer containing 50 mM Tris hydrochloride (Tris-HCl) pH 7.5, 500 mM sodium chloride (NaCl), 20 mM imidazole, 10% glycerol, 1 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and protease inhibitor tablet (Pierce) (Buffer A). Cells were lysed using an S-450D sonifier (Emmerson industrial) on ice. Cell lysate was centrifuged at 15,000 times gravity for 30 minutes at 4°C. Soluble material was applied to nickel-nitrilotriacetic acid (Ni-NTA) coupled Sepharose CL-6B (Qiagen) for 1 hour with gentle rocking at 4°C. After transfer to a gravity flow poly-prep column (Bio-Rad), resin was washed extensively in buffer A. Protein was eluted from the column in a buffer containing 20 mM Tris-HCl pH 7.5, 150mM NaCl, 300 mM imidazole, 10% glycerol and 0.5 mM TCEP. To remove the 6xHIS-SUMO tag eluted protein was mixed with TEV protease (Sigma) at a ratio of 1:250 (w:w) and dialyzed overnight against a buffer containing 20 mM Tris-HCl pH 7.5, 150mM NaCl, 5 mM imidazole, 10% glycerol and 0.5 mM TCEP. TEV protease and 6xHIS-SUMO tags were depleted by the addition of Ni-NTA resin (Qiagen) to the sample, purified STING amino acids 140-379 was collected by removal of the resin using a poly-prep column. STING AA140-379 was concentrated with a 10,000 Dalton molecular weight cutoff centrifuge concentrator (EMD Millipore) to a final concentration

of approximately 10 mg/ml. Protein was aliquoted, flash frozen in liquid nitrogen and stored at -80°C until use.

[00502] Differential scanning fluorometry (DSF) is a technique that measures the ability of ligands to bind to and stabilize purified proteins (Niesen, et al, (2007) Nature protocols 2.9, 2212-2221). The protein is heated in the presence of a dye that binds to and fluoresces in hydrophobic environments. The protein is thermally denatured by heating resulting in increased dye binding to the unfolded protein and fluorescence. The temperature midpoint (T_m) of a proteins denaturation is established by calculating the half maximal value of the denaturation curve. The temperature midpoint of the protein in the presence of a ligand is directly related to the affinity of the ligand for the protein and therefore its ability to stabilize the protein at higher temperatures.

[00503] DSF was performed in a 20 μ L reaction comprising 20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1:500 dilution of SYPRO Orange (Life Technologies), 1 mg/ml purified STING AA140-379 protein and ligand at a concentration of 1 mM. Each of wild type hSTING, HAQ allele hSTING and REF allele hSTING were used with each of the reference compounds and compounds of the invention as listed in Table 4. Samples were placed in hard shell PCR plate (Bio-Rad). The fluorescence as a function of temperature was recorded in a CFX 96 real time PCR machine (Bio-Rad) reading on the HEX channel, excitation 450–490, emission 560–580 nm. The temperature gradient was from 15–80°C ramping 0.5°C per 15 seconds and recording every 0.5°C. After subtraction of the background signal from a sample lacking protein and ligand. The midpoint temperature (T_m) was calculated by fitting the curves of the fluorescence as a function of temperature to a Boltzmann sigmoidal function (Graph Pad Prism). The change in thermal stability of STING AA140-379 in the presence of ligand (T_m Shift) was calculated by subtracting the T_m (Protein and Ligand) from T_m (Protein alone).

[00504] Table 4: T_m shifts in hSTING WT, HAQ allele and REF allele

Example no. Compound no. Compound name	hSTING T_m Shift (°C)		
	WT	HAQ	REF
Reference Compound 2'3'-(G)(A)	16.1	26.8	7.0
Reference Compound 2'3'-RR-(A)(A)	11.2	18.5	7.3
Reference Compound 2'3'-RR-(G)(A)	20.5	34.1	12.6

Example no. Compound no. Compound name	hSTING T _m Shift (°C)		
	WT	HAQ	REF
Example 2 Compound 5a 3'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A)	16.8	27.6	10.6
Example 2 Compound 5b 3'3'-RS-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A)	11.3	18.3	8.7
Example 2 Compound 4a 3'3'-RR-(2'O,4'C-LNA-BzA)(2'O,4'C-LNA-BzA)	4.3	5.6	4.4
Example 3 Compound 10a 2'3'-RR-(A)(2'O,4'C-LNA-A)	10.4	16.1	7.5
Example 3 Compound 10b 2'3'-RS-(A)(2'O,4'C-LNA-A)	8.7	13.9	7.4
Example 3 Compound 10c 2'3'-SR-(A)(2'O,4'C-LNA-A)	6.5	10.4	5.0
Example 3 Compound 10d 2'3'-SS-(A)(2'O,4'C-LNA-A)	4.1	5.0	3.2
Example 3 Compound 11a 2'3'-RR-(3'-OTBS-A)(2'O,4'C-LNA-A)	7.0	11.6	5.4
Example 3 Compound 11b 2'3'-RS-(3'-OTBS-A)(2'O,4'C-LNA-A)	-1.8	3.3	-1.5
Example 3 Compound 11d 2'3'-RR-(BzA)(2'O,4'C-LNA-BzA)	2.5	3.7	2.4
Example 3 Compound 11e 2'3'-RS-(BzA)(2'O,4'C-LNA-BzA)	1.1	1.5	1.2
Example 3 Compound 11f 2'3'-SR-(BzA)(2'O,4'C-LNA-BzA)	6.6	7.5	6.5
Example 4 Compound 15a 2'3'-RR-(3'F-G)(2'O,4'C-LNA-A)	20.5	33.6	12.1

Example no. Compound no. Compound name	hSTING T _m Shift (°C)		
	WT	HAQ	REF
Example 4 Compound 15b 2'3'-RS-(3'F-G)(2'O,4'C-LNA-A)	12.1	19.2	8.4
Example 4 Compound 15c 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A)	13.5	21.2	8.6
Example 4 Compound 15d 2'3'-SS-(3'F-G)(2'O,4'C-LNA-A)	7.5	11.6	6.1
Example 5 Compound 21a 2'3'-RR-(3'H-A)(2'O,4'C-LNA-A)	9.5	16.2	6.2
Example 5 Compound 21b 2'3'-RS-(3'H-A)(2'O,4'C-LNA-A)	5.0	10.0	4.2
Example 5 Compound 22a 2'3'-RR-(3'H-BzA)(2'O,4'C-LNA-BzA)	0.6	2.2	0.6
Example 5 Compound 22b 2'3'-RS-(3'H-BzA)(2'O,4'C-LNA-BzA)	4.6	5.9	4.6
Example 6 Compound 27a 2'3'-RR-(3'F-A)(2'O,4'C-LNA-A)	11.8	18.2	10.2
Example 6 Compound 27b 2'3'-RS-(3'F-A)(2'O,4'C-LNA-A)	8.2	13.2	7.1
Example 7 Compound 33a 3'3'-RR-(A)(2'O,4'C-LNA-G)	18.36	29.6	11.9
Example 7 Compound 33b 3'3'-RS-(A)(2'O,4'C-LNA-G)	13.2	19.3	9.3
Example 8 Compound 37a 2'3'-RR-(3'βF-A)(2'O,4'LNA-A)	4.7	11.2	2.1
Example 8 Compound 37b 2'3'-RS-(3'βF-A)(2'O,4'LNA-A)	1.8	6.8	0.5

Example no. Compound no. Compound name	hSTING T _m Shift (°C)		
	WT	HAQ	REF
Example 8 Compound 37c 2'3'-SR-(3'βF-A)(2'O,4'LNA-A)	1.2	5.7	0.8
Example 8 Compound 37d 2'3'-SS-(3'βF-A)(2'O,4'LNA-A)	-0.3	2.4	-0.3
Example 9 Compound 45a 3'3'-RR-(G)(2'O,4'C-LNA-A)	20.3	34.0	13.1
Example 9 Compound 45b 3'3'-RS-(G)(2'O,4'C-LNA-A)	9.4	14.7	7.4

[00505] The LNA-CDN compounds bind to WT, HAQ and REF alleles of STING, with several of the compounds (5a, 5b, 15a, 15b, 15c, 27a, 33a, 33b and 45a) having a T_m shift that is comparable to or greater than that of the reference compound 2'3'-RR-(A)(A) for all three alleles, with greater T_m shift than both 2'3'-RR-(A)(A) and 2'3'-(G)(A) for the REF allele. Compounds 5a, 15a, 33a and 45a demonstrated a greater T_m shift than the natural ligand 2'3'-(G)(A) for all three alleles.

Example 11: Induction of type I interferon by LNA-CDN compounds in hPBMCs.

[00506] The induction of type I interferon was measured in human primary blood mononuclear cells (hPBMCs) to evaluate the potency of the LNA-CDN compounds as described herein. hPBMCs from three unique donors were used: one donor was homozygous for the wild type (WT) STING allele (STING^{WT/WT}), one donor was homozygous for the so-called reference (REF) (R232H) STING allele (STING^{REF/REF}), and the third donor was homozygous for the HAQ (R71H, G230A, R293Q) STING allele (STING^{HAQ/HAQ}). The STING genotype of these donors was determined by PCR amplification and sequencing: genomic DNA was isolated from hPBMCs using Quick Extract DNA Extraction Solution (Epicentre) and was used to amplify regions of exon 3, 6, and 7 of the human STING gene. Primers for amplification and sequencing were: hSTING exon3F GCTGAGACAGGAGCTTTGG (SEQ ID NO: 11), hSTING exon3R AGCCAGAGAGGTTCAAGGA (SEQ ID NO: 12), hSTING exon6F

GGCCAATGACCTGGGTCTCA (SEQ ID NO: 13), hSTING exon6R
 CACCCAGAATAGCATCCAGC (SEQ ID NO: 14), hSTING exon7F
 TCAGAGTTGGGTATCAGAGGC (SEQ ID NO: 15), hSTING exon7R
 ATCTGGTGTGCTGGGAAGAGG (SEQ ID NO: 16). STING variant alleles were assigned according to Yi, et al., 2013, PLoS One, 8(10), e77846 (DOI: 10.1371/journal.pone.0077846).

[00507] Cryopreserved hPBMCs were thawed and 10^6 cells were either left untreated or treated with 10 μ M or 100 μ M of reference compounds 2'3'-RR-(A)(A) or 2'3'-(G)(A) (known activators of STING) and with the LNA-CDNs 3'3'-RR-(2'O,4'C-LNA-A) (2'O,4'C-LNA-A) (Compound 5a, Example 2) and 3'3'-RS-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) (Compound 5b, Example 2) in RPMI media supplemented with 10% fetal bovine serum and 1% Penicillin/Streptomycin. After 2 hours stimulation, cells were harvested by centrifugation and washed once in phosphate-buffered saline. Cellular RNA was isolated using the Aurum Total RNA 96 Kit normalized by concentration across samples, and cDNA was synthesized using the iScript cDNA Synthesis Kit. Gene expression was assessed by real-time qRT-PCR using PrimePCR probe assays and the CFX96 gene cycler (all reagents and equipment from BioRad). IFN β expression was expressed relative to untreated cells. Target genes included type I interferon (IFN β), Th1-associated cytokines (IFN γ , IL-12p35) and NF-kB dependent inflammatory cytokines (TNF α , IL-6). Results for these samples are shown in Figs. 1A-C (IFN β), 2A-C (TNF α), 3A-C (IFN γ), 4A-C (IL-6), and 5A-C (IL-12p35). Additional samples were assayed with 10 μ M or 100 μ M of reference compounds 2'3'-RR-(A)(A) or 2'3'-RR-(G)(A) and LNA-CDNs 3'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) (Compound 5a, Example 2) and 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A) (Compound 15c, Example 4), sampled after 2 or 6 hours of stimulation. Results for these samples are shown in Figs. 6A-C (IFN β), 7A-C (TNF α), 8A-C (IFN γ), 9A-C (IL-6), and 10A-C (IL-12p35). In each Figure, A is WT, B is HAQ and C is REF STING alleles. These results demonstrate the LNA-CDN Compounds are potent STING activators, and that the Rp,Rp isomer of Compound 5a generally shows greater activation of cytokines than the Rp,Sp isomer of Compound 5b. Further, Compound 15c more broadly activates all human alleles than Compound 5a, with Compound 15c potently inducing activation of the most refractory human STING allele (REF/REF).

Example 12: LNA-CDN compounds potently activate human STING signaling in THP1 cells.

[00508] To determine the relative level of type I interferon induced in human cells by each of the LNA-CDN as a signature of adjuvant potency, 100,000 THP1-Dual cells (a human monocyte cell line containing the hSTING HAQ allele transfected with an IRF-3 inducible secreted luciferase reporter gene (Invivogen) which express secreted luciferase under the control of a promoter comprised of five IFN-stimulated response elements) were activated with 30 ng/ml phorbol 12-myristate 13-acetate overnight in a 96-well dish. Cells were washed with fresh media and incubated for 30 min at 37 °C with 5% CO₂ with compounds in 3-fold titration steps from 2,000 to 0.0338 µM in PB buffer (50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 100 mM KCl, 3mM MgCl₂, 0.1mM dithiothreitol, 85 mM sucrose, 1 mM ATP, 0.1 mM GTP and 0.2% bovine serum albumin). After 30 minutes, cells were washed and fresh RPMI media containing 10% FBS was added, and cells were incubated at 37 °C with 5% CO₂. Cell culture supernatants from each sample were collected after overnight incubation, and 10 µL of the cell culture supernatants was added to 50 µL QUANTI-Luc reagent (Invivogen). Type I interferon activation was determined by measuring secreted luciferase levels on a SpectraMax M3 spectrophotometer (Molecular Devices). The EC₅₀ value was determined from the dose-response curve for the 10 concentrations from the serial dilution of the reference compounds and compounds of the invention tested in this assay as listed in Table 5, showing the results without digitonin.

[00509] Table 5: EC₅₀ without digitonin in THP1 cells (HAQ allele).

Example/Compound Compound name	EC ₅₀ (µM)
Reference Compound 2'3'-(G)(A)	252.5
Reference Compound 2'3'-RR-(A)(A)	41.5
Reference Compound 2'3'-RR-(G)(A)	28.6
Example 2 Compound 5a 3'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A)	41.6

Example/Compound Compound name	EC50 (μM)
Example 2 Compound 5b 3'3'-RS-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A)	19.3
Example 2 Compound 4a 3'3'-RR-(2'O,4'C-LNA-BzA)(2'O,4'C-LNA-BzA)	340.9
Example 3 Compound 10a 2'3'-RR-(A)(2'O,4'C-LNA-A)	24.3
Example 3 Compound 10b 2'3'-RS-(A)(2'O,4'C-LNA-A)	110.1
Example 3 Compound 10c 2'3'-SR-(A)(2'O,4'C-LNA-A)	>150
Example 3 Compound 10d 2'3'-SS-(A)(2'O,4'C-LNA-A)	>150
Example 4 Compound 15a 2'3'-RR-(3'F-G)(2'O,4'C-LNA-A)	45.1
Example 4 Compound 15b 2'3'-RS-(3'F-G)(2'O,4'C-LNA-A)	23.4
Example 4 Compound 15c 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A)	9.5
Example 4 Compound 15d 2'3'-SS-(3'F-G)(2'O,4'C-LNA-A)	>200
Example 5 Compound 21a 2'3'-RR-(3'H-A)(2'O,4'C-LNA-A)	26.9
Example 5 Compound 21b 2'3'-RS-(3'H-A)(2'O,4'C-LNA-A)	77.3
Example 6 Compound 27a 2'3'-RR-(3'F-A)(2'O,4'C-LNA-A)	10.0

Example/Compound Compound name	EC50 (μM)
Example 6 Compound 27b 2'3'-RS-(3'F-A)(2'O,4'C-LNA-A)	81.0
Example 7 Compound 33a 3'3'-RR-(A)(2'O,4'C-LNA-G)	30.2
Example 7 Compound 33b 3'3'-RS-(A)(2'O,4'C-LNA-G)	38.0
Example 8 Compound 37a 2'3'-RR-(3'βF-A)(2'O,4'LNA-A)	61.2
Example 8 Compound 37b 2'3'-RS-(3'βF-A)(2'O,4'LNA-A)	>150
Example 8 Compound 37c 2'3'-SR-(3'βF-A)(2'O,4'LNA-A)	>150
Example 8 Compound 37d 2'3'-SS-(3'βF-A)(2'O,4'LNA-A)	>150
Example 9 Compound 45a 3'3'-RR-(G)(2'O,4'C-LNA-A)	5.8
Example 9 Compound 45b 3'3'-RS-(G)(2'O,4'C-LNA-A)	60.0

[00510] The LNA-CDN compounds are very active STING agonists as shown in the THP-1 assay, with several compounds having an EC50 of less than that of the reference compound 2'3'-RR-(A)(A), and compounds 5b, 15c, 27a and 45a having an EC50 of less than 20 μM.

Example 13: Anti-tumor efficacy of LNA-CDN compounds

[00511] To assess the ability of the LNA-CDN molecules to promote anti-tumor immunity in aggressive murine tumor models, 6-8 week old female C57BL/6 mice (8 mice per group) were implanted with B16.SIY melanoma tumor cells (1x10⁶ cells in 100 μL

PBS). Mice were treated with 3'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) (Compound 5a, Example 2) or 2'3'-SR-(A)(2'O,4'C-LNA-A) (Compound 10c, Example 3), both at 0.1, 1, 10 and 100 μ g in a total volume of 40 μ L HBSS, and compared to 2'3'-RR-(A)(A) as a reference compound (1 μ g in a total volume of 40 μ L HBSS) or HBSS vehicle control. Treatments began when tumors reached a volume of approximately 100 mm³, on day 9 post tumor implantation. The compounds were administered by a single subcutaneous injection into the center of the tumor (IT) using a 27 gauge needle. Tumors were measured twice weekly.

[00512] As shown in Figs. 11A-F and 12A-F, in the aggressive B16-SIY melanoma model, the LNA-CDN Compounds 5a and 10c induced potent tumor inhibition as compared to HBSS, in a dose-dependent manner, with both compounds demonstrating comparable tumor efficacy as compared to the reference compound 2'3'-RR-(A)(A) at the 1 μ g dose, with Compound 5a eliciting complete tumor regression at the 100 μ g dose. These data demonstrate the potent anti-tumor effects of these LNA-CDN compounds in an aggressive murine tumor model.

Example 14: Anti-tumor efficacy of LNA-CDN compounds is STING-Dependent

[00513] To assess the ability of the LNA-CDN molecules to promote STING-dependent anti-tumor immunity in an aggressive murine tumor model, 6-8 week old female C57BL/6 (WT) mice or STING-deficient *Goldenticket* (GT) mice were implanted with B16.SIY melanoma cells (1x10⁶ cells in 100 μ L PBS). Treatments began when tumors reached a volume of approximately 100 mm³, on day 7 post tumor implantation. Mice were treated with 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A) (Compound 15c, Example 4), at 100 μ g in a total volume of 40 μ L HBSS (6 mice per group), and compared to HBSS vehicle control (4 mice per group). The compounds were administered by a single subcutaneous injection into the center of the tumor (IT) using a 27-gauge needle. Tumors were measured twice weekly.

[00514] As shown in Figs. 13A-D, in the aggressive B16.SIY melanoma model, the LNA-CDN Compound 15c induces complete tumor rejection in the WT mice, as compared to HBSS vehicle control, while having no anti-tumor effect in the STING-deficient GT mice, similar to HBSS. Further, it was observed that all surviving mice in the WT group treated with Compound 15c presented with patches of vitiligo and alopecia, indicative of a strong CD8⁺ T cell response against endogenous melanocyte and

keratinocyte differentiation antigens, which was not evident in the GT mice treated with Compound 15c (data not shown). These data demonstrate the profound anti-tumor immunity of this LNA-CDN compound in an aggressive murine tumor model.

Example 15: Systemic pro-inflammatory cytokines induced by LNA-CDN compounds

[00515] To assess the relative induction of systemic serum cytokines by the LNA-CDN compounds as a measure of safety and tolerability, mice from Example 13 were bled six hours post-IT injection and their serum was assessed by Mouse Inflammation Cytometric Bead Array (CBA, BD Biosciences). As shown in Figs. 14A-D (Comp. 5a) and 15A-D (Comp. 10c), concentrations of pro-inflammatory cytokines TNF- α (A), IFN- γ (B), IL-6 (C), and MCP-1 (D) at the 1 μ g dose were comparable between 2'3'-RR-(A)(A) and the LNA-CDN Compounds 5a and 10c, with Compound 10c demonstrating significantly lower systemic cytokines than 2'3'-RR-(A)(A) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, student's t -test). These data demonstrate a comparable safety profile for Compound 5a, as compared to reference compound 2'3'-RR-(A)(A), and a superior safety profile for Compound 10c.

Example 16: T cell mediated anti-tumor immunity induced by LNA-CDN compounds

[00516] To demonstrate that the anti-tumor effect is mediated by adaptive T cell immune responses, mice from Example 13 were euthanized on day 7 post IT injection and spleens were harvested and splenocytes prepared. Splenocytes (2×10^5) were (A) stimulated overnight in an IFN γ ELISPOT assay with media alone or with 1 μ M SIYRYYYGL (SIY) peptide. IFN- γ ELISPOT plates were developed and quantified using a CTL plate reader and ImmunoSpot software; or (B) pre-incubated with anti-CD16/32 monoclonal antibody to block potential nonspecific binding, and labeled with PE-MHC class I pentamer (Proimmune) consisting of murine H-2Kb complexed to SIYRYYYGL (SIY) peptide, anti-TCR β -AF700 (H57-597), anti-CD8-Pacific Blue (53-6.7), anti-CD4-Pacific Orange (RM4-5) (all antibodies from BioLegend) and the Fixable Viability Dye eFluor 450 (eBioscience). Stained cells were analyzed using FACS Versa cytometer with FACSDiva software (BD). Data analysis was conducted with FlowJo software (Tree Star).

[00517] As shown in Fig. 16A, Compound 5a elicits significantly higher T cell production of IFN γ in response to SIY peptide stimulation at the 0.1, 1 and 10 μ g doses, as compared to HBSS, and comparable T cell responses at 1 μ g to the reference compound 2'3'-RR-(A)(A) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, student's t -test). As shown in Figure 16B, Compound 10c demonstrates comparable responses at the 1 μ g dose compared to the reference compound 2'3'-RR-(A)(A), and significantly higher responses at all doses tested compared to HBSS, demonstrating an even broader range of efficacy than Compound 5a. To further confirm that these responses were due to SIY-specific CD8 $^{+}$ T cells, flow cytometry analysis was conducted using an SIY pentamer and antibodies specific to CD8 $^{+}$ T cells. As shown in Figs. 17A and B (A-Comp.5a, B-Comp. 10c), similar trends in SIY-specific CD8 $^{+}$ T cell frequencies were observed for both compounds, demonstrating the ability of these LNA-CDN compounds to elicit functional T cell-mediated anti-tumor immunity, in an antigen-specific manner. Further, Compound 10c demonstrated a broader range of dose responses, significantly improving the therapeutic window for inducing anti-tumor immunity.

[00518] One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The examples provided herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

[00519] It is to be understood that the invention is not limited in its application to the details of construction and to the arrangements of the components set forth in the following description or illustrated in the drawings. The invention is capable of embodiments in addition to those described and of being practiced and carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein, as well as the abstract, are for the purpose of description and should not be regarded as limiting.

[00520] As such, those skilled in the art will appreciate that the conception upon which this disclosure is based may readily be utilized as a basis for the designing of other structures, methods and systems for carrying out the several purposes of the present invention. It is important, therefore, that the claims be regarded as including such

equivalent constructions insofar as they do not depart from the spirit and scope of the present invention.

[00521] While the invention has been described and exemplified in sufficient detail for those skilled in this art to make and use it, various alternatives, modifications, and improvements should be apparent without departing from the spirit and scope of the invention. The examples provided herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the invention and are defined by the scope of the claims.

[00522] It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

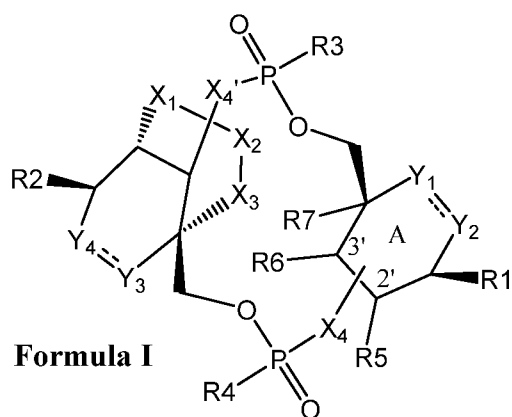
[00523] All patents and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains.

[00524] The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms “comprising”, “consisting essentially of” and “consisting of” may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[00525] Other embodiments are set forth within the following claims.

We claim:

1. A compound having the structure of Formula I:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof,

wherein:

R1 and R2 are independently a pyrimidine or purine nucleic acid base or analog or derivative thereof;

R3 and R4 are independently -OH, -OR^a, -SH or -SR^a;

Y₁ is -O-, -S- or -CRR- and Y₂ is absent, wherein each R is independently -H or -C₁₋₆alkyl, or the two R together with the carbon to which they are attached form a 3-6 membered heterocycloalkyl or C₃₋₆cycloalkyl spirocyclic ring; or

Y₁ is -O-, -CH₂- or -CH= and Y₂ is -CH₂- or =CH-, selected to provide -Y₁-Y₂- as -O-CH₂-, -CH₂-CH₂- or -CH=CH-;

Y₃ is -O-, -S- or -CRR- and Y₄ is absent, wherein each R is independently -H or -C₁₋₆alkyl, or the two R together with the carbon to which they are attached form a 3-6 membered heterocycloalkyl or C₃₋₆cycloalkyl spirocyclic ring; or

Y₃ is -O-, -CH₂- or -CH= and Y₄ is -CH₂- or =CH-, selected to provide -Y₃-Y₄- as -O-CH₂-, -CH₂-CH₂- or -CH=CH-;

X₁, X₂, and X₃ are all present, X₁ and X₂ are -C(R^bR^c)-, -N(R^d), -O- or -S(=O)₂-, and X₃ is -C(R^bR^c)- or -O-, selected to provide -X₁-X₂-X₃- as -C(R^bR^c)-C(R^bR^c)-C(R^bR^c)-, -C(R^bR^c)-N(R^d)-C(R^bR^c)-, -C(R^bR^c)-O-C(R^bR^c)-, -C(R^bR^c)-C(R^bR^c)-O-, -O-C(R^bR^c)-C(R^bR^c)-, -O-N(R^d)-C(R^bR^c)-, -S(=O)₂-N(R^d)-C(R^bR^c)-, -N(R^d)-C(R^bR^c)-C(R^bR^c)-, -N(R^d)-O-C(R^bR^c)- or -N(R^d)-S(=O)₂-C(R^bR^c)-; or

X₂ is absent, X₁ and X₃ are both present, X₁ is -C(R^bR^c)-, -N(R^d) or -O- and X₃ is

-C(R^bR^c)- or -S(=O)₂-, selected to provide -X₁-X₃- as -C(R^bR^c)-C(R^bR^c)-,

-O-C(R^bR^c)-, -N(R^d)-C(R^bR^c)- or -N(R^d)-S(=O)₂-;

X₄' is -O- or -NH-;

X₄ is -O- or -NH- bonded to either the 2' or the 3' carbon atom of the indicated ring A;

wherein:

when X₄ is -O- or -NH- bonded to the 2' carbon atom of ring A:

R₅ is absent, R₆ is selected from the group consisting of -H, -OH, halogen and

-OR^e, and R₇ is -H;

when X₄ is -O- or -NH- bonded to the 3' carbon atom of ring A:

R₆ is absent, R₅ is selected from the group consisting of -H, -OH, halogen and

-OR^e, and R₇ is -H; or

R₆ is absent, and R₅ and R₇ join to form -X₅-X₆-X₇-, resulting in a fused ring system with X₅ bonded to the 2' carbon atom of ring A and X₇ bonded to the carbon atom of ring A to which R₇ is attached,

wherein:

X₅, X₆, and X₇ are all present, X₅ and X₆ are -C(R^bR^c)-, -N(R^d), -O- or

-S(=O)₂-, and X₇ is -C(R^bR^c)- or -O-, selected to provide -X₅-X₆-X₇- as

-C(R^bR^c)-C(R^bR^c)-C(R^bR^c)-, -C(R^bR^c)-N(R^d)-C(R^bR^c)-,

-C(R^bR^c)-O-C(R^bR^c)-,

-C(R^bR^c)-C(R^bR^c)-O-, -O-C(R^bR^c)-C(R^bR^c)-, -O-N(R^d)-C(R^bR^c)-,

-S(=O)₂-N(R^d)-C(R^bR^c)-, -N(R^d)-C(R^bR^c)-C(R^bR^c)-, -N(R^d)-O-C(R^bR^c)- or

-N(R^d)-S(=O)₂-C(R^bR^c)-; or

X₆ is absent, X₅ and X₇ are both present, X₅ is -C(R^bR^c)-, -N(R^d) or -O- and X₇

is -C(R^bR^c)- or -S(=O)₂-, selected to provide -X₅-X₇- as

-C(R^bR^c)-C(R^bR^c)-, -O-C(R^bR^c)-, -N(R^d)-C(R^bR^c)- or -N(R^d)-S(=O)₂-;

R^a at each occurrence is independently selected from the group consisting of alkyl,

alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl and

-C(=O)R^f; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^m, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -Rⁿ;

R^b and R^c, at each occurrence, are independently selected from the group consisting of

-H, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R' and -L₁-R^g; or R^b and R^c together on a carbon are =O, =CR^hR^h,

$=N-OR^i$, $=N-R^i$, or $=N-NR^iR^i$; or R^b and R^c together with the carbon to which they are attached form a C_{3-6} cycloalkyl or 3 to 6 membered heterocycloalkyl spirocyclic ring; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^n$;

R^d at each occurrence is independently selected from the group consisting of $-H$, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-R''$ and $-L_2-R^j$; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^n$; or R^d is a suitable nitrogen protecting group.

R^e is selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl and $-C(=O)R^f$; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^n$; or R^e is a suitable oxygen protecting group;

R^f at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^n$;

R' at each occurrence is selected from the group consisting of $-CN$, $-NO_2$, $-OH$, $-SH$, $-NH_2$, $-C(=W)-WH$, $-C(=W)-NH_2$, $-C(=W)-NH-WH$, $-C=N-OH$, $-C(=NH)-NH_2$, $-W-C(=W)-WH$, $-W-C(=W)-NH_2$, $-S(=O)_2-NH_2$, $-S(=O)_2-NH-W-$, $-NH-NH_2$, $-NH-WH$, $-NH-C(=W)-WH$, $-NH-C(=W)-NH_2$, $-NH-S(=O)_2-NH_2$, and $-NH-C(=NH)-NH_2$;

R'' at each occurrence is selected from the group consisting of $-OH$, $-SH$, $-NH_2$, $-C(=W)-WH$, $-C(=W)-NH_2$, $-C(=NH)-NH_2$, and $-S(=O)_2-NH_2$;

L_1 is selected from the group consisting of $-O-$, $-S-$, $-S(=O)-$, $-S(=O)_2-$, $-C(=W)-$, $-C(=W)-W-$, $-C(=W)-NR^k-$, $-C(=W)-NR^k-W-$, $-C=N-O-$, $-C(=NR^k)NR^k-$, $-C(NR^kR^k)=N-$, $-W-C(=W)-$, $-W-C(=W)-W-$, $-W-C(=W)-NR^k-$, $-S(=O)_2-NR^k-$, $-S(=O)_2-NR^k-W-$, $-NR^k-$, $-N=CR^h-$, $-NR^k-W-$, $-NR^k-NR^k-$, $-NR^k-C(=NR^k)NR^k-$, $-NR^k-C(NR^kR^k)=N-$,

$-\text{NR}^k-\text{C}(=\text{W})-$, $-\text{NR}^k-\text{C}(=\text{W})-\text{W}-$, $-\text{NR}^k-\text{C}(=\text{W})-\text{NR}^k-$, $-\text{NR}^k-\text{S}(=\text{O})_2-\text{NR}^k-$ and $-\text{NR}^k-\text{S}(=\text{O})_2-$;

L_2 is selected from the group consisting of $-\text{O}-$, $-\text{S}-$, $-\text{NR}^k-$, $-\text{C}(=\text{W})-$, $-\text{C}(=\text{W})-\text{W}-$, $-\text{C}(=\text{W})-\text{NR}^k-$, $-\text{C}(=\text{NR}^k)\text{NR}^k-$, $-\text{C}(\text{NR}^k\text{R}^k)=\text{N}-$, $-\text{S}(=\text{O})_2-\text{NR}^k-$, and $-\text{S}(=\text{O})_2-$;

R^g and R^j , at each occurrence, are independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-\text{R}^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-\text{R}^n$;

R^h at each occurrence is independently selected from the group consisting of $-\text{H}$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-\text{R}^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-\text{R}^n$;

R^i and R^k , at each occurrence, are independently selected from the group consisting of $-\text{H}$, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-\text{R}^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-\text{R}^n$;

R^m at each occurrence is independently selected from the group consisting of $=\text{O}$, halogen, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-\text{R}'$ and $-\text{L}_3-\text{R}^o$, when substituted on an available carbon atom, and carbocyclyl, heterocyclyl, aryl, heteroaryl, $-\text{R}''$ and $-\text{L}_4-\text{R}^p$, when substituted on an available nitrogen atom; wherein carbocyclyl, heterocyclyl, aryl and heteroaryl are optionally independently substituted with one or more $-\text{R}^n$.

R^n at each occurrence is independently selected from the group consisting of $=\text{O}$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-\text{R}'$ and $-\text{L}_3-\text{R}^o$, when substituted on an available carbon atom, and alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-\text{R}''$ and $-\text{L}_4-\text{R}^p$, when substituted on an available nitrogen atom;

L_3 is selected from the group consisting of $-\text{O}-$, $-\text{S}-$, $-\text{S}(=\text{O})-$, $-\text{S}(=\text{O})_2-$, $-\text{C}(=\text{W})-$, $-\text{C}(=\text{W})-\text{W}-$, $-\text{C}(=\text{W})-\text{NR}^q-$, $-\text{C}(=\text{W})-\text{NR}^q-\text{W}-$, $-\text{C}=\text{N}-\text{O}-$, $-\text{C}(=\text{NR}^q)\text{NR}^q-$, $-\text{C}(\text{NR}^q\text{R}^q)=\text{N}-$, $-\text{W}-\text{C}(=\text{W})-$, $-\text{W}-\text{C}(=\text{W})-\text{W}-$, $-\text{W}-\text{C}(=\text{W})-\text{NR}^q-$, $-\text{S}(=\text{O})_2-\text{NR}^q-$, $-\text{S}(=\text{O})_2-\text{NR}^q-\text{W}-$, $-\text{NR}^q-$,

-N=CR^r-, -NR^q-W-, -NR^q-NR^q-, -NR^q-C(=NR^q)NR^q-, -NR^q-C(NR^qR^q)=N-,
 -NR^q-C(=W)-, -NR^q-C(=W)-W-, -NR^q-C(=W)-NR^q-, -NR^q-S(=O)₂-NR^q-, and
 -NR^q-S(=O)₂-;

L₄ is selected from the group consisting of -O-, -S-, -NR^q-, -C(=W)-, -C(=W)-W-,
 -C(=W)-NR^q-, -C(=NR^q)NR^q-, -C(NR^qR^q)=N-, -S(=O)₂-NR^q-, and -S(=O)₂-;
 each W is independently O or S;

R^o and R^p, at each occurrence, are independently selected from the group consisting of
 alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl;
 wherein alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently
 substituted with one or more -R^s; carbocyclyl or heterocyclyl are optionally
 independently substituted with one or more -R^t; and aryl or heteroaryl are optionally
 independently substituted with one or more -R^u;

R^q at each occurrence is independently selected from the group consisting of -H, alkyl,
 alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein
 alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently substituted with
 one or more -R^s; carbocyclyl or heterocyclyl are optionally independently substituted
 with one or more -R^t; and aryl or heteroaryl are optionally independently substituted
 with one or more -R^u;

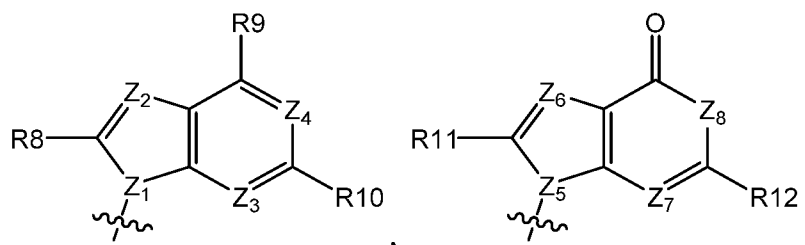
R^r at each occurrence is independently selected from the group consisting of -H, halogen,
 alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl;
 wherein alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently
 substituted with one or more -R^s; carbocyclyl or heterocyclyl are optionally
 independently substituted with one or more -R^t; and aryl or heteroaryl are optionally
 independently substituted with one or more -R^u;

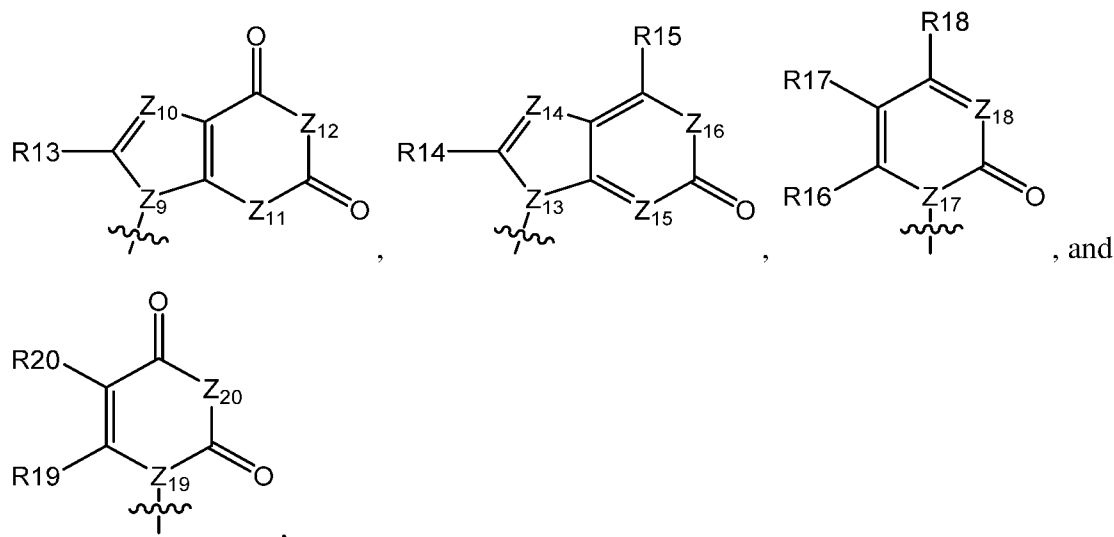
R^s at each occurrence is independently selected from the group consisting of halogen, =O,
 -OH, -SH, -NH₂, -CN, C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl,
 C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino, and N-linked-heterocycloalkyl;
 wherein C₁₋₆alkyl, alone or as part of C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl,
 C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, or C₁₋₆dialkylamino, is optionally substituted with
 one or more substituents independently selected from the group consisting of -OH,
 -SH, -NH₂, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylthio, C₁₋₆haloalkylthio,
 C₁₋₆alkylsulfinyl, C₁₋₆haloalkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆haloalkylsulfonyl,
 C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl;

R^t at each occurrence is independently selected from the group consisting of halogen, =O, -OH, -SH, -NH₂, -CN, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl; wherein C₂₋₆alkenyl, C₂₋₆alkynyl, and C₁₋₆alkyl, alone or as part of C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, or C₁₋₆dialkylamino, is optionally substituted with one or more substituents independently selected from the group consisting of -OH, -SH, -NH₂, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylthio, C₁₋₆haloalkylthio, C₁₋₆alkylsulfinyl, C₁₋₆haloalkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆haloalkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl; and

R^u at each occurrence is independently selected from the group consisting of halogen, -OH, -SH, -NH₂, -CN, -NO₂, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino, and N-linked-heterocycloalkyl; wherein C₂₋₆alkenyl, C₂₋₆alkynyl, and C₁₋₆alkyl, alone or as part of C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, or C₁₋₆dialkylamino, is optionally substituted with one or more substituents independently selected from the group consisting of -OH, -SH, -NH₂, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylthio, C₁₋₆haloalkylthio, C₁₋₆alkylsulfinyl, C₁₋₆haloalkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆haloalkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl.

2. The compound according to claim 1, wherein R1 and R2 are independently selected from the group consisting of:





wherein:



indicates the bond of R1 or R2 to the ring as shown in Formula I;

$Z_1, Z_5, Z_9, Z_{13}, Z_{17}$ and Z_{19} are independently $-\text{CH}-$ or $-\text{N}-$;

$Z_8, Z_{11}, Z_{12}, Z_{16}$ and Z_{20} are independently $-\text{CR}^v\text{R}^w-$, $-\text{O}-$ or $-\text{NR}^x-$;

$Z_2, Z_3, Z_4, Z_6, Z_7, Z_{10}, Z_{14}, Z_{15}$ and Z_{18} are independently $=\text{CR}^v-$ or $=\text{N}-$;

R^v and R^w , at each occurrence, are independently selected from the group consisting of

$-\text{H}$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl,

heteroaryl, $-\text{R}'$ and $-\text{L}_1-\text{R}^g$; or R^v and R^w together are $=\text{O}$, $=\text{CR}^h\text{R}^h$, $=\text{N}-\text{OR}^i$, $=\text{N}-\text{R}^i$, or

$=\text{N}-\text{NR}^i\text{R}^i$; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently

substituted with one or more $-\text{R}^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are

optionally independently substituted with one or more $-\text{R}^n$;

each R^x is independently selected from the group consisting of $-\text{H}$, alkyl, alkenyl, alkynyl,

heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-\text{R}''$ and $-\text{L}_2-\text{R}^j$; wherein alkyl,

alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or

more $-\text{R}^m$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally

independently substituted with one or more $-\text{R}^n$;

$\text{R}_8, \text{R}_9, \text{R}_{10}, \text{R}_{11}, \text{R}_{12}, \text{R}_{13}, \text{R}_{14}, \text{R}_{15}, \text{R}_{16}, \text{R}_{17}, \text{R}_{18}, \text{R}_{19}$ and R_{20} are independently

selected from the group consisting of $-\text{H}$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl,

carbocyclyl, heterocyclyl, aryl, heteroaryl, $-\text{R}'$ and $-\text{L}_1-\text{R}^g$; wherein alkyl, alkenyl,

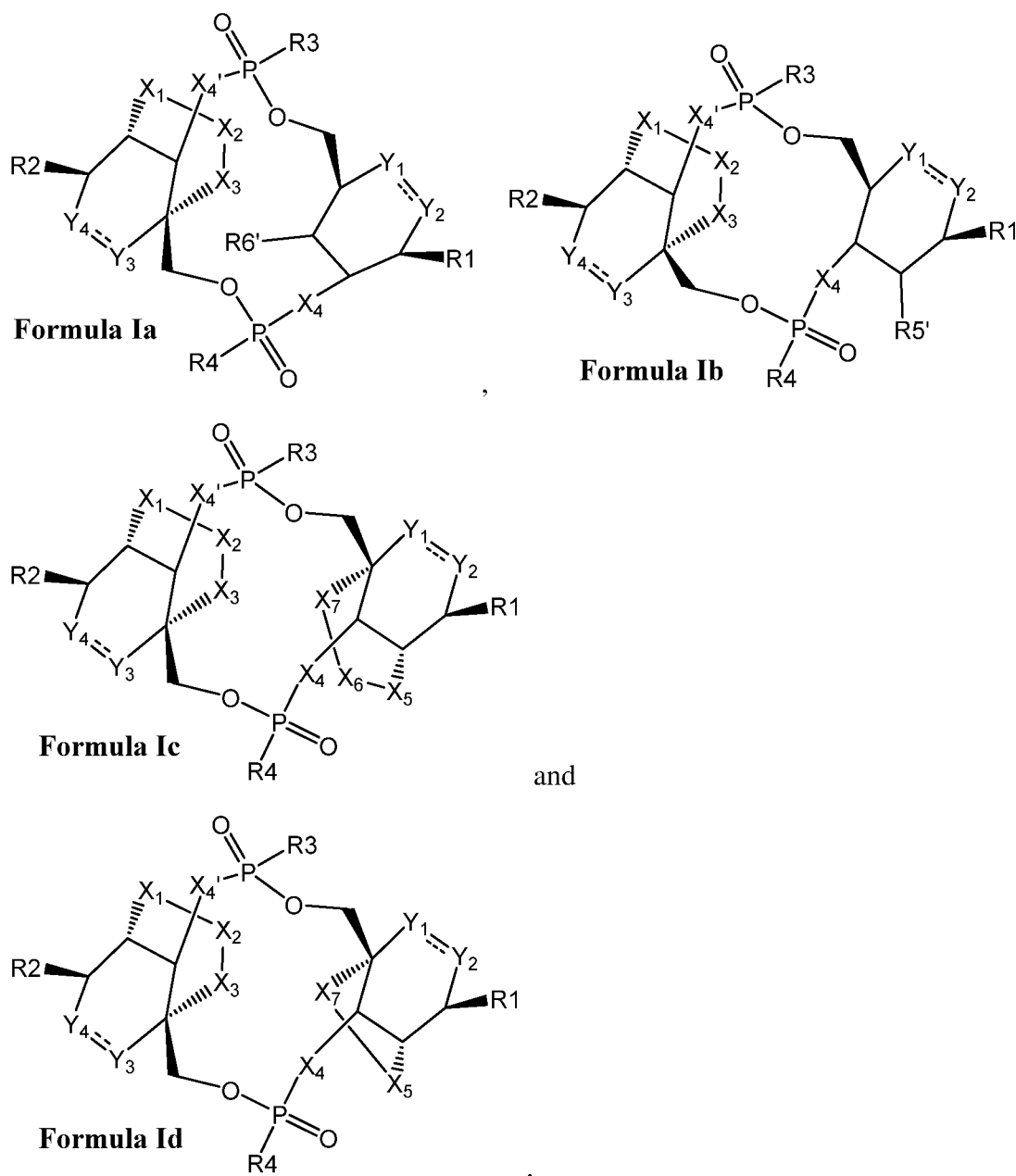
alkynyl or heteroalkyl are optionally independently substituted with one or more $-\text{R}^m$,

and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently

substituted with one or more $-\text{R}^n$;

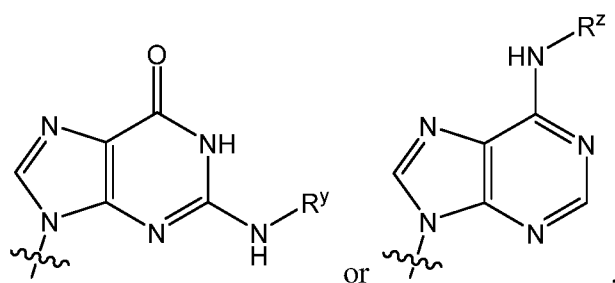
$R^{g'}$ is R^g or a suitable nitrogen protecting group when L_1 is $-NH-$; and R' , R'' , R^g , R^h , R^i , R^j , R^m , R^n , L_1 and L_2 are as defined in claim 1.

3. The compound according to claim 1 or 2, wherein the compound is selected from the group consisting of a compound of Formula Ia, a compound of Formula Ib, a compound of Formula Ic and a compound of Formula Id:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein $R5'$ and $R6'$ are selected from the group consisting of $-H$, $-OH$, halogen and $-OR^e$ and $R1$, $R2$, $R3$, $R4$, R^e , $Y1$, $Y2$, $Y3$, $Y4$, $X1$, $X2$, $X3$, $X4$, $X4'$, $X5$, $X6$ and $X7$ are as defined in claim 1.

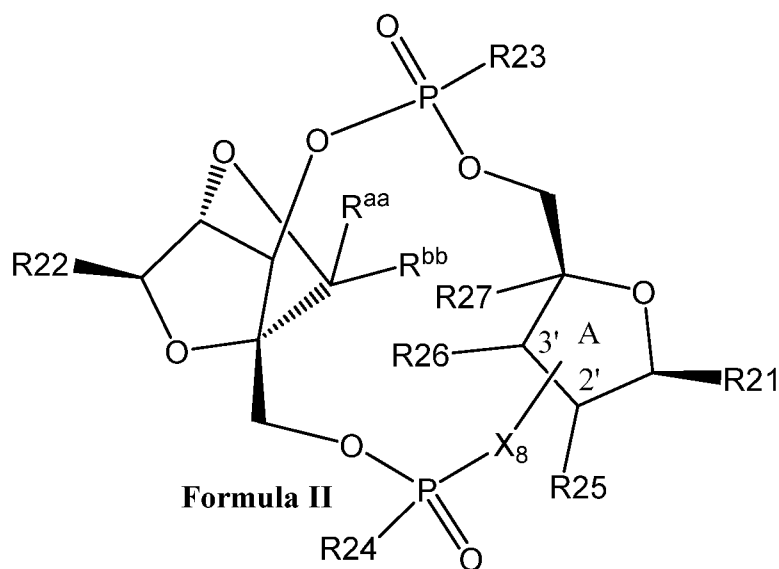
4. The compound according to claim 3, wherein, when present, R5' and R6' are selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl.
5. The compound according to claim 4, wherein, when present, R5' and R6' are -H, -OH or -F.
6. The compound according to any one of claims 1-5, wherein R1 and R2 are independently



wherein indicates the bond of R1 or R2 to the ring of Formula I and sub-generic formulae thereof, and wherein R^y and R^z are independently -H, -C(=O)-C₁₋₆ alkyl, -C(=O)-phenyl or other suitable nitrogen protecting group.

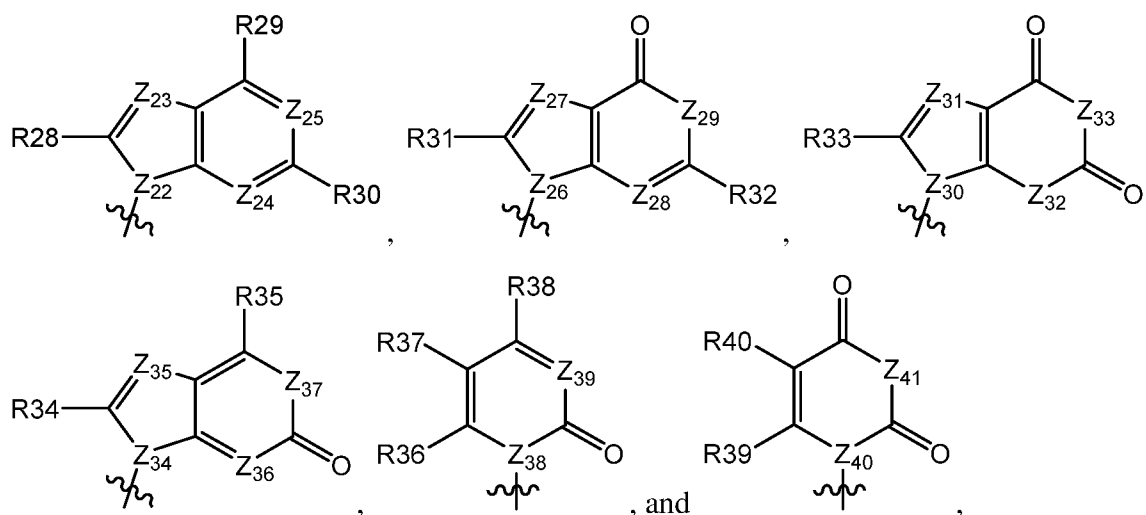
7. The compound according to claim 6, wherein R^y and R^z are both -H.
8. The compound according to any one of claims 1-7, wherein R3 and R4 are -SH.
9. The compound according to claim 8, wherein relative to the stereochemistry at each phosphorous atom the compound is the substantially pure Rp,Rp isomer, the substantially pure Rp,Sp isomer, the substantially pure Sp,Rp isomer, or the substantially pure Sp,Sp isomer.

10. The compound according to claim 9, wherein $-X_1-X_2-X_3-$ or $-X_1-X_3-$, and when present $-X_5-X_6-X_7-$ or $-X_5-X_7-$, are independently $-O-C(R^{b'}R^{c'})-$, where $R^{b'}$, $R^{c'}$ at each occurrence are independently $-H$, $-CH_3$, $-CH_2F$, $-CHF_2$, $-CF_3$, or $-CH_2OCH_3$, or together form $=CH_2$, or together with the carbon to which they are attached form a cyclopropyl spirocyclic ring.
11. The compound according to claim 10, wherein $R^{b'}$ and $R^{c'}$ at each occurrence are both $-H$.
12. The compound according to any one of claims 1-11, wherein X_4 and X_4' are both $-O-$.
13. The compound according to claim 1, wherein the compound is a compound of Formula II:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof,
wherein:

R21 and R22 are independently selected from the group consisting of:



wherein  indicates the bond of R21 or R22 to the ring as shown in Formula I;

R23 and R24 are independently -OH, -OR^{cc}, -SH or -SR^{cc};

X₈ is -O- bonded to either the 2' or the 3' carbon atom of the indicated ring A;

wherein:

when X₈ is -O- bonded to the 2' carbon atom of ring A:

R25 is absent, R26 is selected from the group consisting of -H, -OH, halogen and -OR^{dd}, and R27 is -H;

when X₈ is -O- bonded to the 3' carbon atom of ring A:

R26 is absent, R25 is selected from the group consisting of -H, -OH, halogen and -OR^{dd}, and R27 is -H; or

R26 is absent, and R25 and R27 join to form -O-C(R^{aa}R^{bb})-, resulting in a fused ring system with -O- bonded to the 2' carbon atom of ring A and -C(R^{aa}R^{bb})- bonded to the carbon atom of ring A to which R27 is attached;

R^{aa} and R^{bb}, at each occurrence, are independently selected from the group consisting of -H, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R' and -L₅-R^{ee}; or R^{aa} and R^{bb} together on a carbon are =O, =CR^{ff}R^{ff}, =N-OR^{gg}, =N-R^{gg}, or =N-NR^{gg}R^{gg}; or R^{aa} and R^{bb} together with the carbon to which they are attached form a C₃₋₆cycloalkyl or 3 to 6 membered heterocycloalkyl spirocyclic ring; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

- R^{cc} at each occurrence are independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl and $-C(=O)R^{hh}$; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^{jj}$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^{kk}$;
- R^{dd} is selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl and $-C(=O)R^{hh}$; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^{jj}$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^{kk}$; or R^{dd} is a suitable oxygen protecting group;
- R^{ee} at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^{jj}$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^{kk}$;
- R^{ff} at each occurrence is independently selected from the group consisting of $-H$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^{jj}$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^{kk}$;
- R^{gg} at each occurrence is independently selected from the group consisting of $-H$, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^{jj}$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^{kk}$;
- R^{hh} at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^j$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^{kk}$;
- R' at each occurrence is selected from the group consisting of $-CN$, $-NO_2$, $-OH$, $-SH$, $-NH_2$, $-C(=W)-WH$, $-C(=W)-NH_2$, $-C(=W)-NH-WH$, $-C=N-OH$, $-C(=NH)-NH_2$, $-W-C(=W)-WH$, $-W-C(=W)-NH_2$, $-S(=O)_2-NH_2$, $-S(=O)_2-NH-W-$, $-NH-NH_2$,

-NH-WH, -NH-C(=W)-WH, -NH-C(=W)-NH₂, -NH-S(=O)₂-NH₂, and
-NH-C(=NH)-NH₂;

L₅ is selected from the group consisting of -O-, -S-, -S(=O)-, -S(=O)₂-, -C(=W)-, -C(=W)-W-, -C(=W)-NRⁱⁱ-, -C(=W)-NRⁱⁱ-W-, -C=N-O-, -C(=NRⁱⁱ)NRⁱⁱ-, -C(NRⁱⁱRⁱⁱ)=N-, -W-C(=W)-, -W-C(=W)-W-, -W-C(=W)-NRⁱⁱ-, -S(=O)₂-NRⁱⁱ-, -S(=O)₂-NRⁱⁱ-W-, -NRⁱⁱ-, -N=CR^{ff}-, -NRⁱⁱ-W-, -NRⁱⁱ-NRⁱⁱ-, -NRⁱⁱ-C(=NRⁱⁱ)NRⁱⁱ-, -NRⁱⁱ-C(NRⁱⁱRⁱⁱ)=N-, -NRⁱⁱ-C(=W)-, -NRⁱⁱ-C(=W)-W-, -NRⁱⁱ-C(=W)-NRⁱⁱ-, -NRⁱⁱ-S(=O)₂-NRⁱⁱ- and -NRⁱⁱ-S(=O)₂-;

Rⁱⁱ at each occurrence is independently selected from the group consisting of -H, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

R^{jj} at each occurrence is independently selected from the group consisting of =O, halogen, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R' and -L₆-R^{mmm}, when substituted on an available carbon atom, and carbocyclyl, heterocyclyl, aryl, heteroaryl, -R'' and -L₇-R^{mm}, when substituted on an available nitrogen atom; wherein carbocyclyl, heterocyclyl, aryl and heteroaryl are optionally independently substituted with one or more -R^{kk}.

R^{kk} at each occurrence is independently selected from the group consisting of =O, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R' and -L₆-R^{mmm}, when substituted on an available carbon atom, and alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R'' and -L₇-R^{mm}, when substituted on an available nitrogen atom;

R'' at each occurrence is selected from the group consisting of -OH, -SH, -NH₂, -C(=W)-WH, -C(=W)-NH₂, -C(=NH)-NH₂, and -S(=O)₂-NH₂;

L₆ is selected from the group consisting of -O-, -S-, -S(=O)-, -S(=O)₂-, -C(=W)-, -C(=W)-W-, -C(=W)-NR^{oo}-, -C(=W)-NR^{oo}-W-, -C=N-O-, -C(=NR^{oo})NR^{oo}-, -C(NR^{oo}R^{oo})=N-, -W-C(=W)-, -W-C(=W)-W-, -W-C(=W)-NR^{oo}-, -S(=O)₂-NR^{oo}-, -S(=O)₂-NR^{oo}-W-, -NR^{oo}-, -N=CR^{pp}-, -NR^{oo}-W-, -NR^{oo}-NR^{oo}-, -NR^{oo}-C(=NR^{oo})NR^{oo}-, -NR^{oo}-C(NR^{oo}R^{oo})=N-, -NR^{oo}-C(=W)-, -NR^{oo}-C(=W)-W-, -NR^{oo}-C(=W)-NR^{oo}-, -NR^{oo}-S(=O)₂-NR^{oo}-, and -NR^{oo}-S(=O)₂-;

L₇ is selected from the group consisting of -O-, -S-, -NR^{oo}-, -C(=W)-, -C(=W)-W-,
 -C(=W)-NR^{oo}-, -C(=NR^{oo})NR^{oo}-, -C(NR^{oo}R^{oo})=N-, -S(=O)₂-NR^{oo}-, and -S(=O)₂-;
 each W is independently O or S;

R^{mm} and Rⁿⁿ, at each occurrence, are independently selected from the group consisting of
 alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl;
 wherein alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently
 substituted with one or more -R^{qq}; carbocyclyl or heterocyclyl are optionally
 independently substituted with one or more -R^{rr}; and aryl or heteroaryl are optionally
 independently substituted with one or more -R^{ss};

R^{oo} at each occurrence is independently selected from the group consisting of -H, alkyl,
 alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein
 alkyl, alkenyl, alkynyl and heteroalkyl, are optionally independently substituted with
 one or more -R^{qq}; carbocyclyl or heterocyclyl are optionally independently substituted
 with one or more -R^{rr}; and aryl or heteroaryl are optionally independently substituted
 with one or more -R^{ss};

R^{pp} at each occurrence is independently selected from the group consisting of -H,
 halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and
 heteroaryl; wherein alkyl, alkenyl, alkynyl and heteroalkyl, are optionally
 independently substituted with one or more -R^{qq}; carbocyclyl or heterocyclyl are
 optionally independently substituted with one or more -R^{rr}; and aryl or heteroaryl are
 optionally independently substituted with one or more -R^{ss};

R^{qq} at each occurrence is independently selected from the group consisting of halogen,
 =O, -OH, -SH, -NH₂, -CN, C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl,
 C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino, and N-linked-heterocycloalkyl;
 wherein C₁₋₆alkyl, alone or as part of C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl,
 C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, or C₁₋₆dialkylamino, is optionally substituted with
 one or more substituents independently selected from the group consisting of -OH,
 -SH, -NH₂, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylthio, C₁₋₆haloalkylthio,
 C₁₋₆alkylsulfinyl, C₁₋₆haloalkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆haloalkylsulfonyl,
 C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl;

R^{rr} at each occurrence is independently selected from the group consisting of halogen,
 =O, -OH, -SH, -NH₂, -CN, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₁₋₆alkoxy,
 C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino
 and N-linked-heterocycloalkyl; wherein C₂₋₆alkenyl, C₂₋₆alkynyl, and C₁₋₆alkyl, alone

or as part of C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, or C₁₋₆dialkylamino, is optionally substituted with one or more substituents independently selected from the group consisting of -OH, -SH, -NH₂, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylthio, C₁₋₆haloalkylthio, C₁₋₆alkylsulfinyl, C₁₋₆haloalkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆haloalkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl; and

R^{ss} at each occurrence is independently selected from the group consisting of halogen, -OH, -SH, -NH₂, -CN, -NO₂, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino, and N-linked-heterocycloalkyl; wherein C₂₋₆alkenyl, C₂₋₆alkynyl, and C₁₋₆alkyl, alone or as part of C₁₋₆alkoxy, C₁₋₆alkylthio, C₁₋₆alkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆alkylamino, or C₁₋₆dialkylamino, is optionally substituted with one or more substituents independently selected from the group consisting of -OH, -SH, -NH₂, halogen, C₁₋₆alkoxy, C₁₋₆haloalkoxy, C₁₋₆alkylthio, C₁₋₆haloalkylthio, C₁₋₆alkylsulfinyl, C₁₋₆haloalkylsulfinyl, C₁₋₆alkylsulfonyl, C₁₋₆haloalkylsulfonyl, C₁₋₆alkylamino, C₁₋₆dialkylamino and N-linked-heterocycloalkyl;

Z₂₂, Z₂₆, Z₃₀, Z₃₄, Z₃₈ and Z₄₀ are independently $\text{---}\underset{|}{\text{CH}}\text{---}$ or $\text{---}\underset{|}{\text{N}}\text{---}$;

Z₂₉, Z₃₂, Z₃₃, Z₃₇ and Z₄₁ are independently -CR^{tt}R^{uu}-, -O- or -NR^{vv}-;

Z₂₃, Z₂₄, Z₂₅, Z₂₇, Z₂₈, Z₃₁, Z₃₅, Z₃₆ and Z₃₉ are independently =CR^{tt}- or =N-;

R^{tt} and R^{uu}, at each occurrence, are independently selected from the group consisting of -H, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R' and -L₅-R^{ee}; or R^{tt} and R^{uu} together are =O, =CR^{ff}R^{ff}, =N-OR^{gg}, =N-R^{gg}, or =N-NR^{gg}R^{gg}; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

each R^{vv} is independently selected from the group consisting of -H, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, -R'' and -L₈-R^{ww}; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more -R^{jj}, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more -R^{kk};

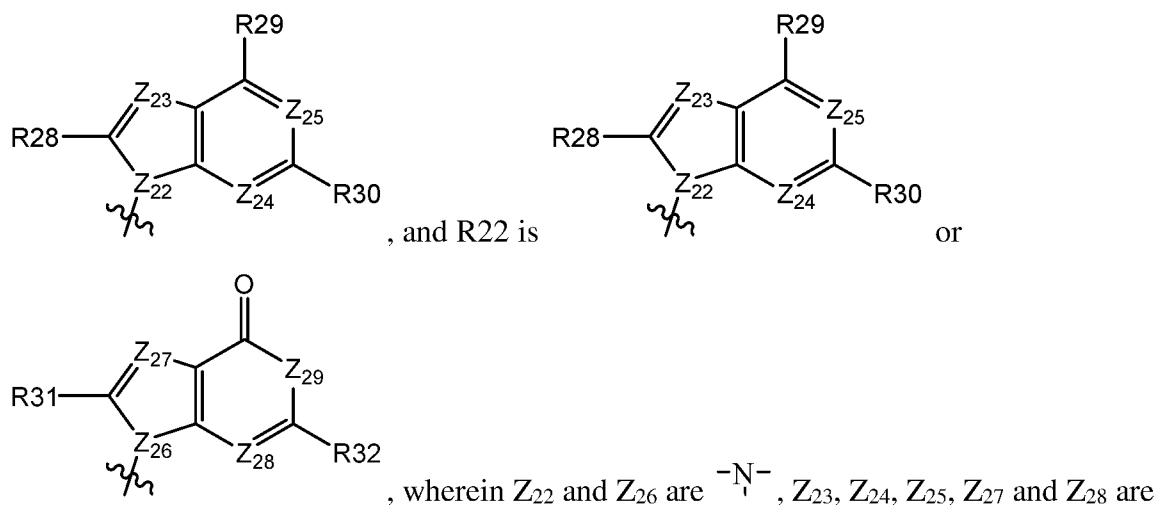
L₈ is selected from the group consisting of -O-, -S-, -NR^{xx}-, -C(=W)-, -C(=W)-W-, -C(=W)-NR^{xx}-, -C(=NR^{xx})NR^{xx}-, -C(NR^{xx}R^{xx})=N-, -S(=O)₂-NR^{xx}-, and -S(=O)₂-;

R^{ww} at each occurrence is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^{jj}$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^{kk}$;

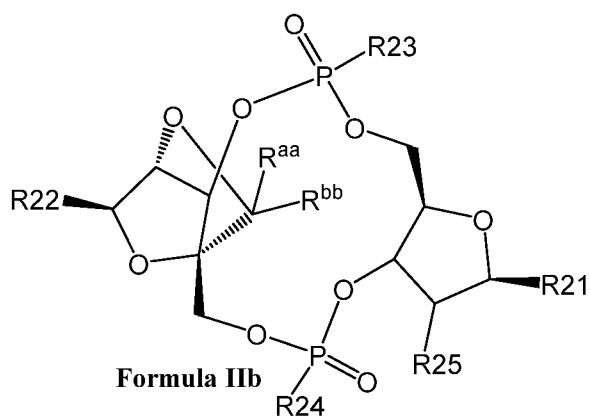
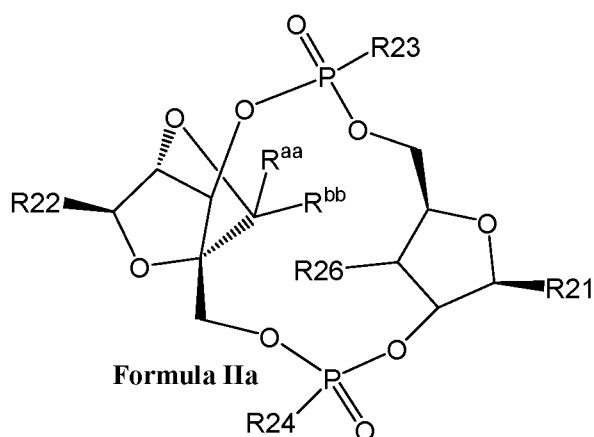
R^{xx} at each occurrence is independently selected from the group consisting of $-H$, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl and heteroaryl; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^{jj}$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^{kk}$;

R_{28} , R_{29} , R_{30} , R_{31} , R_{32} , R_{33} , R_{34} , R_{35} , R_{36} , R_{37} , R_{38} , R_{39} and R_{40} are independently selected from the group consisting of $-H$, halogen, alkyl, alkenyl, alkynyl, heteroalkyl, carbocyclyl, heterocyclyl, aryl, heteroaryl, $-R'$ and $-L_5-R^{ee'}$; wherein alkyl, alkenyl, alkynyl or heteroalkyl are optionally independently substituted with one or more $-R^{jj}$, and carbocyclyl, heterocyclyl, aryl or heteroaryl are optionally independently substituted with one or more $-R^{kk}$, wherein $R^{ee'}$ is R^{ee} or a suitable nitrogen protecting group when L_5 is $-NH-$;

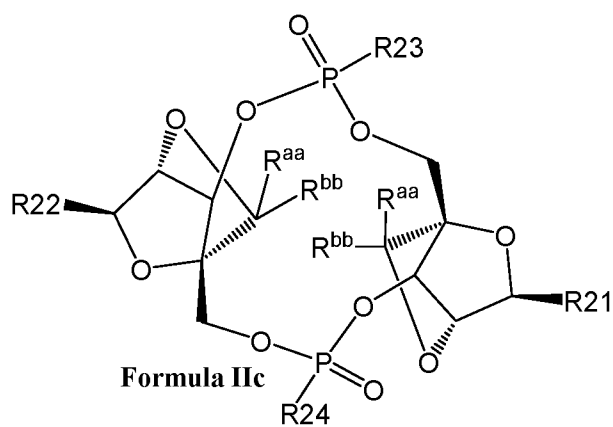
provided that when X_8 is $-O-$ bonded to the 2' carbon atom of ring A, R_{21} is



14. The compound according to claim 13, wherein the compound is selected from the group consisting of a compound of Formula IIa, a compound of Formula IIb and a compound of Formula IIc:



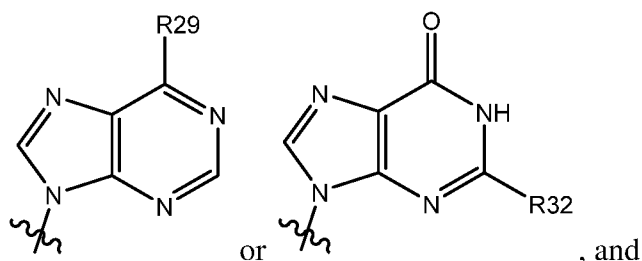
and



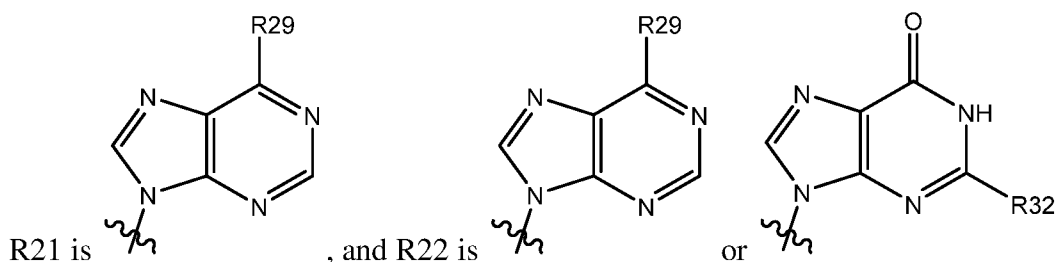
;

or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R21, R22, R23, R24, R25, R26, R^{aa} and R^{bb} are as defined in claim 13.

15. The compound according to claim 13, wherein when X₈ is -O- bonded to the 3' carbon atom of ring A, R21 and R22 are independently



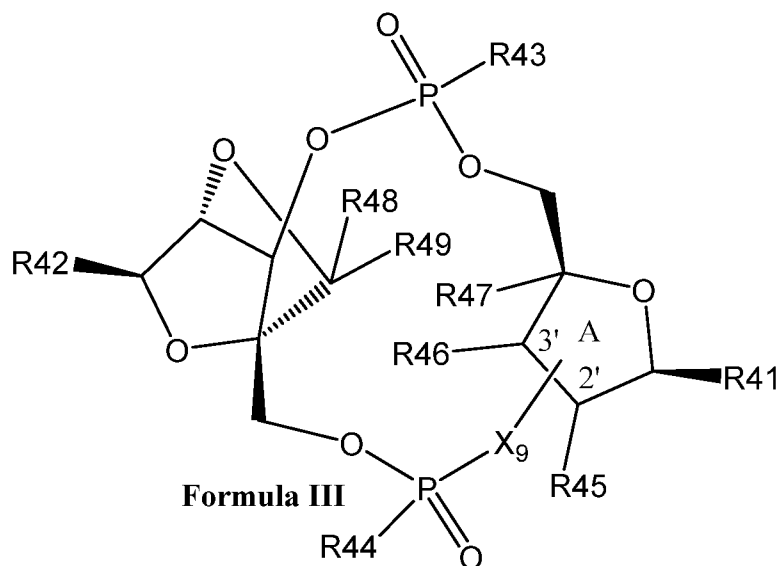
when X₈ is -O- bonded to the 2' carbon atom of ring A,



wherein R29 and R32 are independently -NH₂ or -NH-C₁₋₆alkyl, wherein -NH₂ is optionally substituted with a suitable nitrogen protecting group.

16. The compound according to claim 15, wherein R25 and R26 are selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl.
17. The compound according to claim 16, wherein R25 and R26 are -H, -OH, -OTBS or -F.
18. The compound according to any one of claims 13-17, wherein R3 and R4 are -SH.
19. The compound according to claim 18, wherein relative to the stereochemistry at each phosphorous atom the compound is the substantially pure Rp,Rp isomer, the substantially pure Rp,Sp isomer, the substantially pure Sp,Rp isomer, or the substantially pure Sp,Sp isomer.
20. The compound according to claim 19, wherein R^{aa} and R^{bb} at each occurrence are independently -H, -CH₃, -CH₂F, -CHF₂, -CF₃, or -CH₂OCH₃, or together form =CH₂, or together with the carbon to which they are attached form a cyclopropyl spirocyclic ring.
21. The compound according to claim 20, wherein R^{aa} and R^{bb} at each occurrence are -H.

22. The compound according to claim 1, wherein the compound is a compound of Formula III:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof,

wherein:

R43 and R44 are independently selected from the group consisting of -OH, -SH,

-O-C₁₋₁₄alkyl, -S-C₁₋₁₄alkyl, -O-(C=O)-C₁₋₁₄alkyl and -S-C(=O)-C₁₋₁₄alkyl;

X₉ is -O- bonded to either the 2' or the 3' carbon atom of the indicated ring A;

wherein:

when X₉ is -O- bonded to the 2' carbon atom of ring A:

R45 is absent, R46 is selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen,

-O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl and R47 is -H;

when X₉ is -O- bonded to the 3' carbon atom of ring A:

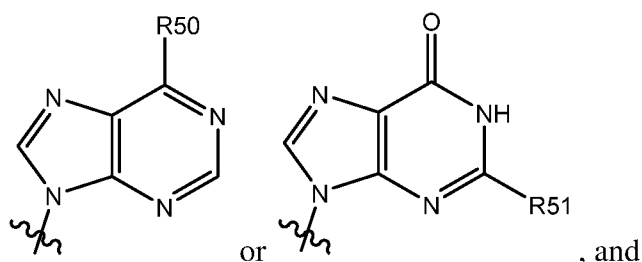
R46 is absent, R45 is selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, halogen, -O-C₁₋₁₄alkyl and -O-(C=O)-C₁₋₁₄alkyl, and R47 is -H; or

R46 is absent, and R45 and R47 join to form -O-C(R48R49)-, resulting in a fused ring system with -O- bonded to the 2' carbon atom of ring A and -C(R48R49)- is bonded to the carbon atom of ring A to which R47 is attached;

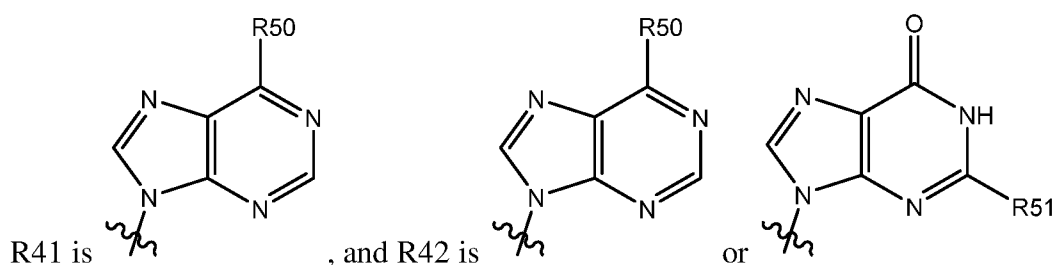
R48 and R49 at each occurrence are independently -H or -C₁₋₆alkyl, wherein alkyl is optionally substituted with 1, 2 or 3 -F or with -C₁₋₆alkoxy; or R48 and R49 on the same carbon together form a C₃₋₆cycloalkyl spirocyclic ring;

and

when X₉ is -O- bonded to the 3' carbon atom of ring A, R41 and R42 are independently

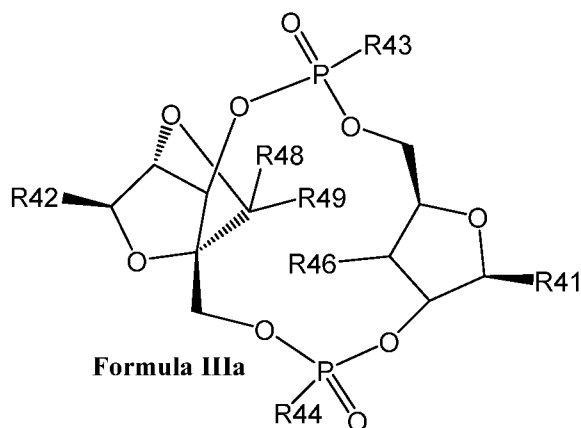


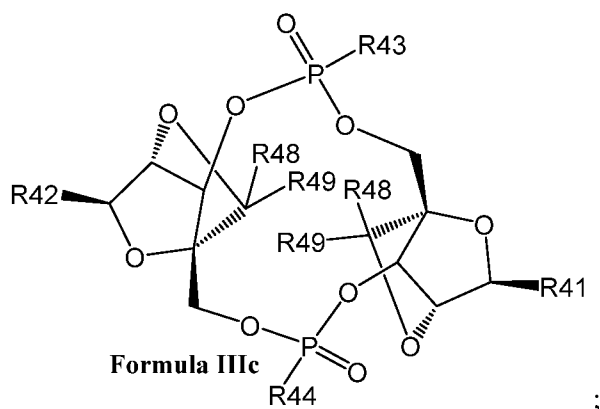
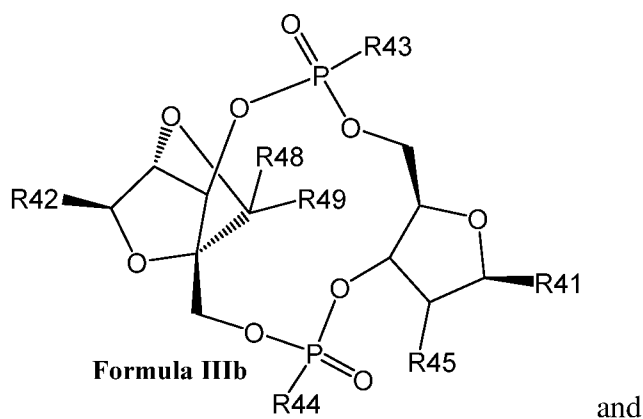
when X₉ is -O- bonded to the 2' carbon atom of ring A,



wherein  indicates the bond of R41 or R42 to the ring as shown in Formula III, and wherein R50 and R51 are independently -NH₂ or -NH-C₁₋₆alkyl, wherein -NH₂ is optionally substituted with a suitable nitrogen protecting group.

23. The compound according to claim 22, wherein the compound is selected from the group consisting of a compound of Formula IIIa, a compound of Formula IIIb and a compound of Formula IIIc:





or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R41, R42, R43, R44, R45, R46, R48 and R49 are as defined claim 22.

24. The compound according to claim 22 or 23, wherein R45 and R46 are -H, -OH, -OTBS or -F.

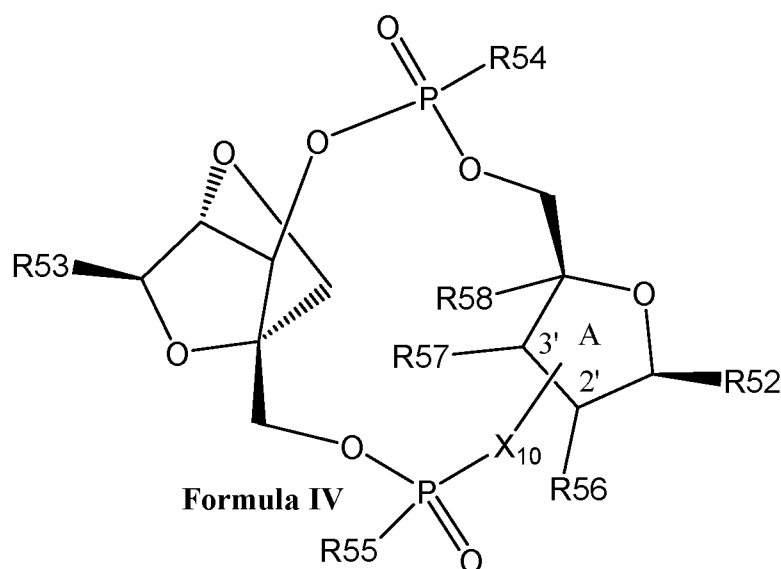
25. The compound according to any one of claims 22-24, wherein R50 and R51 are independently -NH₂, -NH-C₁₋₆alkyl, -N=CH-N(CH₃)₂, -NH-C(=O)-C₁₋₆alkyl or -NH-C(=O)-phenyl.

26. The compound according to claim 25, wherein R50 and R51 are both -NH₂.

27. The compound according to any one of claims 22-26, wherein R43 and R44 are -SH.

28. The compound according to claim 27, wherein relative to the stereochemistry at each phosphorous atom the compound is the substantially pure Rp,Rp isomer, the substantially pure Rp,Sp isomer, the substantially pure Sp,Rp isomer, or the substantially pure Sp,Sp isomer.

29. The compound according to claim 1, wherein the compound is a compound of Formula IV:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof,

wherein:

R54 and R55 are independently -OH or -SH;

X₁₀ is -O- bonded to either the 2' or the 3' carbon atom of the indicated ring A;

wherein:

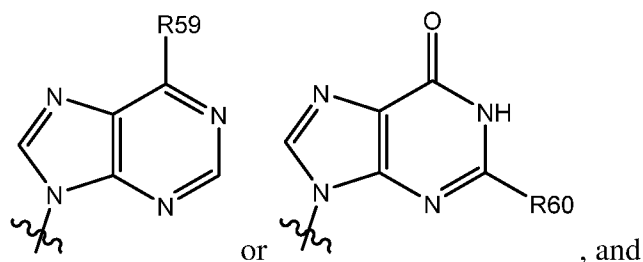
when X₁₀ is -O- bonded to the 2' carbon atom of ring A:

R56 is absent, R57 is selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, and halogen, and R58 is -H;

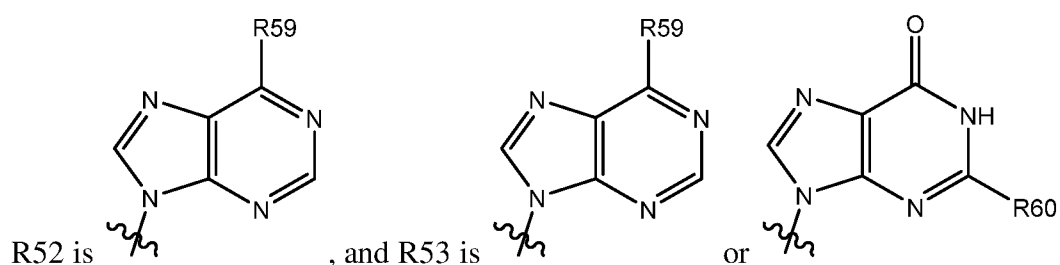
when X₁₀ is -O- bonded to the 3' carbon atom of ring A:

R57 is absent, R56 is selected from the group consisting of -H, -OH optionally substituted with a suitable oxygen protecting group, and halogen, and R58 is -H; or

R57 is absent, and R56 and R58 join to form -O-CH₂-, resulting in a fused ring system with -O- bonded to the 2' carbon atom of ring A and -CH₂- bonded to the carbon atom of ring A to which R58 is attached; and when X₁₀ is -O- bonded to the 3' carbon atom of ring A, R52 and R53 are independently

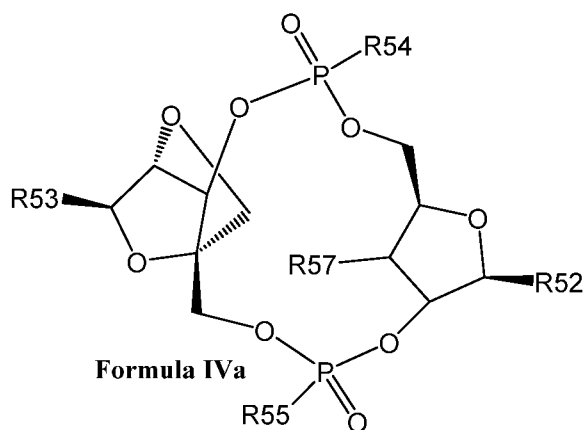


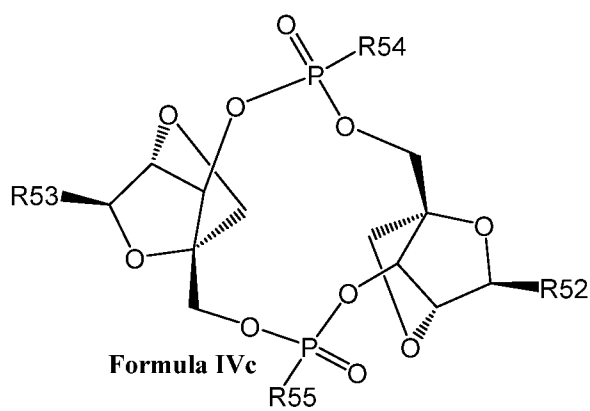
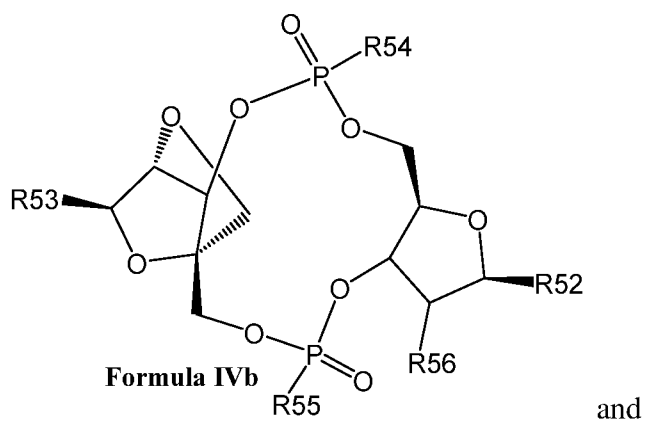
when X₁₀ is -O- bonded to the 2' carbon atom of ring A,



wherein  indicates the bond of R52 or R53 to the ring as shown in Formula IV, and wherein R59 and R60 are independently -NH₂ optionally substituted with a suitable nitrogen protecting group.

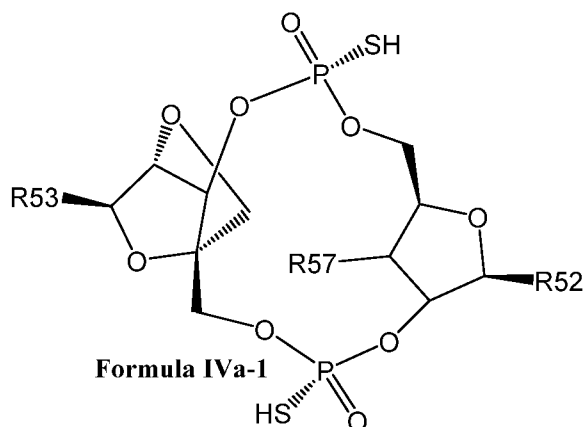
30. The compound according to claim 29, wherein the compound is selected from the group consisting of a compound of Formula IVa, a compound of Formula IVb and a compound of Formula IVc:

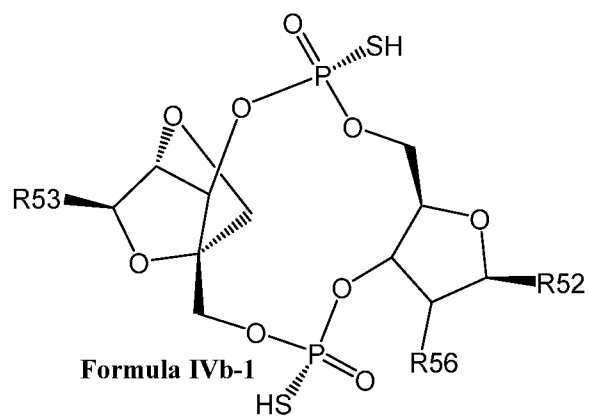
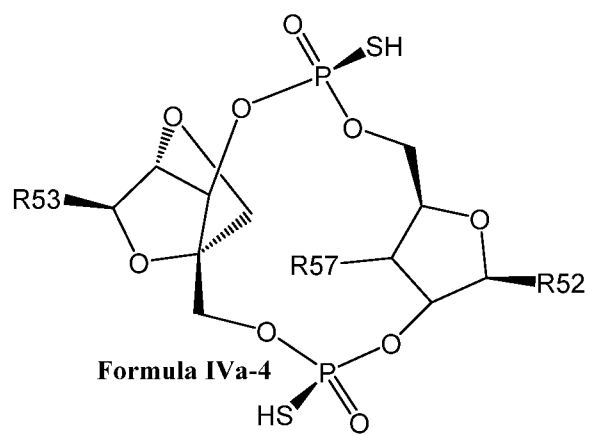
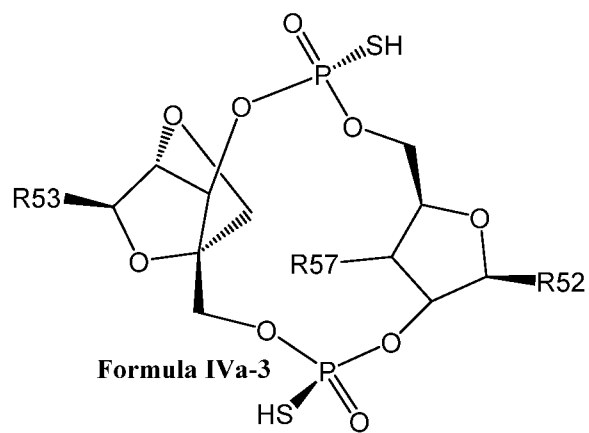
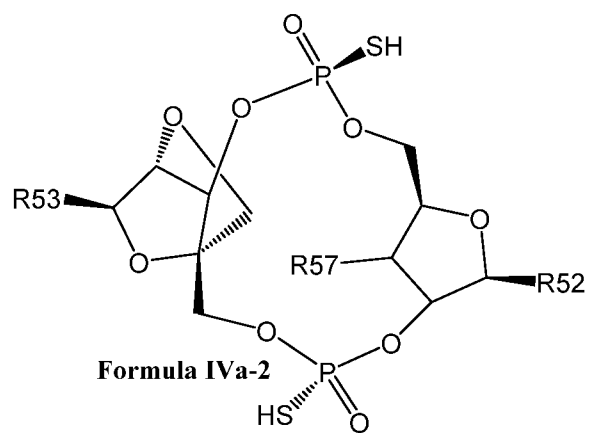


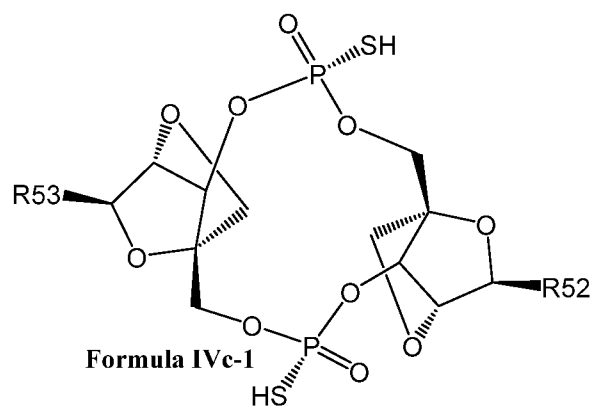
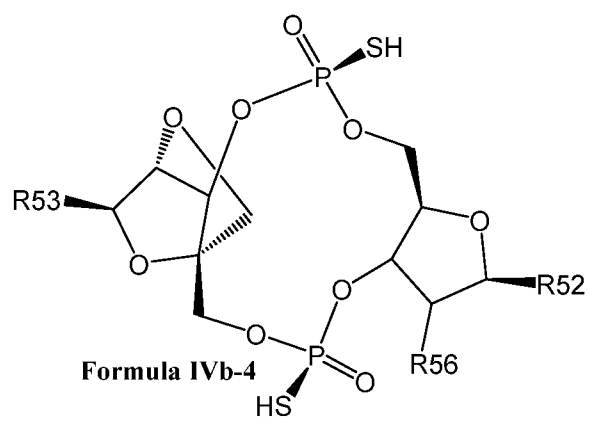
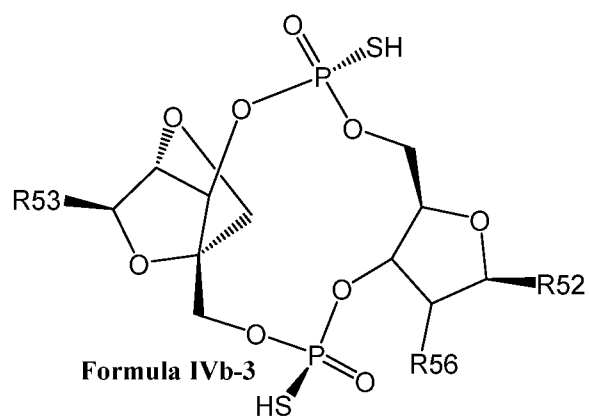
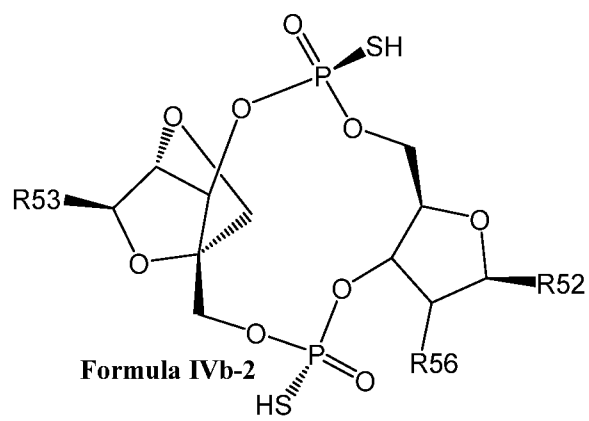


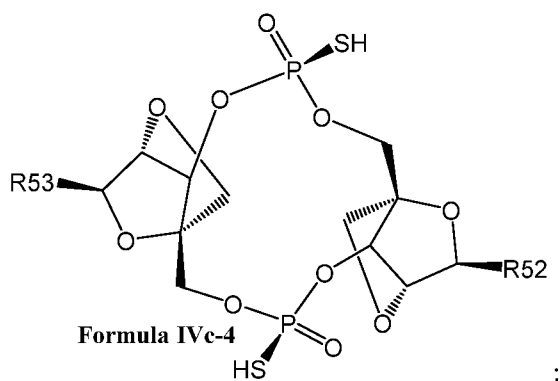
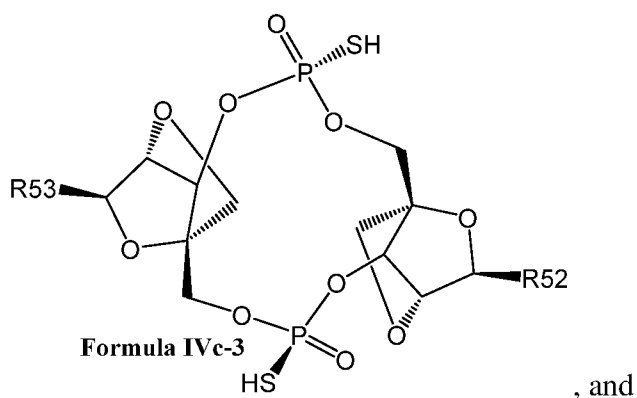
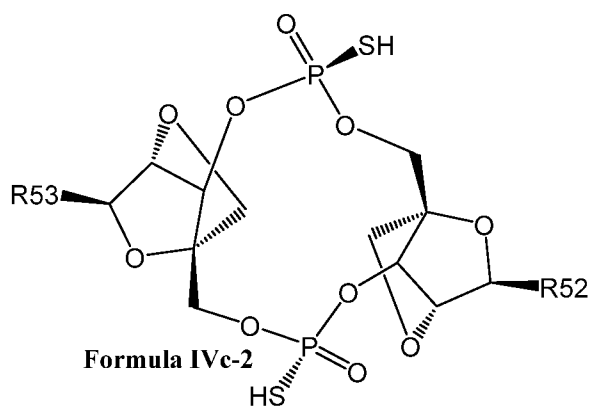
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R52, R53, R54, R55, R56 and R57 are as defined in claim 29.

31. The compound according to claim 29, wherein the compound is selected from the group consisting of a compound of Formula IVa-1, a compound of Formula IVa-2, a compound of Formula IVa-3, a compound of Formula IVa-4, a compound of Formula IVb-1, a compound of Formula IVb-2, a compound of Formula IVb-3, a compound of Formula IVb-4, a compound of Formula IVc-1, a compound of Formula IVc-2, a compound of Formula IVc-3, and a compound of Formula IVc-4:









or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof, wherein R52, R53, R56 and R57 are as defined in claim 29.

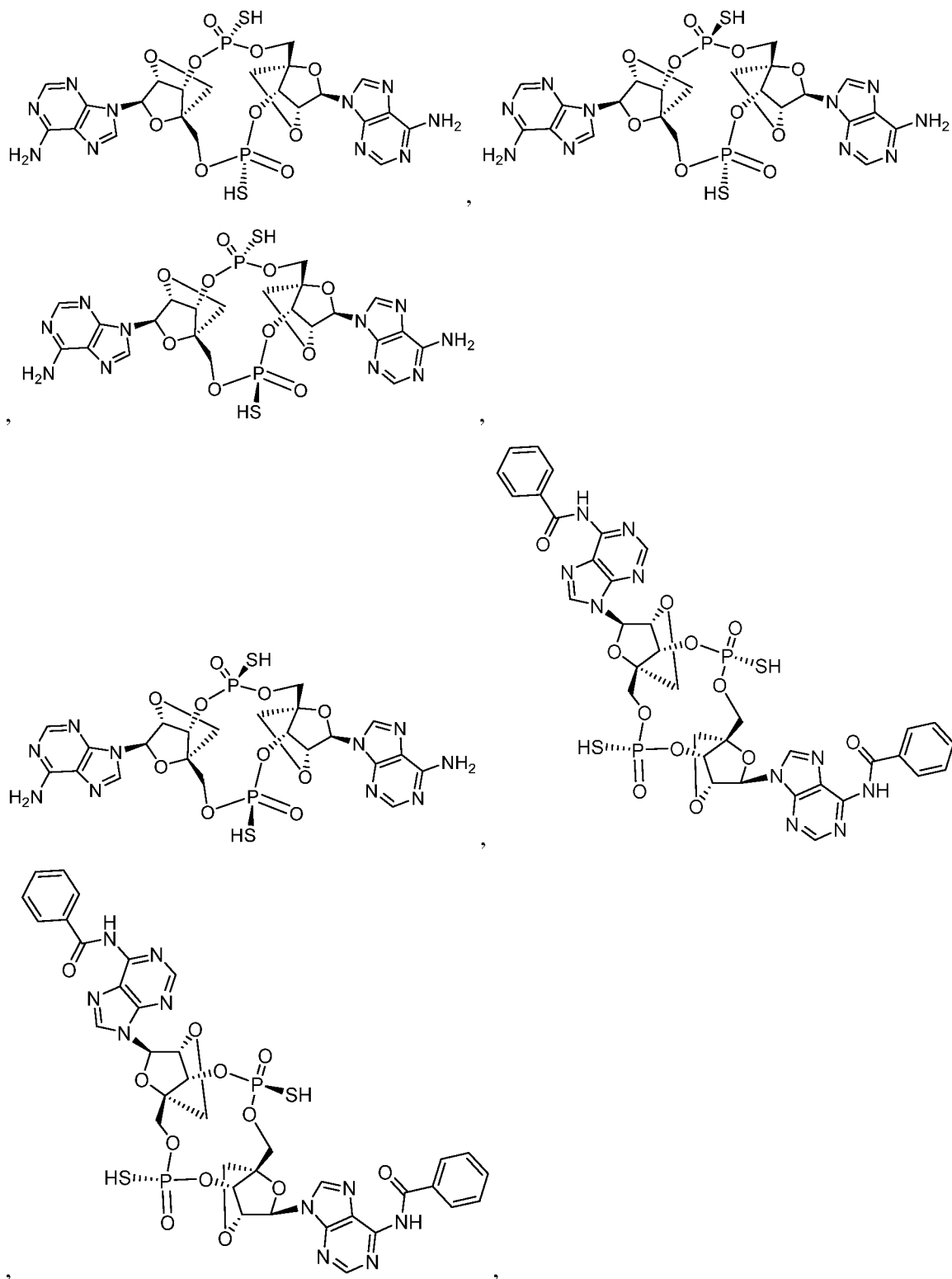
32. The compound according to claim 31, wherein R56 and R57 are -H, -OH, -OTBS or -F.

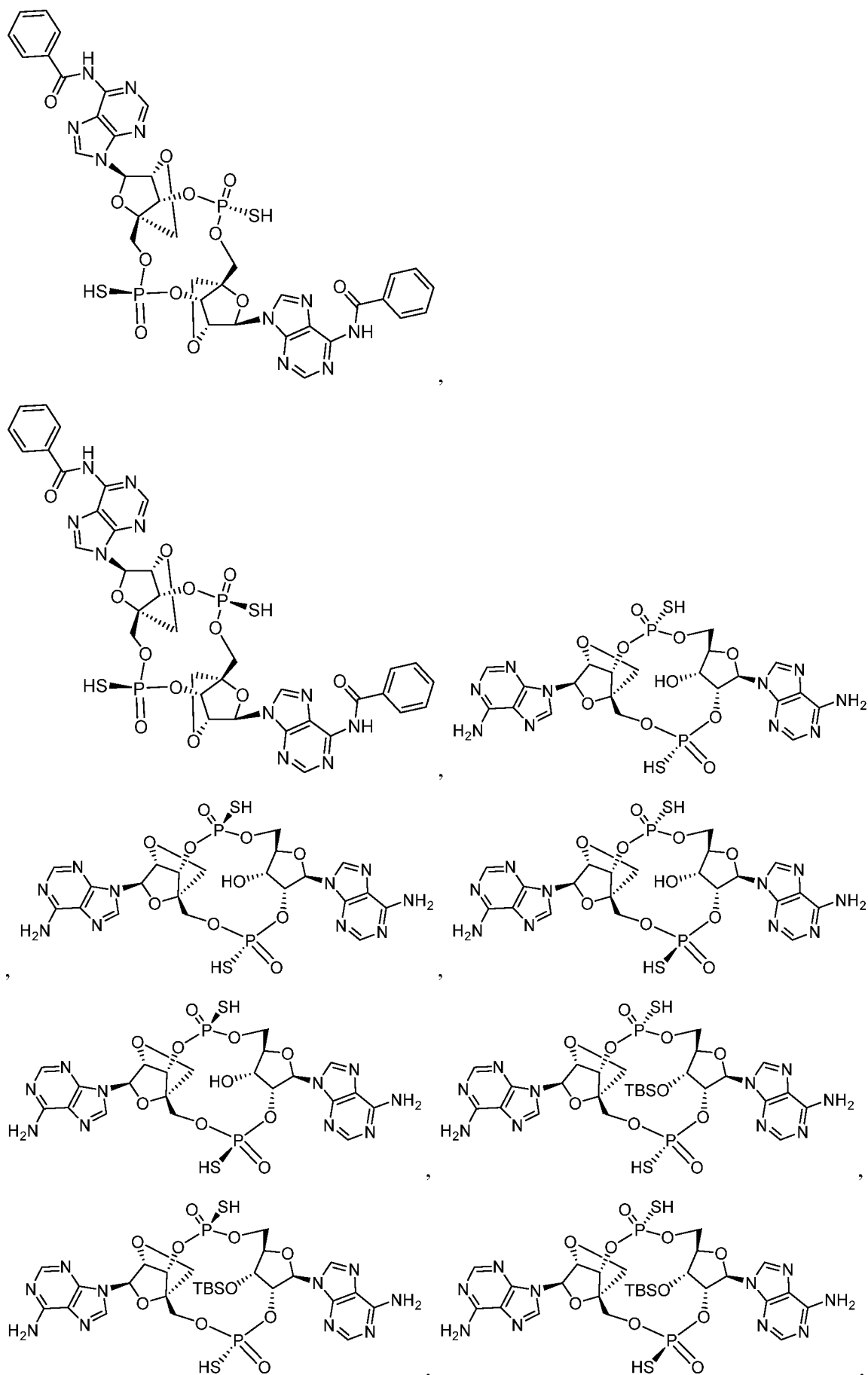
33. The compound according to claim 32, wherein R56 and R57 are -OH or -F.

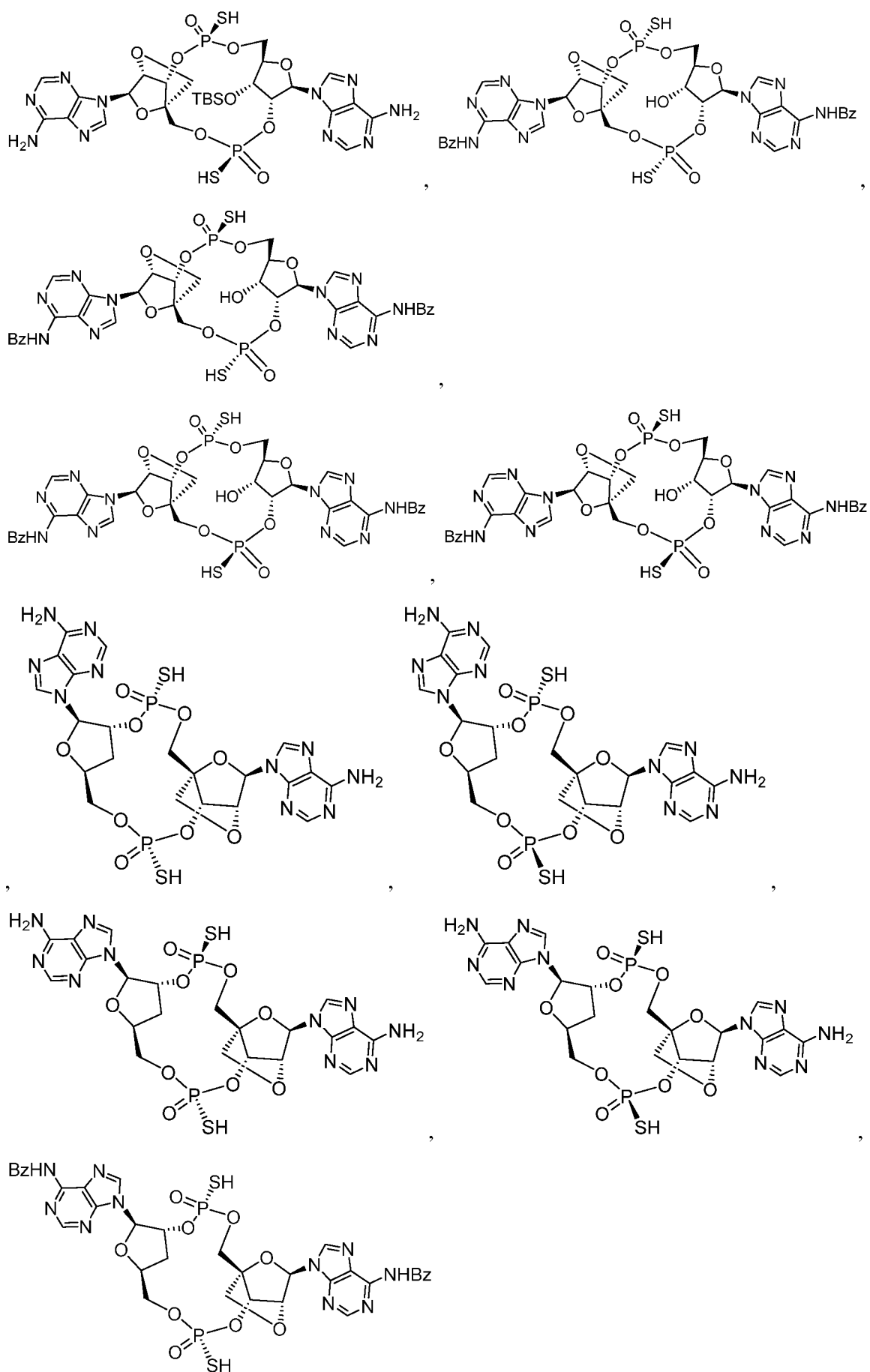
34. The compound according to claim 31, wherein relative to the stereochemistry at each phosphorous atom the compound is the substantially pure Rp,Rp isomer, the substantially

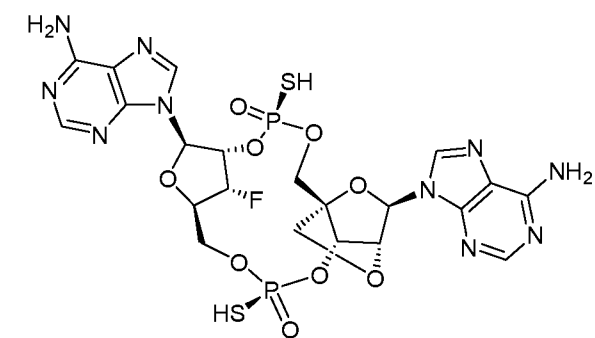
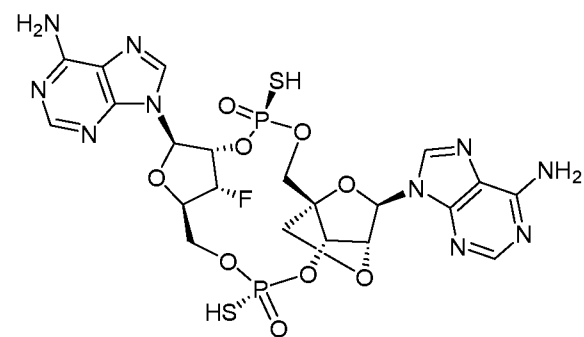
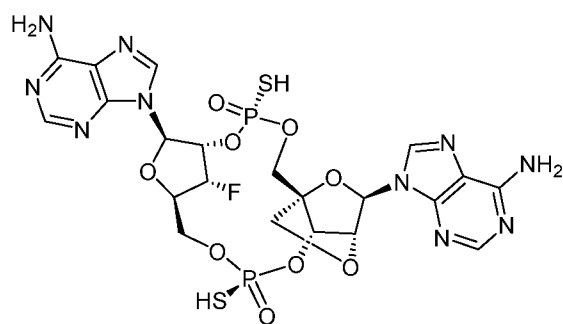
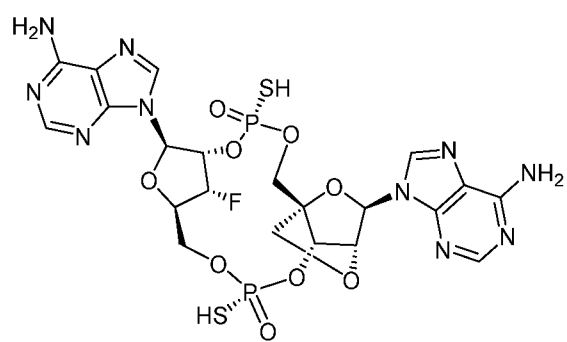
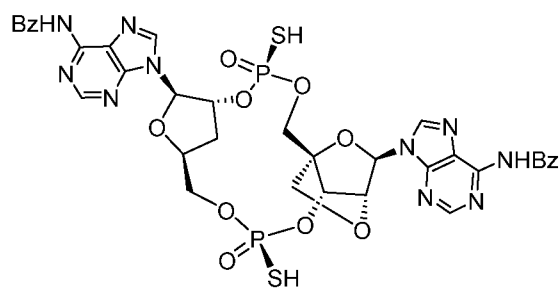
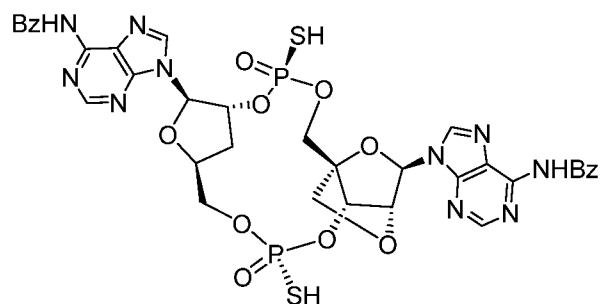
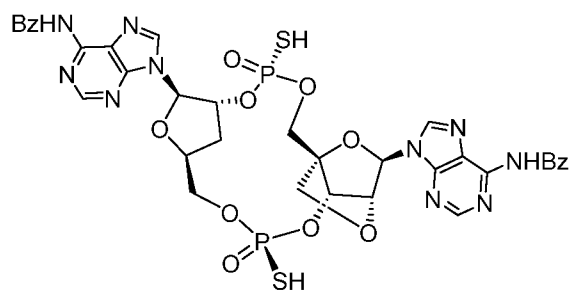
pure Rp,Sp isomer, the substantially pure Sp,Rp isomer, or the substantially pure Sp,Sp isomer.

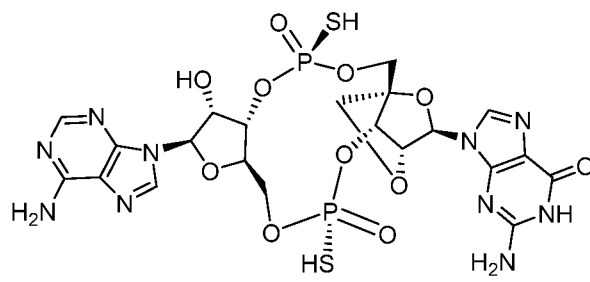
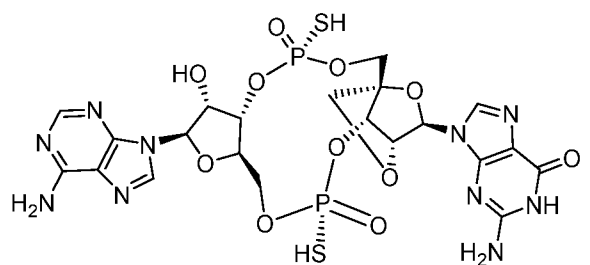
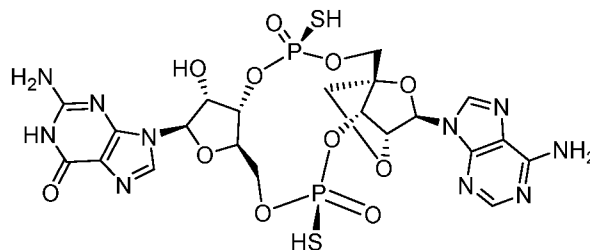
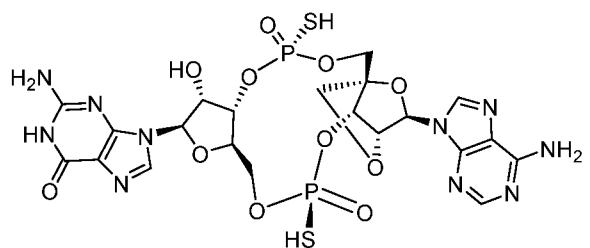
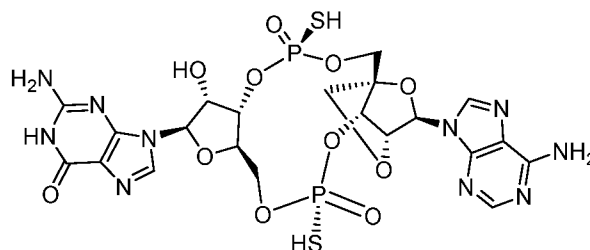
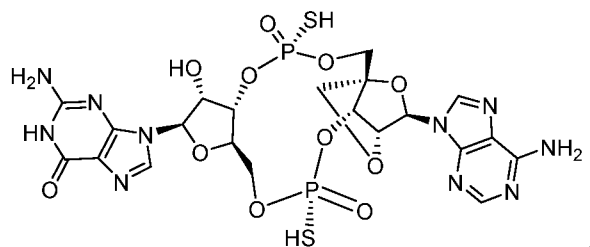
35. The compound according to claim 1, selected from the group consisting of:

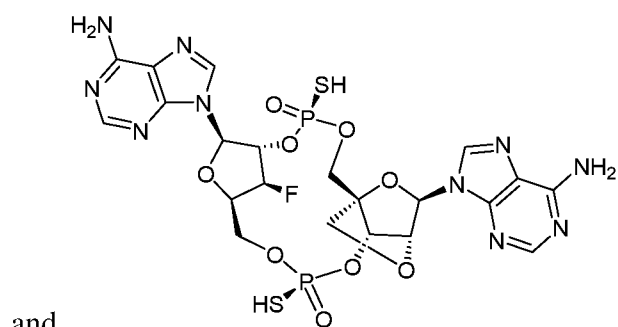
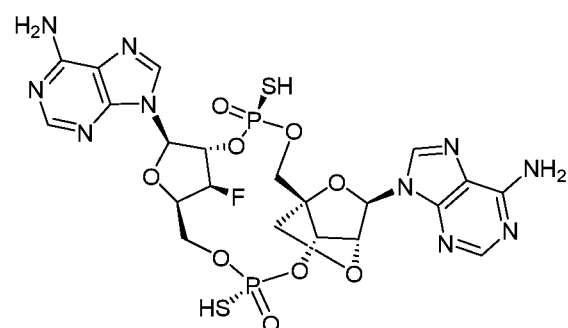
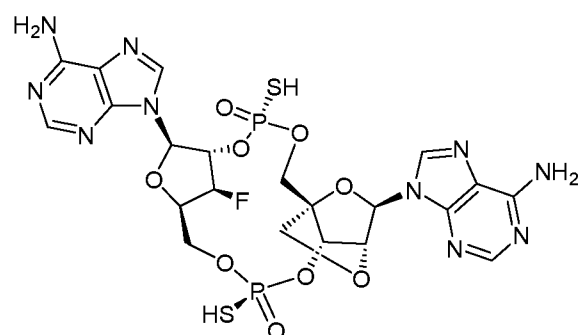
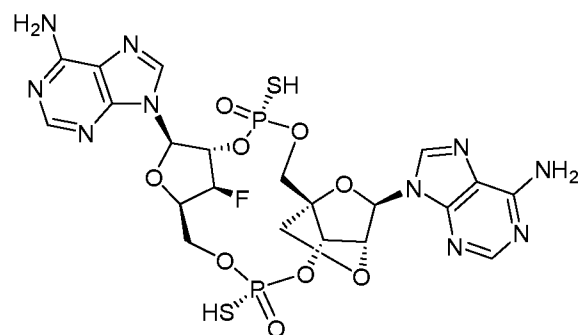
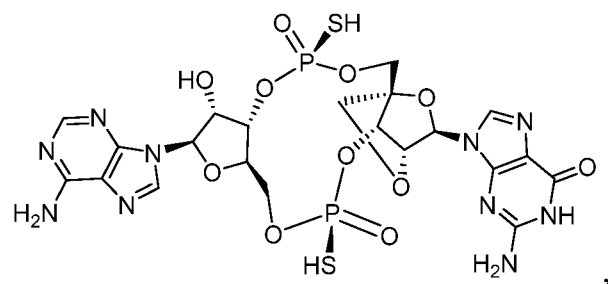
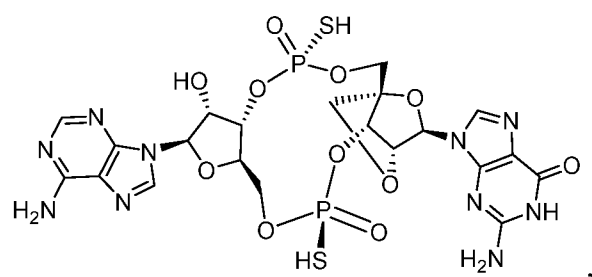








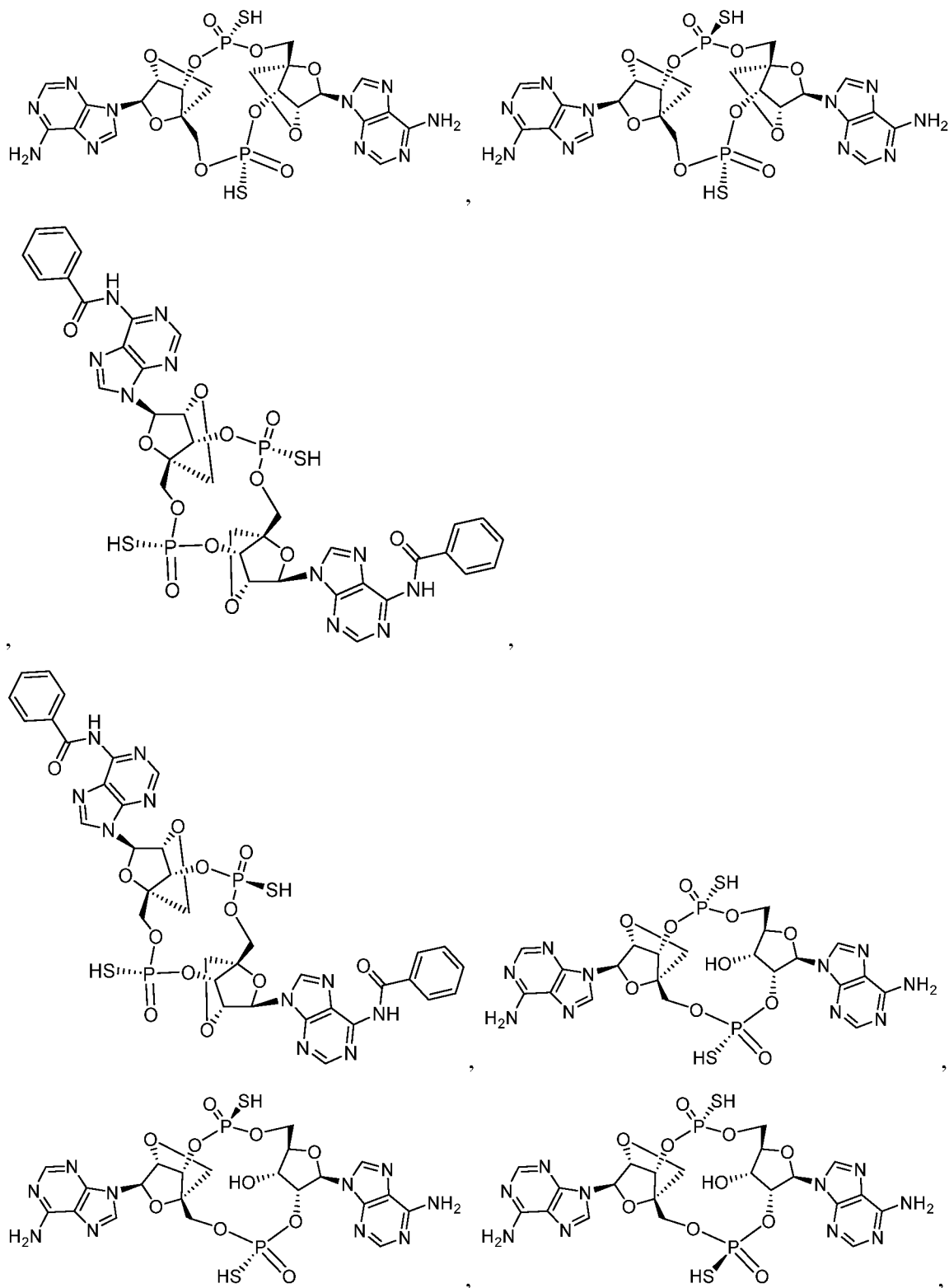


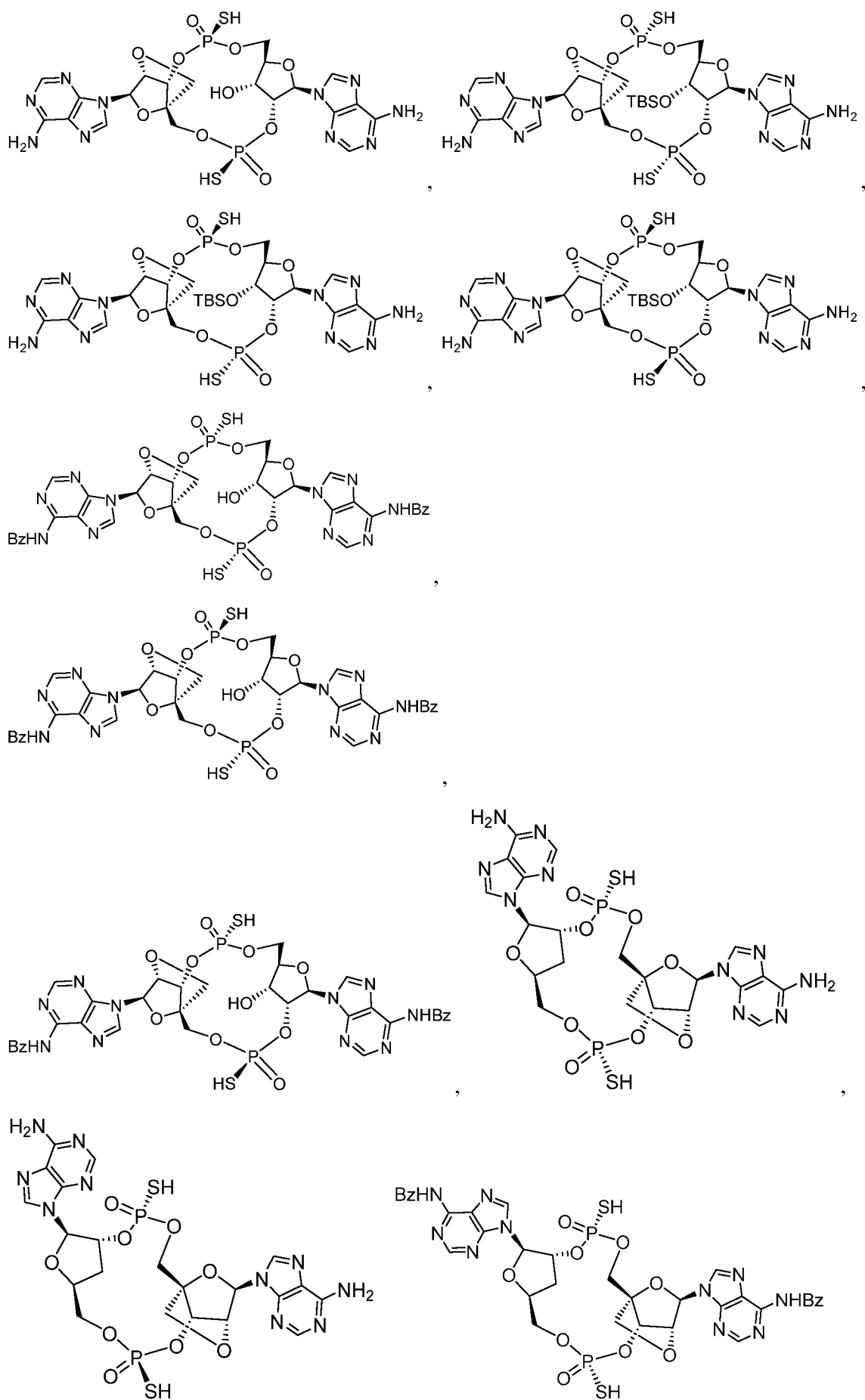


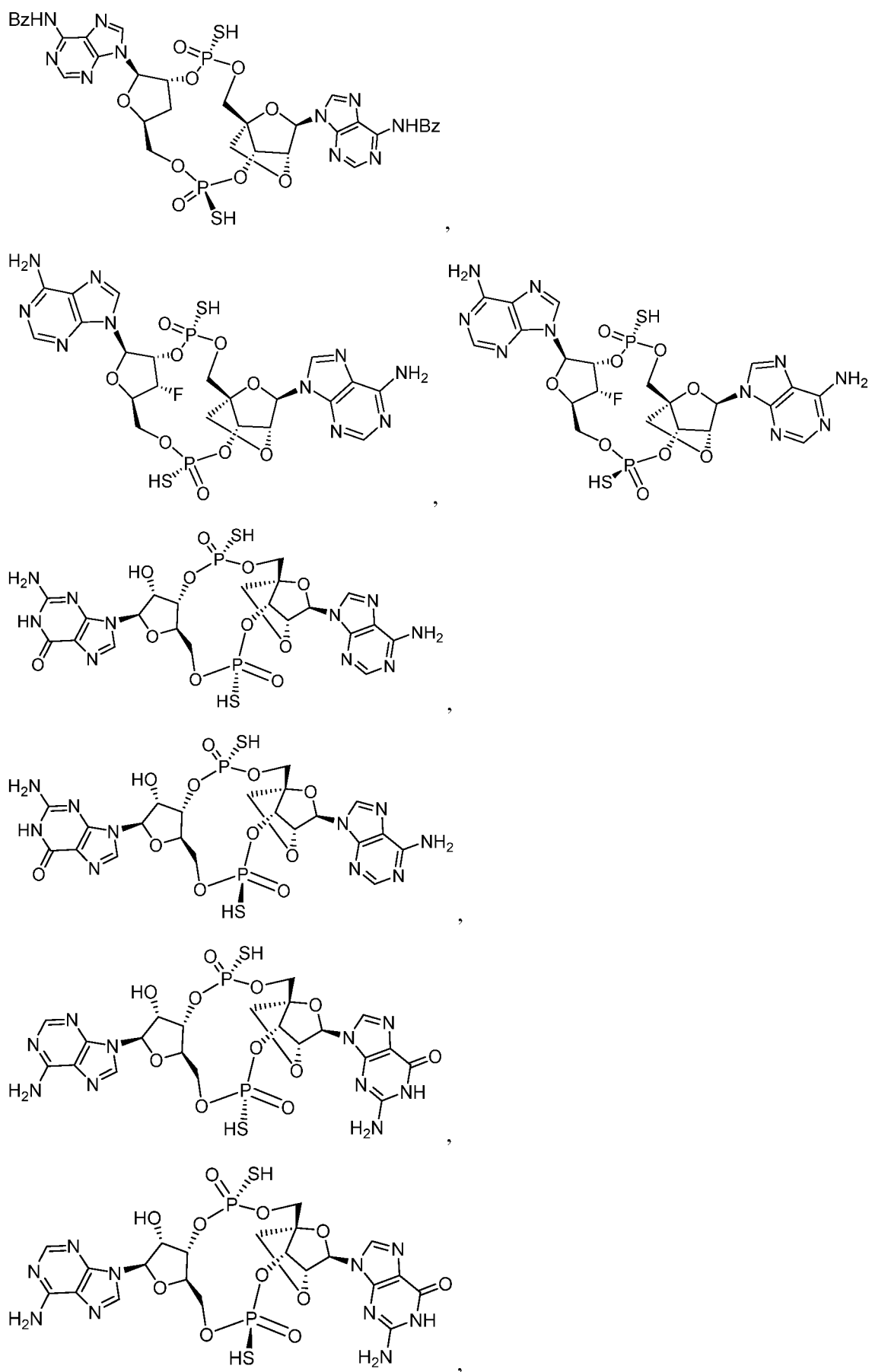
and

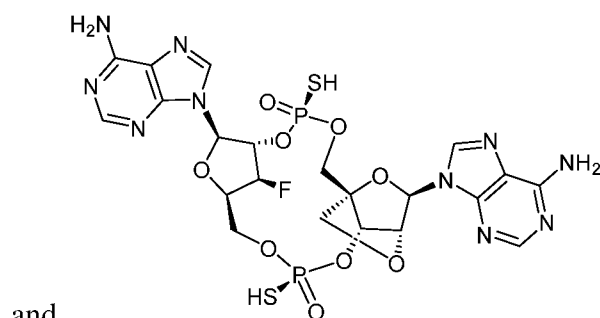
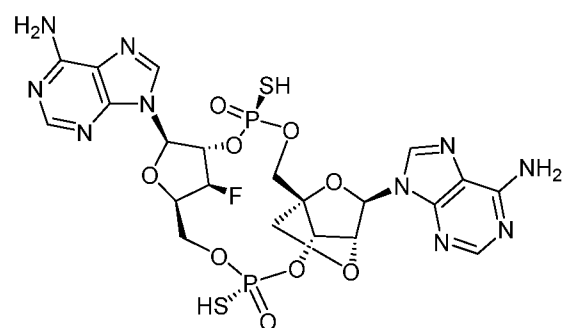
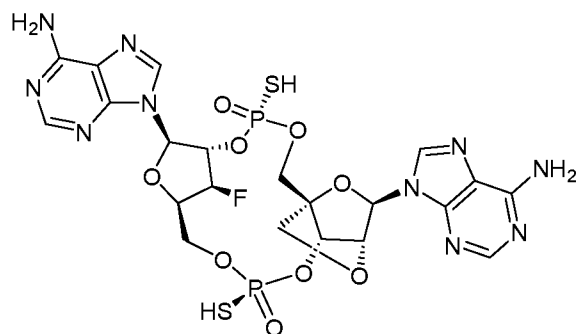
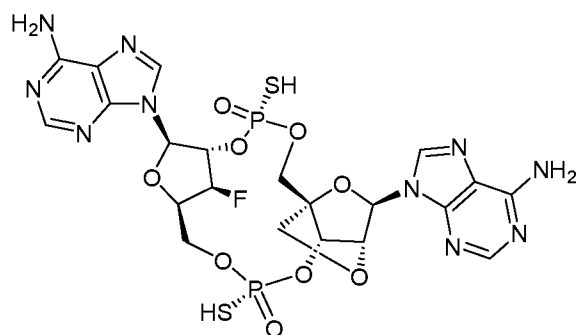
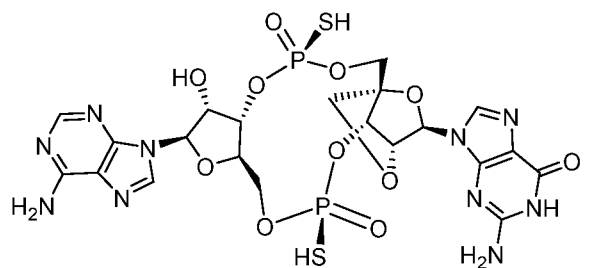
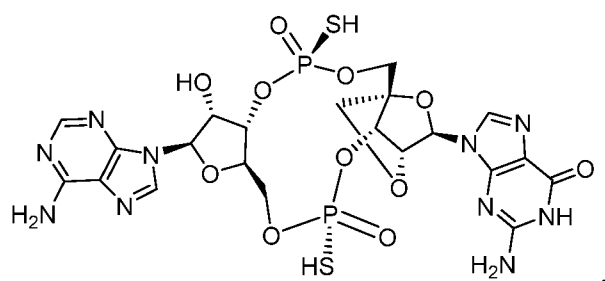
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

36. The compound according to claim 1, selected from the group consisting of:





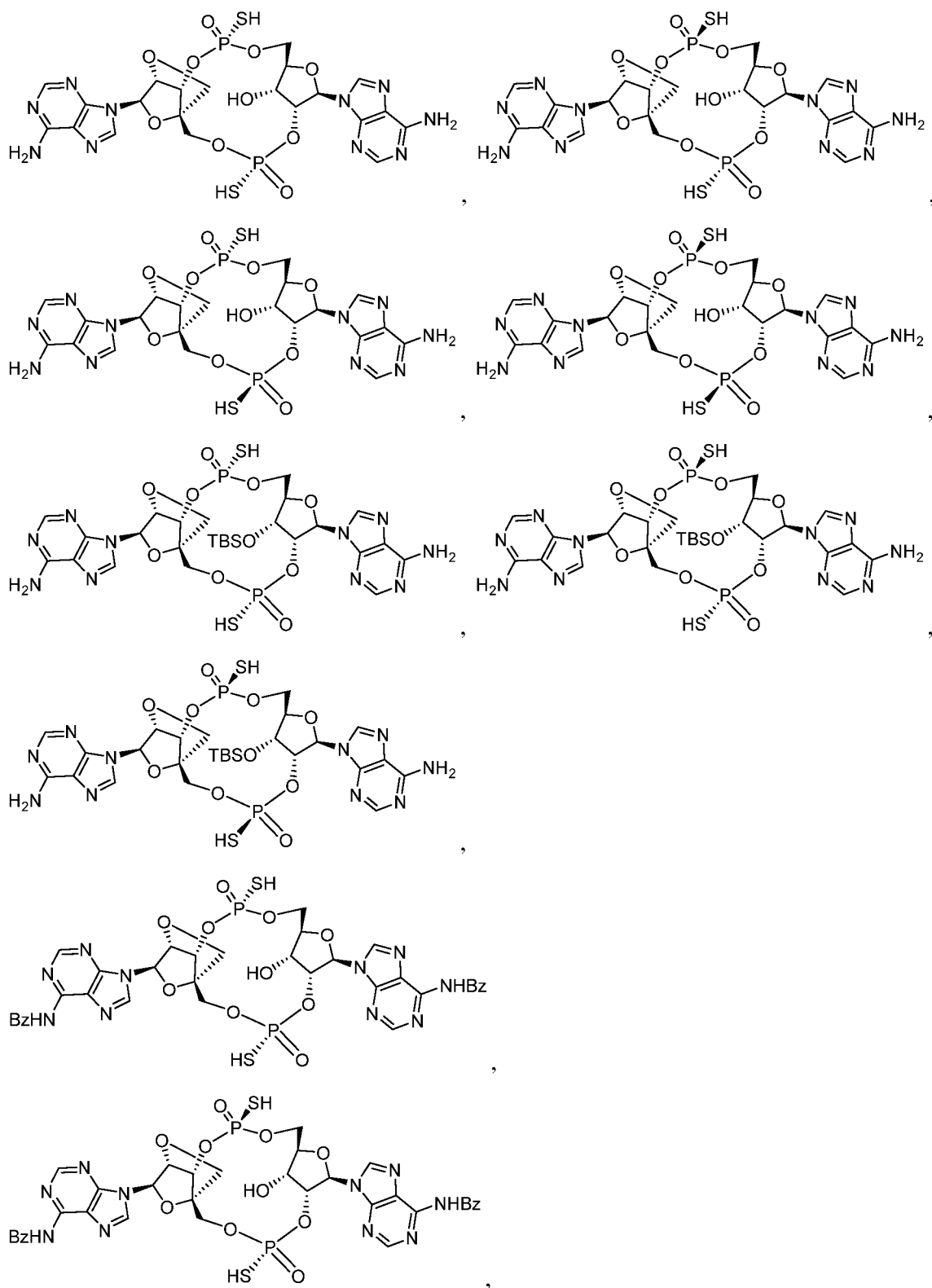


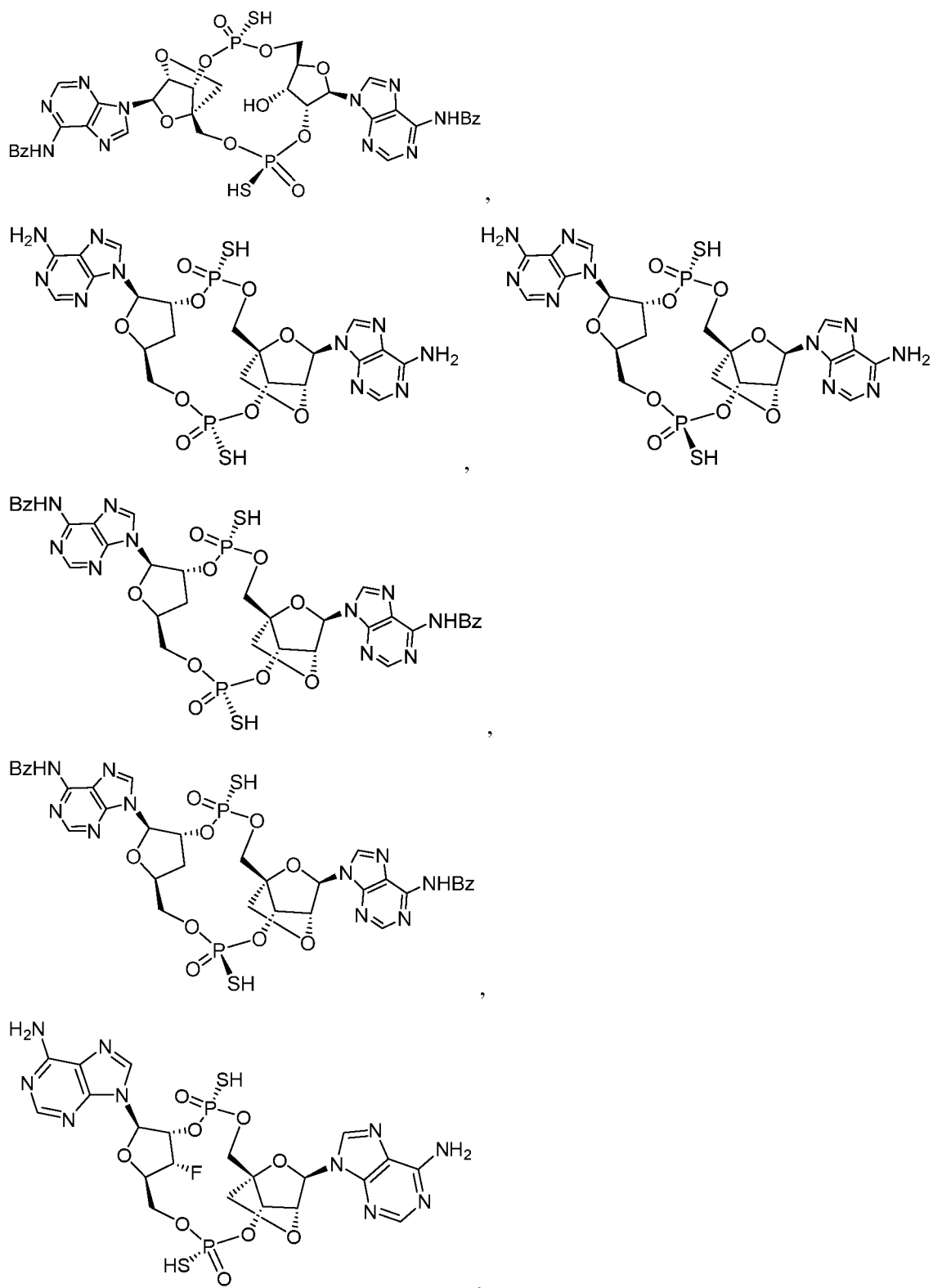


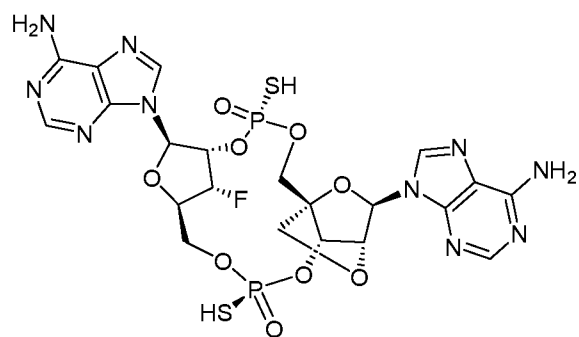
and

or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

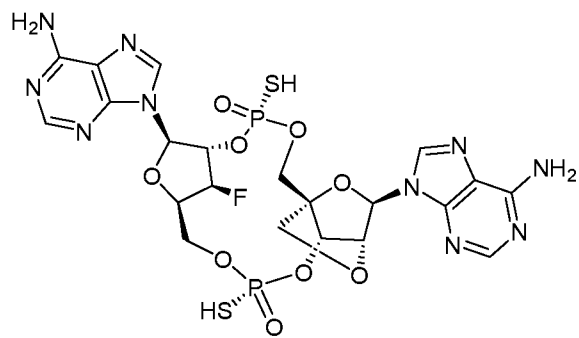
37. The compound according to claim 1, selected from the group consisting of:



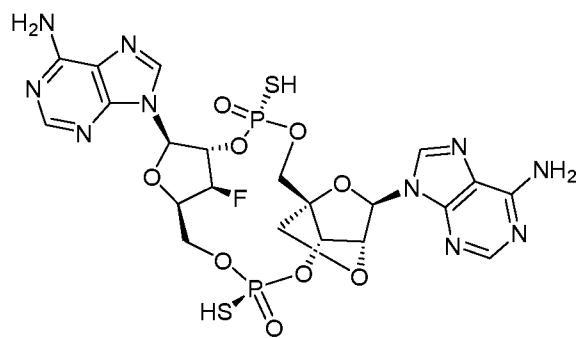




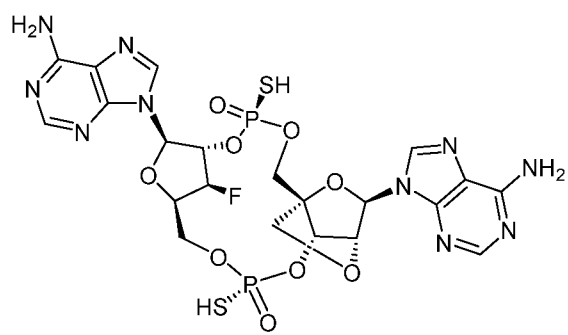
,



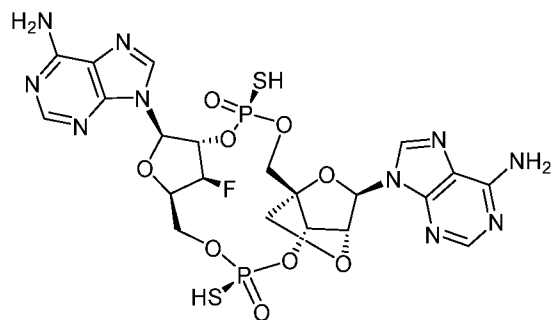
,



,



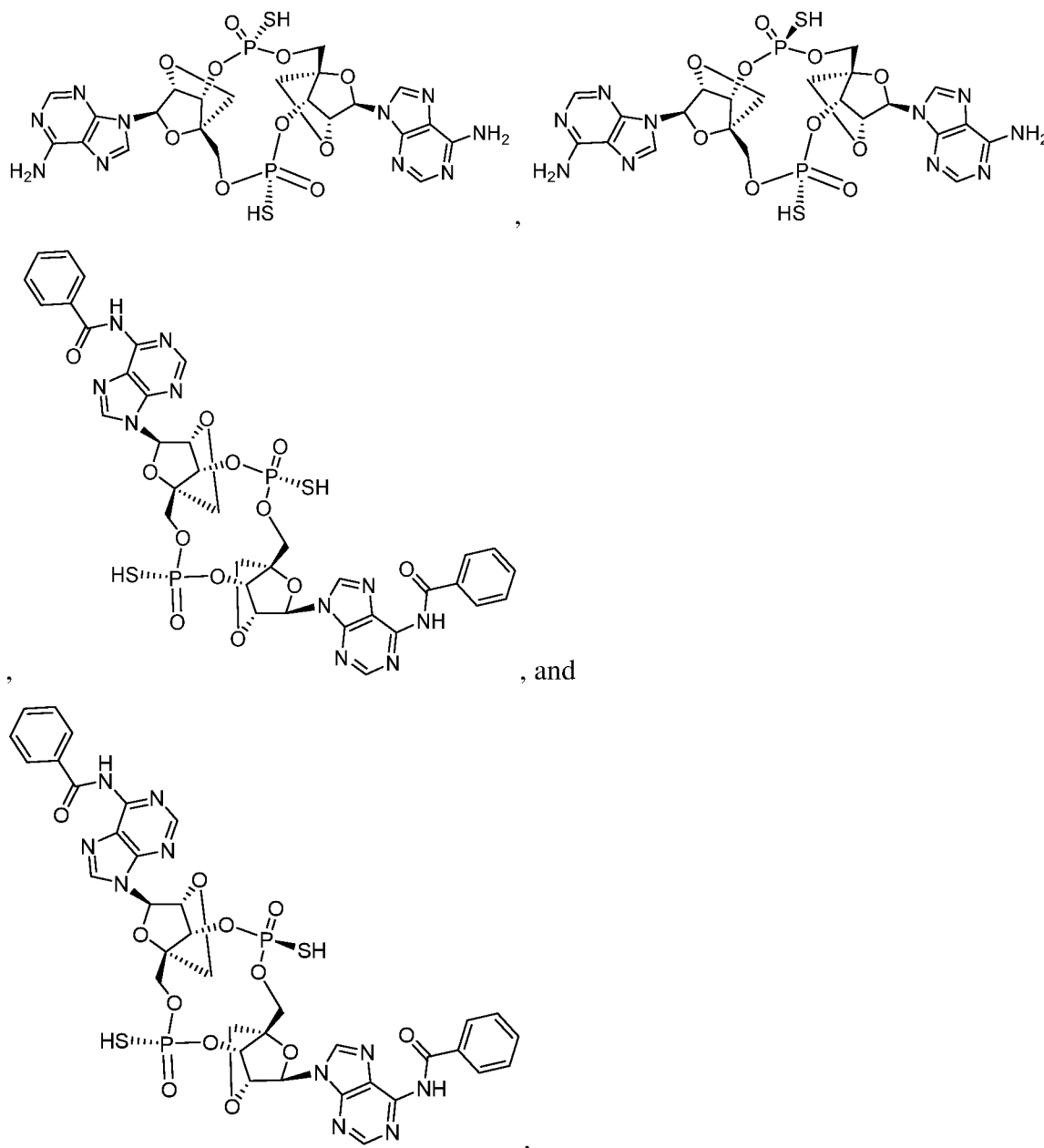
, and



,

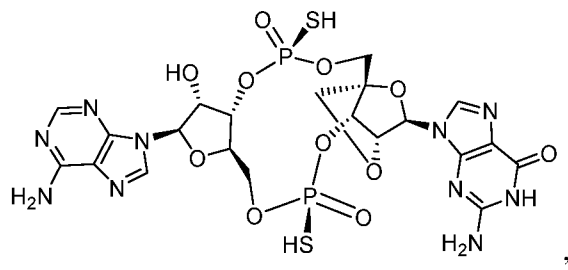
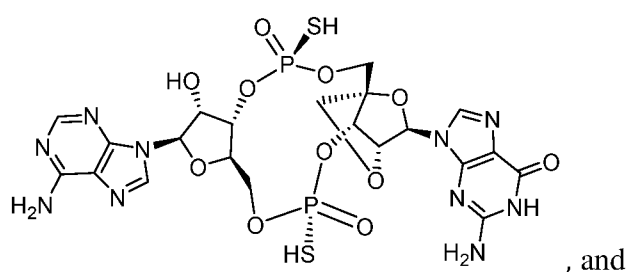
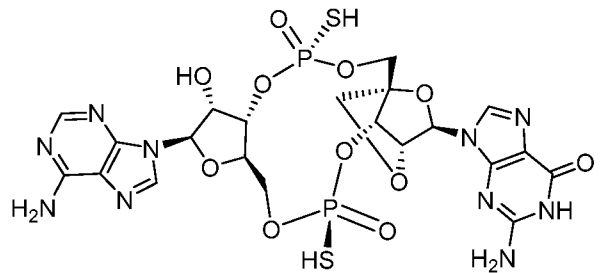
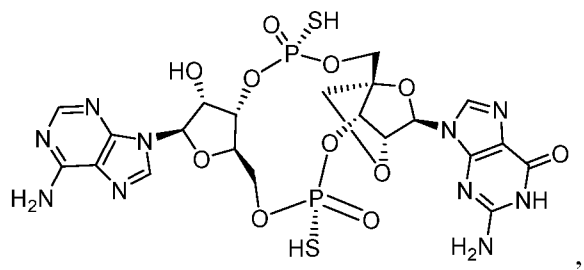
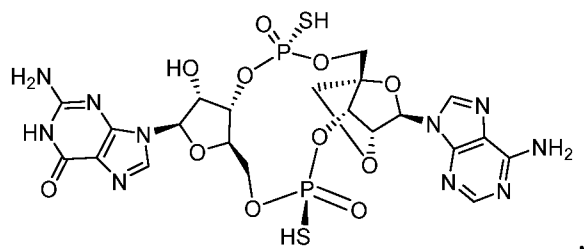
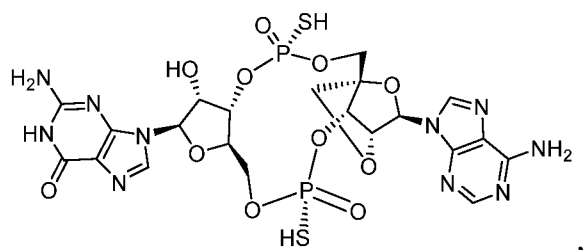
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

38. The compound according to claim 1, selected from the group consisting of:



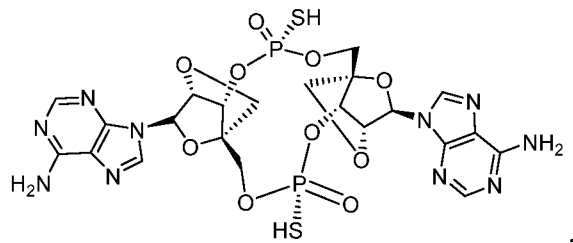
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

39. The compound according to claim 1, selected from the group consisting of:



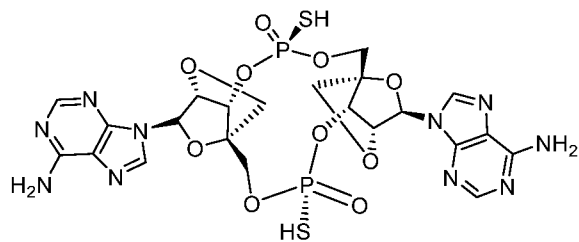
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

40. A compound 3'3'-RR-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) having the structure:



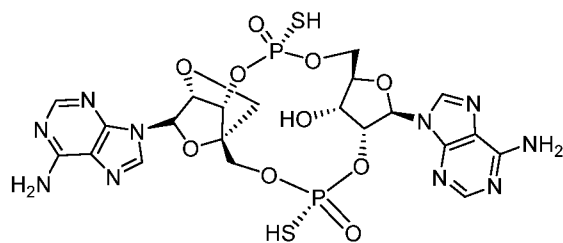
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

41. A compound 3'3'-RS-(2'O,4'C-LNA-A)(2'O,4'C-LNA-A) having the structure:



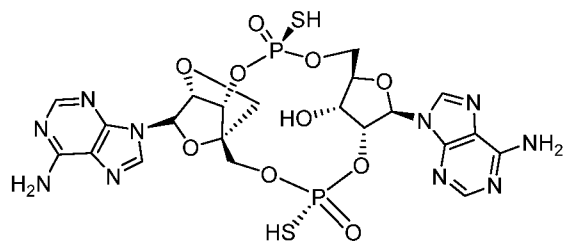
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

42. A compound 2'3'-RR-(A)(2'O,4'C-LNA-A) having the structure:



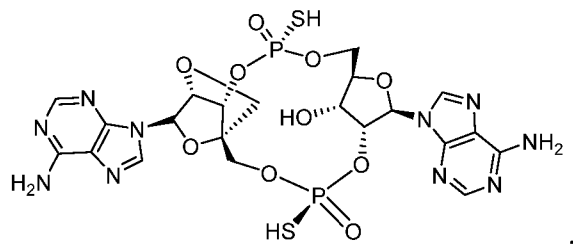
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

43. A compound 2'3'-RS-(A)(2'O,4'C-LNA-A) having the structure:



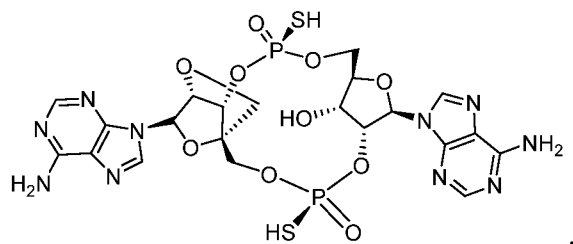
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

44. A compound 2'3'-SR-(A)(2'O,4'C-LNA-A) having the structure:



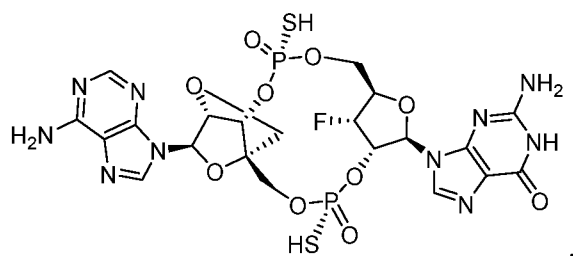
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

45. A compound 2'3'-SS-(A)(2'O,4'C-LNA-A) having the structure:



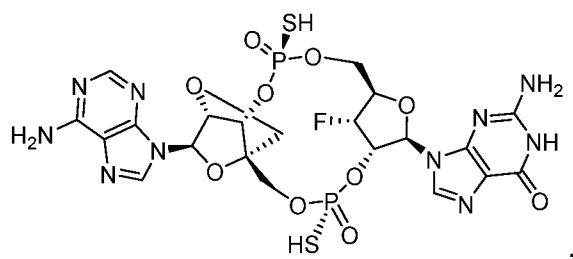
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

46. A compound 2'3'-RR-(3'F-G)(2'O,4'C-LNA-A) having the structure:



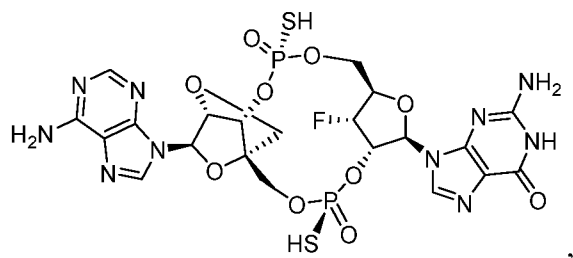
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

47. A compound 2'3'-RS-(3'F-G)(2'O,4'C-LNA-A) having the structure:



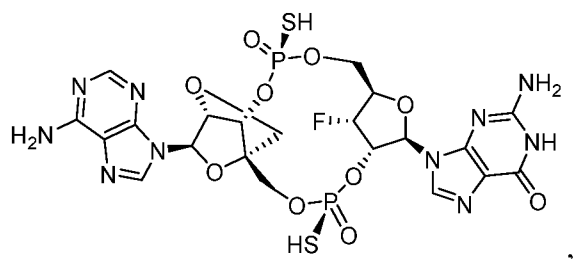
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

48. A compound 2'3'-SR-(3'F-G)(2'O,4'C-LNA-A) having the structure:



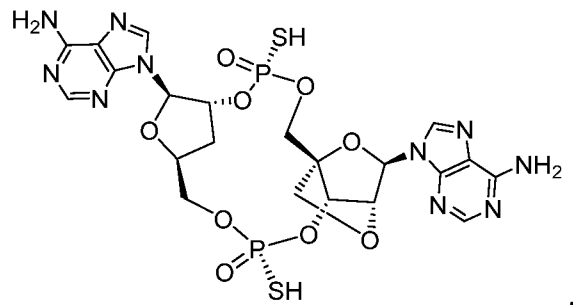
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

49. A compound 2'3'-SS-(3'F-G)(2'O,4'C-LNA-A) having the structure:



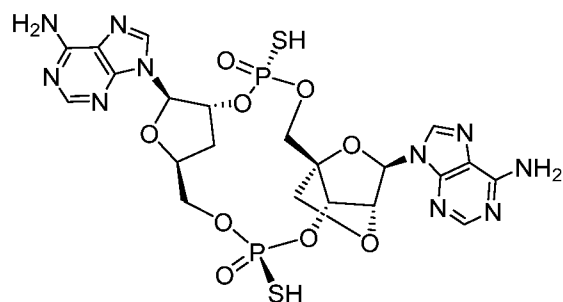
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

50. A compound 2'3'-RR-(3'H-A)(2'O,4'C-LNA-A) having the structure:



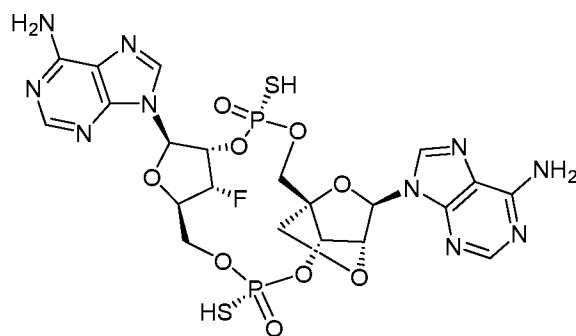
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

51. A compound 2'3'-RS-(3'H-A)(2'O,4'C-LNA-A) having the structure:



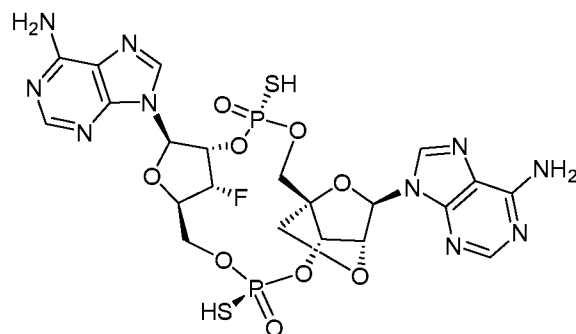
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

52. A compound 2'3'-RR-(3'F-A)(2'O,4'C-LNA-A) having the structure:



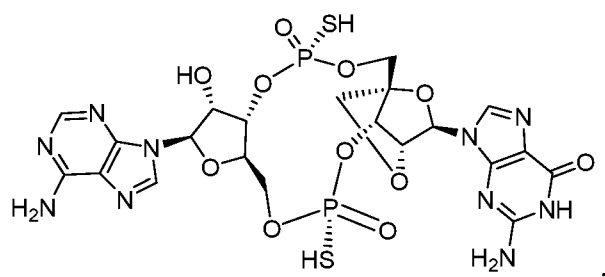
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

53. A compound 2'3'-RS-(3'F-A)(2'O,4'C-LNA-A) having the structure:



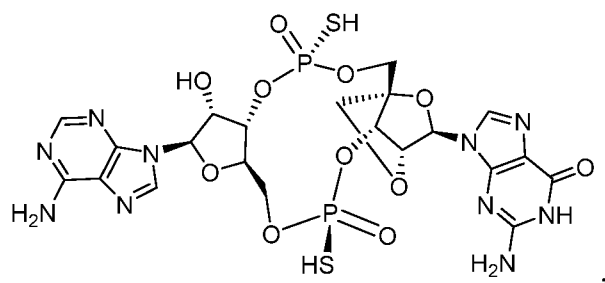
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

54. A compound 3'3'-RR-(A)(2'O,4'C-LNA-G) having the structure:



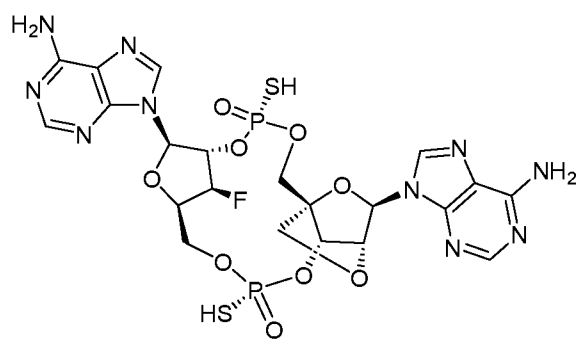
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

55. A compound 3'3'-RS-(A)(2'O,4'C-LNA-G) having the structure:



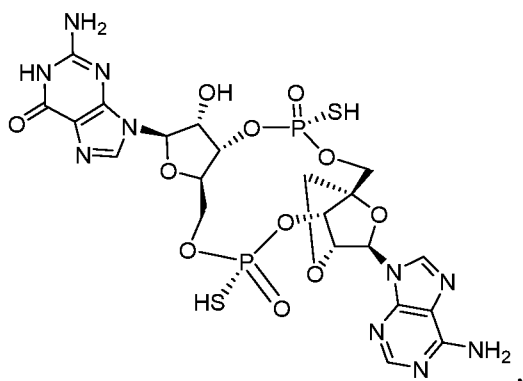
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

56. A compound 2'3'-RR-(3'βF-A)(2'O,4'LNA-A) having the structure:



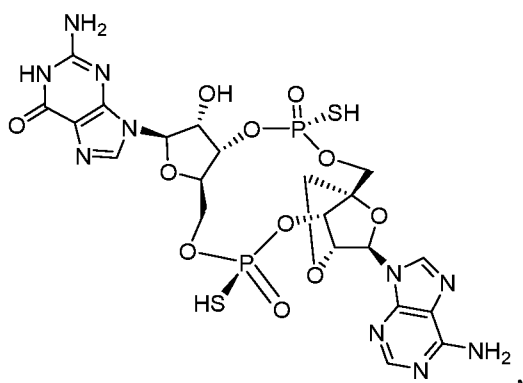
or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

57. A compound 3'3'-RR-(G)(2'O,4'C-LNA-A) having the structure:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

58. A compound 3'3'-RS-(G)(2'O,4'C-LNA-A) having the structure:



or a prodrug, pharmaceutically acceptable salt, pharmaceutically acceptable solvate or pharmaceutically acceptable hydrate thereof.

59. The compound according to any one of claims 1-58, wherein the compound is more active in a cellular assay that measures the induction of human STING dependent IFN- β production as compared to one or more reference compounds, wherein the reference compound is 2'3'-(G)(A) or 2'3'-RR-(A)(A).

60. A composition comprising one or more compounds according to any one of claims 1-59 and a pharmaceutically acceptable excipient.

61. A composition according to claim 60, wherein the composition does not include an agent that enhances cellular permeability of the one or more compounds or an agent that enhances uptake of the one or more compounds into a cell.

62. A composition comprising one or more compounds according to any one of claims 1-59 and a delivery vehicle which enhances cellular uptake and/or stability of the compound.

63. The composition according to claim 62, wherein the delivery vehicle comprises one or more agents selected from the group consisting of lipids, liposomes, interbilayer crosslinked multilamellar vesicles, biodegradable poly(D,L-lactic-co-glycolic acid) [PLGA]-based or poly anhydride-based nanoparticles or microparticles, and nanoporous particle-supported lipid bilayers.

64. A composition comprising one or more compounds according to any one of claims 1-59 and one or more agents selected from the group consisting of an immune checkpoint inhibitor; a Toll-like Receptor (TLR) agonist; a composition that mediates innate immune activation via TLRs, via (NOD)-like receptors (NLRs), via Retinoic acid inducible gene-based (RIG)-I-like receptors (RLRs), via C-type lectin receptors (CLRs), or via pathogen-associated molecular patterns ("PAMPs"); and a chemotherapeutic agent.

65. The composition according to claim 64, wherein the one or more agents is an immune checkpoint inhibitor.

66. The composition according to claim 65, wherein the immune checkpoint inhibitor is selected from the group consisting of a CTLA-4 pathway antagonist, a PD-1 pathway antagonist, a Tim-3 pathway antagonist, a Vista pathway antagonist, a BTLA pathway antagonist, a LAG-3 pathway antagonist, and a TIGIT pathway antagonist.

67. The composition according to claim 64, wherein the one or more agents is a histone deacetylase inhibitor.

68. The composition according to claim 67, wherein the histone deacetylase inhibitor is selected from the group consisting of panobinostat, vorinostat, romidepsin, chidamide, valproic acid, belinostat, pyroxamide, mocetinostat, abexinostat, entinostat, pracinostat, resminostat, givinostat, quisinostat, ricolinostat, CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, and CG200745.

69. A composition comprising one or more compounds according to any one of claims 1-59 and an inactivated tumor cell which expresses and secretes one or more cytokines which stimulate dendritic cell induction, recruitment and/or maturation, or one or more heat shock proteins.

70. The composition according to claim 69, wherein the inactivated tumor cell expresses and secretes one or more cytokines selected from the group consisting of GM-CSF, CCL20, CCL3, IL-12p70 and FLT-3 ligand.

71. The composition according to claim 69, wherein the inactivated tumor cell expresses and secretes a gp96-Ig fusion protein.

72. The composition according to any one of claims 69-71, wherein the tumor cell is inactivated by treatment with radiation.

73. The composition according to any one of claims 60-72, further comprising one or more antigens selected for purposes of inducing an immune response against the antigen(s) when the composition is administered to an individual.

74. The composition according to claim 73, wherein the antigen is one or more recombinant protein antigens related to an infectious disease, a malignancy, or an allergan.

75. A method for treating an individual suffering from cancer, comprising: administering to the individual in need thereof an effective amount of the compound according to any one of claims 1-59, or the composition according to any one of claims 60-74.

76. The method according to claim 75, wherein the cancer is selected from the group consisting of a colorectal cancer, an aero-digestive squamous cancer, a lung cancer, a brain cancer, a liver cancer, a stomach cancer, a bladder cancer, a thyroid cancer, an adrenal cancer, a gastrointestinal cancer, an oropharyngeal cancer, an esophageal cancer, a head and neck cancer, an ovarian cancer, a uterine cancer, a cervical cancer, an endometrial cancer, a breast cancer, a melanoma, a prostate cancer, a pancreatic

carcinoma, a renal carcinoma, a sarcoma, a leukemia, a Merkel-cell carcinoma, a lymphoma and a multiple myeloma.

77. The method according to claim 75 or 76, wherein the administration is intra-tumoral, peri-tumoral, or directly into the tumor-draining lymph node(s).

78. The method according to any one of claims 75-77, wherein the method further comprises administering one or more additional cancer therapies to the individual.

79. The method according to claim 78, wherein the one or more additional cancer therapies is selected from the group consisting of radiation therapy, surgery, a chemotherapy, or an immunotherapy.

80. The method according to claim 78, wherein the one or more additional cancer therapies comprises administering one or more therapeutic antibodies to the individual.

81. The method according to claim 78, wherein the one or more additional cancer therapies comprises administering one or more checkpoint inhibitors to the individual.

82. The method according to claim 81, wherein the immune checkpoint inhibitor(s) comprise an agent selected from the group consisting of an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-CTLA-4 antibody, an anti-TIM-3 antibody, an anti-B7-H3 antibody, an anti-CD70 antibody, an anti-CD40 antibody, an anti-CD137 antibody, an anti-GITR antibody, an anti-OX40 antibody, an anti-KIR antibody, and an anti-LAG-3 antibody.

83. The method according to claim 78, wherein the one or more additional cancer therapies comprises administering an inactivated tumor cell that expresses and secretes one or more cytokines or one or more heat shock proteins to the individual.

84. The method according to claim 83, wherein the inactivated tumor cell expresses and secretes one or more cytokines selected from the group consisting of GM-CSF, CCL20, CCL3, IL-12p70, and FLT-3 ligand.

85. The method according to claim 83, wherein the inactivated tumor cell expresses and secretes a gp96-Ig fusion protein.

86. The method according to claim 78, wherein the one or more additional cancer therapies comprises administering a histone deacetylase inhibitor to the individual, wherein the histone deacetylase inhibitor is selected from the group consisting of panobinostat, vorinostat, romidepsin, chidamide, valproic acid, belinostat, pyroxamide, mocetinostat, abexinostat, entinostat, pracinostat, resminostat, givinostat, quisinostat, ricolinostat, CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, and CG200745.

88. The method according to one of claims 78-87, wherein the administration of the compound according to any one of claims 1-59, or the composition according to any one of claims 60-74 is intra-tumoral, peri-tumoral or into the tumor-draining lymph node(s).

89. The method according to one of claims 78-88, wherein the compound according to any one of claims 1-59 or the composition according to any one of claims 60-74 is administered by a different route and/or at different times, relative to administration of the one or more additional cancer therapies.

90. A method of treating a disease in an individual, comprising: administering to the individual in need thereof i) an effective amount of the compound according to any one of claims 1-59, or the composition according to any one of claims 60-74; and ii) an effective amount of one or more therapeutic antibodies that induce antibody-dependent cellular cytotoxicity and/or antibody-dependent cellular phagocytosis, wherein the disease is selected from the group consisting of a cancer, acute rejection of an organ transplant, Type I diabetes mellitus, rheumatoid arthritis, psoriasis, Crohn's disease, restenosis and allergic asthma.

91. The method according to claim 90, wherein the one or more therapeutic antibodies that induce antibody-dependent cellular cytotoxicity and/or antibody-dependent cellular phagocytosis is selected from the group consisting of muromonab-CD3, infliximab, daclizumab, omalizumab, abciximab, rituximab, ibritumomab, tositumomab, cetuximab,

trastuzumab, brentuximab vedotin, alemtuzumab, oncolym, ibilimumab, vitaxin, and bevacizumab.

92. The method according to one of claims 90 or 91, wherein the disease is a cancer selected from the group consisting of lymphoma, breasts cancer, chronic lymphocytic leukemia, colorectal cancer, melanoma, non-small cell lung carcinoma, small cell lung cancer, bladder cancer, prostate cancer and other solid tumors and wherein the one or more therapeutic antibodies that induce antibody-dependent cellular cytotoxicity is selected from the group consisting of rituximab, ibritumomab, tositumomab, cetuximab, trastuzumab, brentuximab vedotin, alemtuzumab, oncolym, ibilimumab, vitaxin, and bevacizumab.

93. The method according to one of claims 90-92, wherein the administration of the composition is intra-tumoral, peri-tumoral or into the tumor-draining lymph node(s).

94. The method according to one of claims 90-93, wherein the composition is administered by a different route and/or at different times, relative to administration of the therapeutic antibodies that induce antibody-dependent cellular cytotoxicity and/or antibody-dependent cellular phagocytosis.

95. A method of treating an infection or infectious disease in a human subject, comprising administering to the subject an effective amount of the compound according to any one of claims 1-59, or the composition according to any one of claims 60-74, optionally in association with a further therapeutic agent or therapeutic procedure.

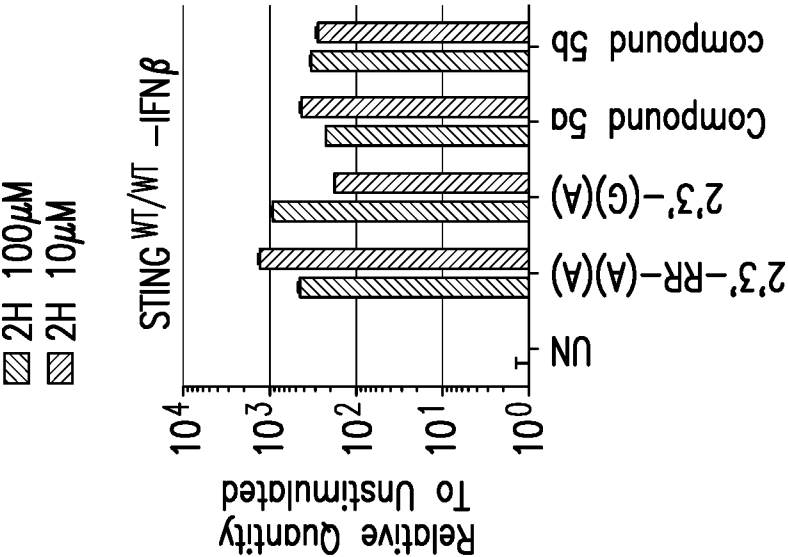


FIG.1A

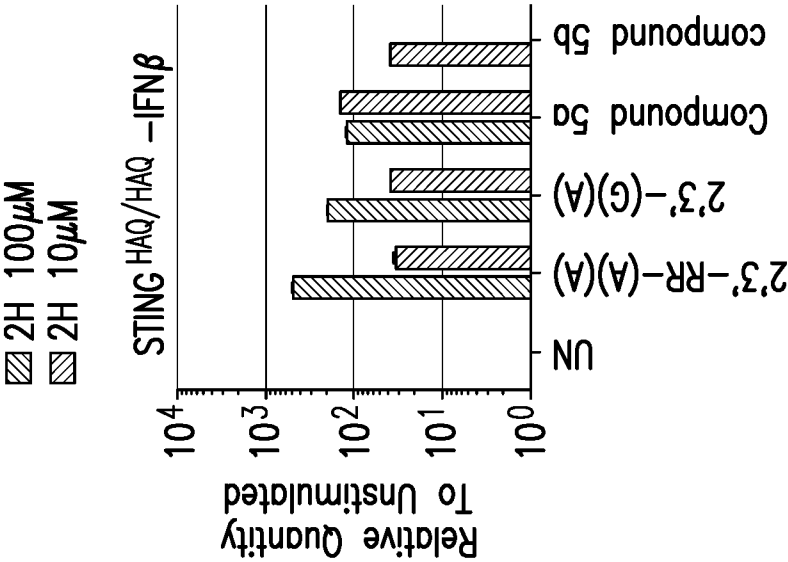


FIG.1B

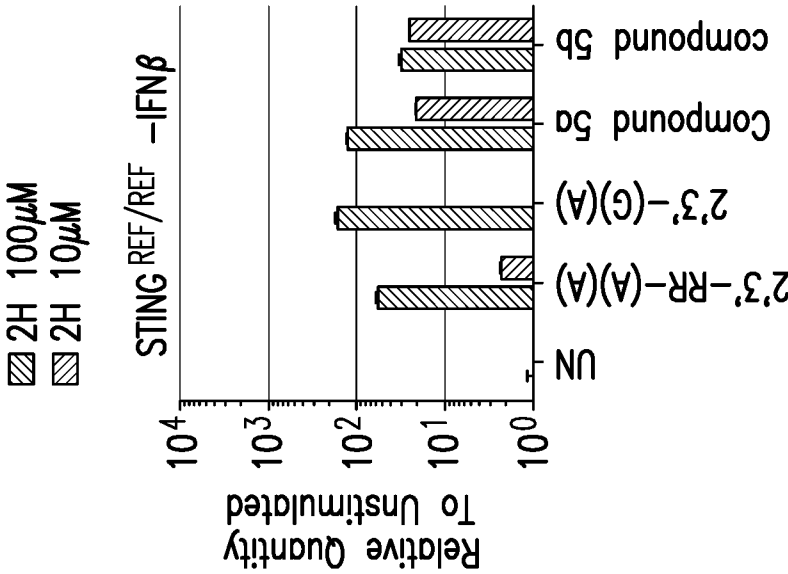


FIG.1C

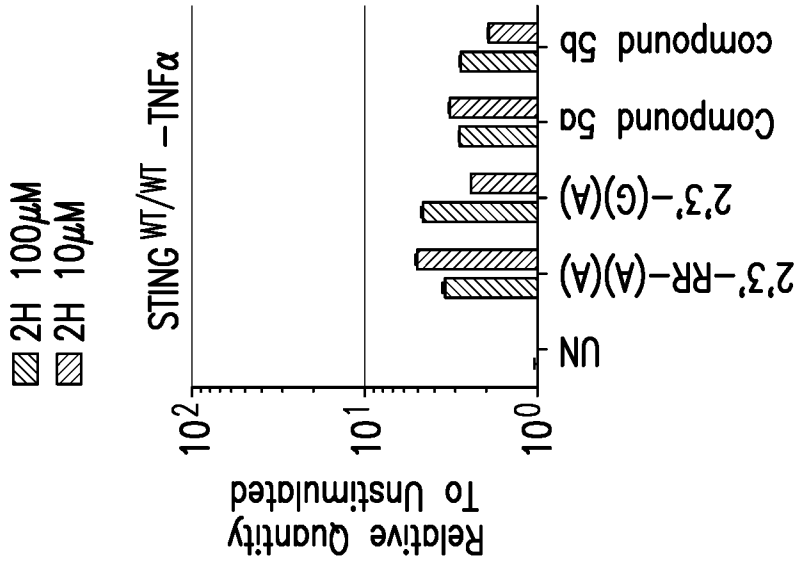


FIG.2A

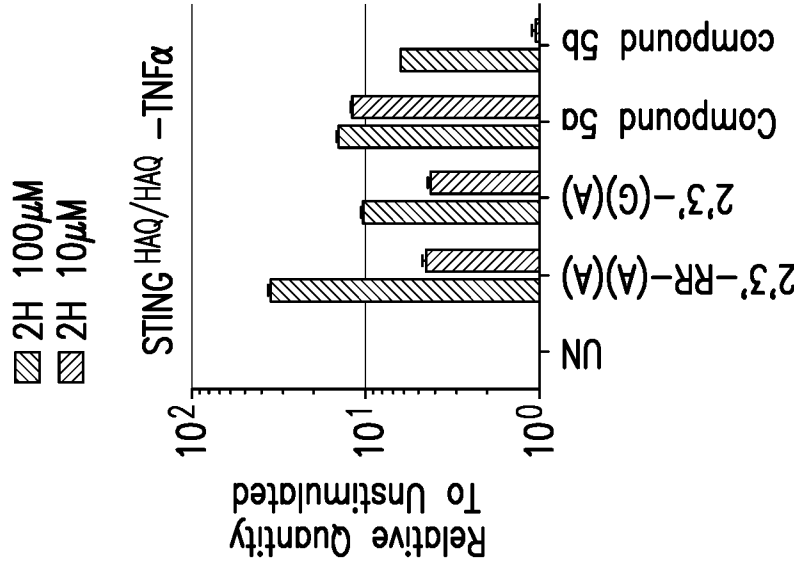


FIG.2B

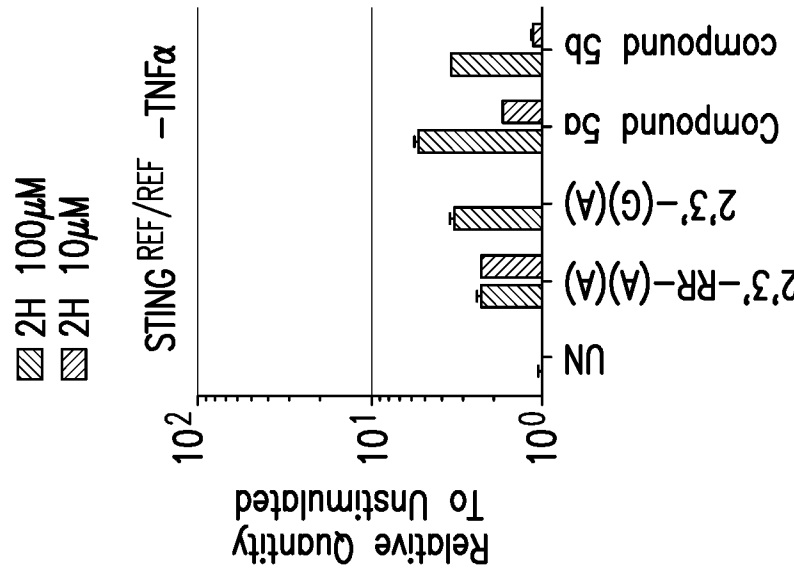


FIG.2C

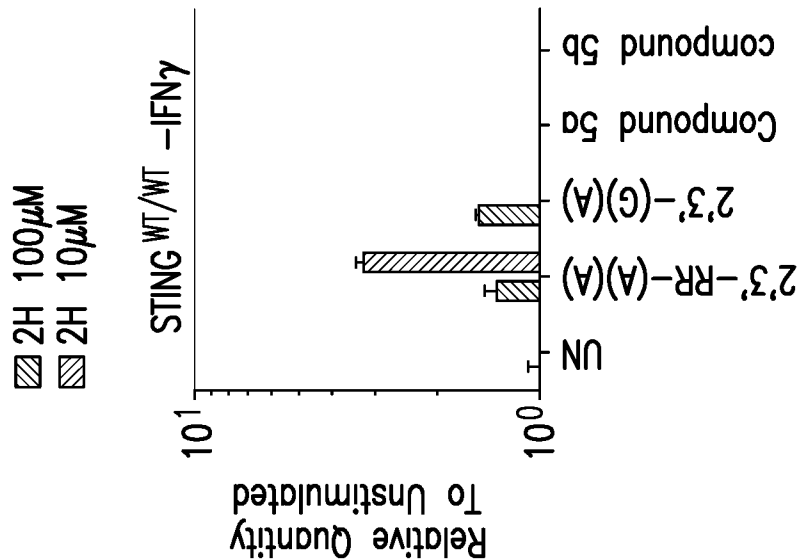


FIG.3A

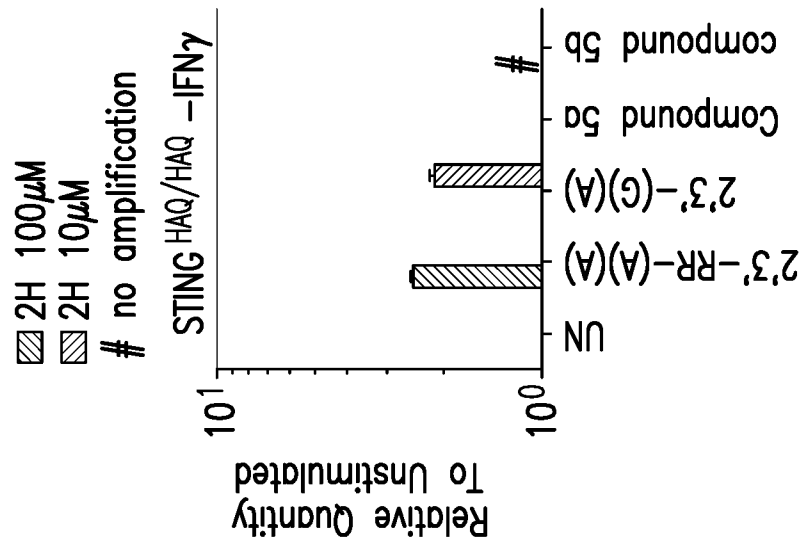


FIG.3B

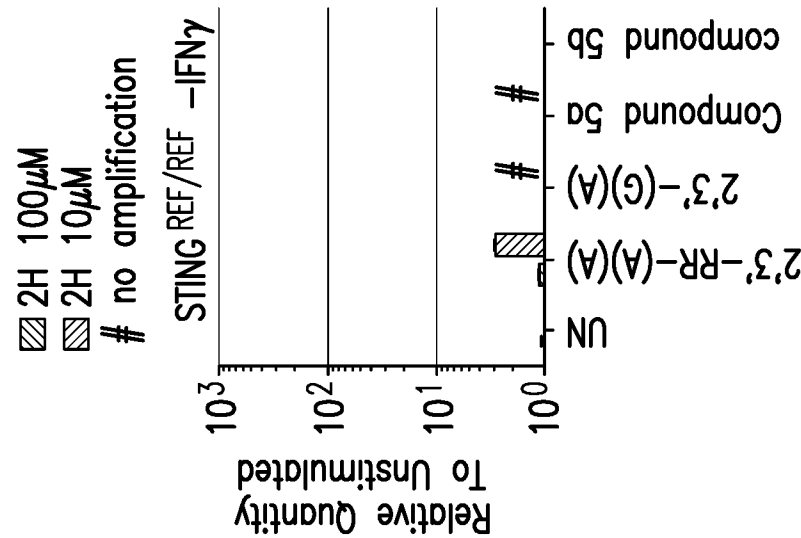


FIG.3C

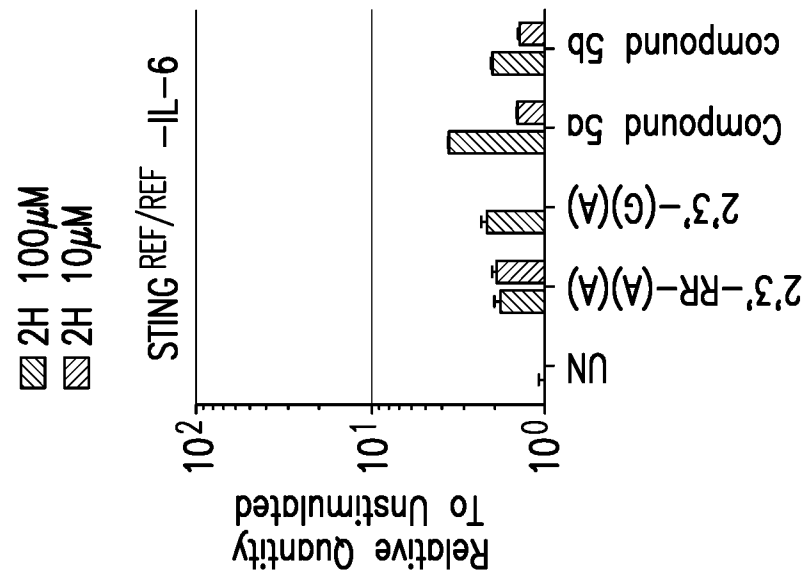


FIG. 4C

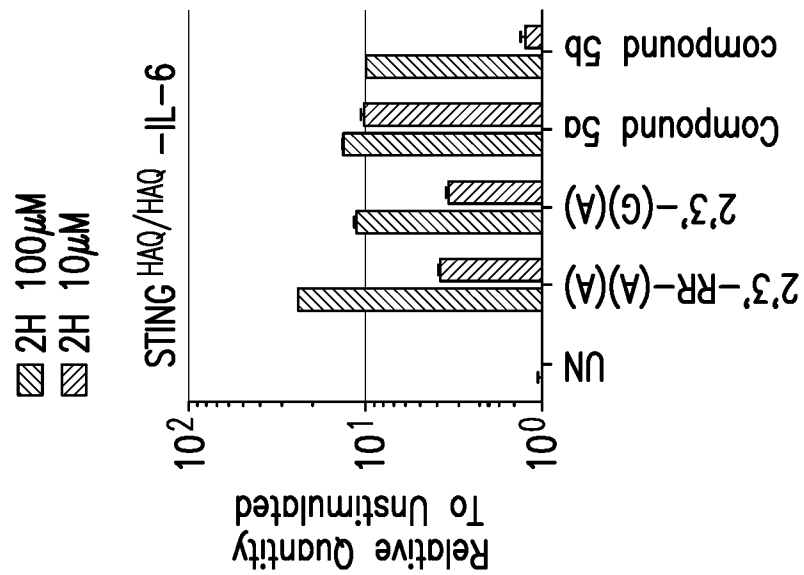


FIG. 4B

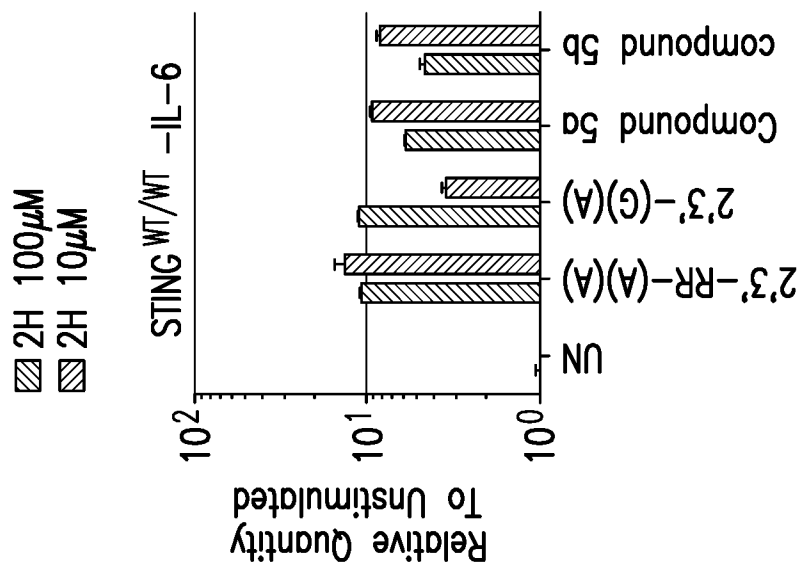
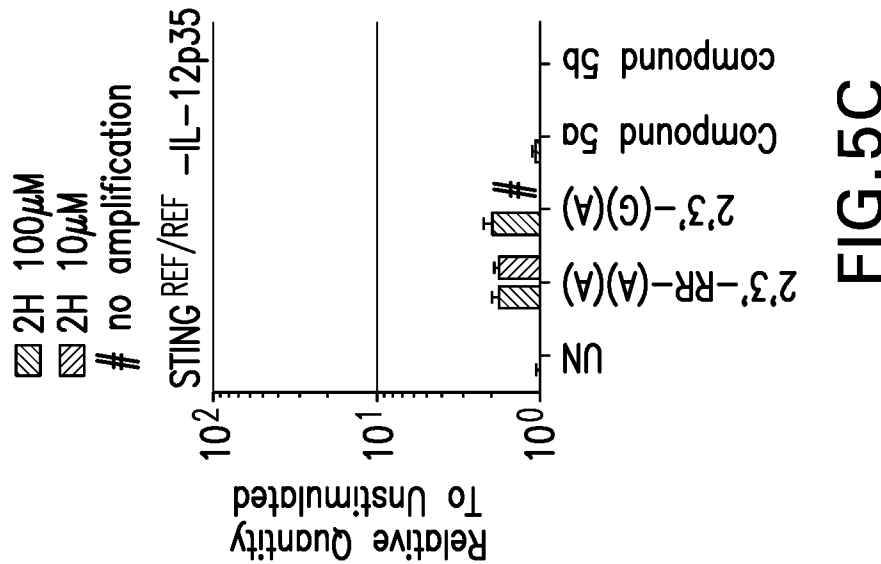
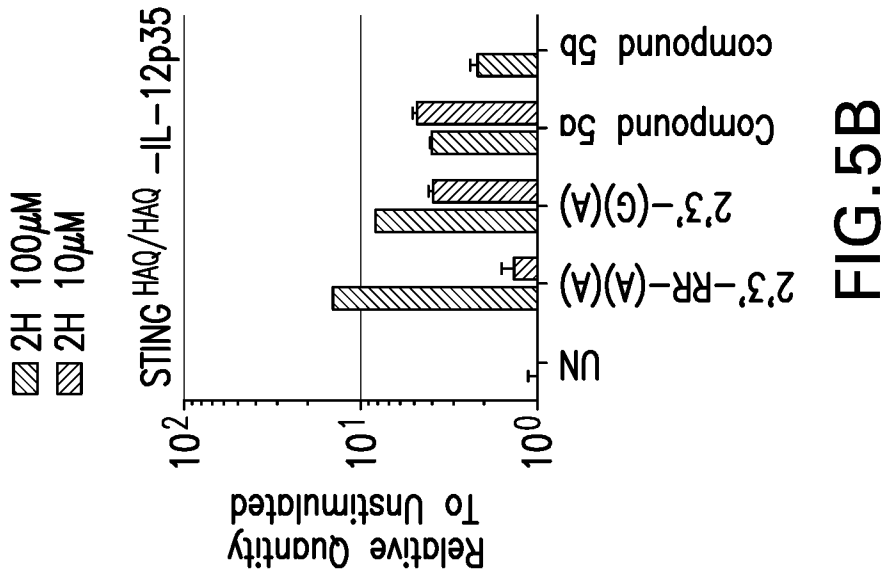
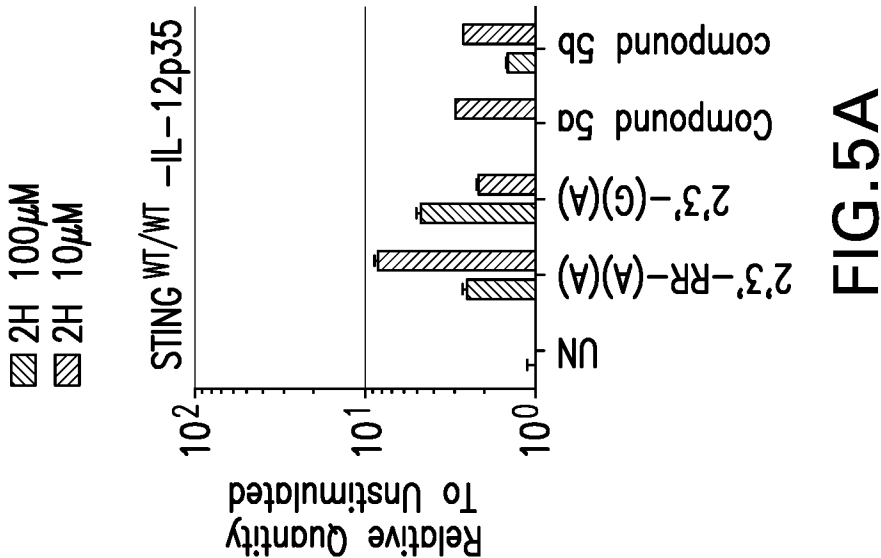


FIG. 4A



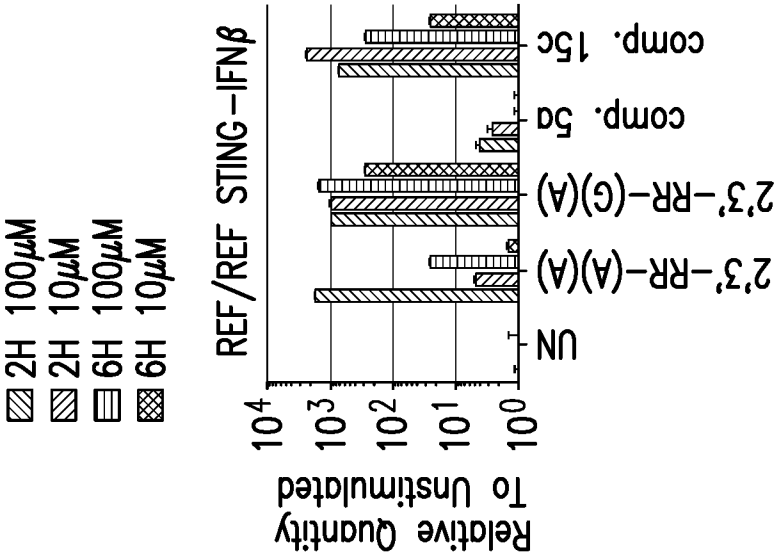


FIG. 6A

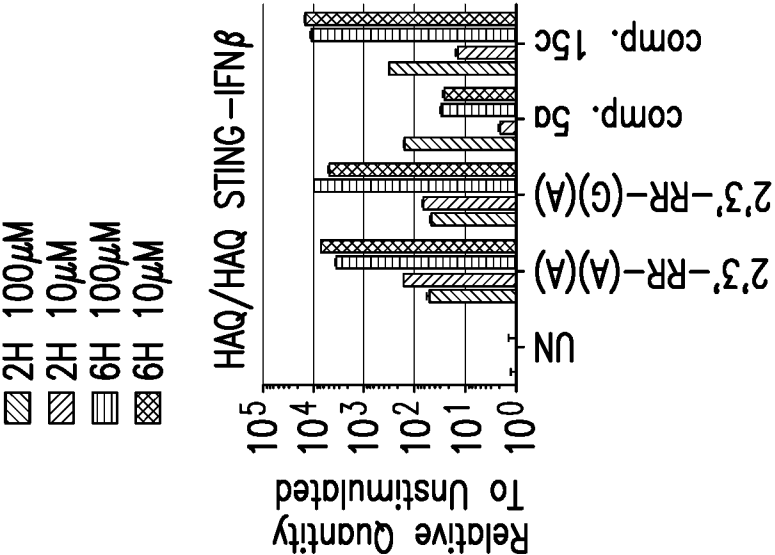


FIG. 6B

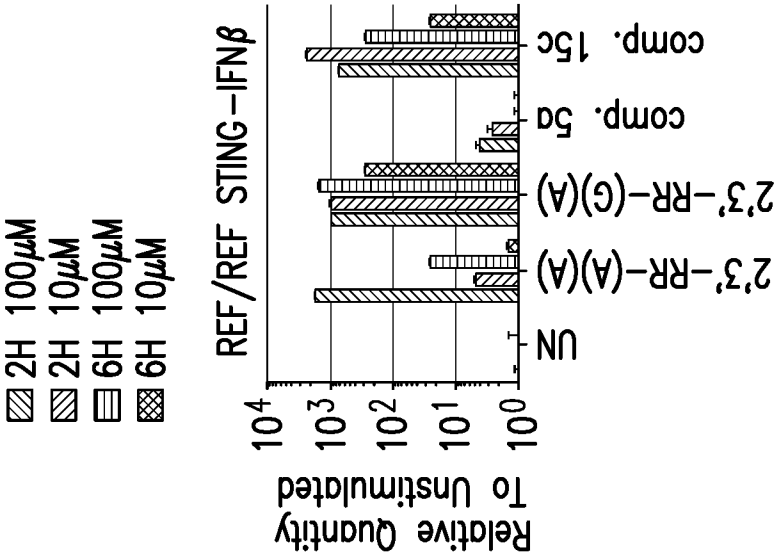


FIG. 6C

2H 100μM
2H 10μM
6H 100μM
6H 10μM

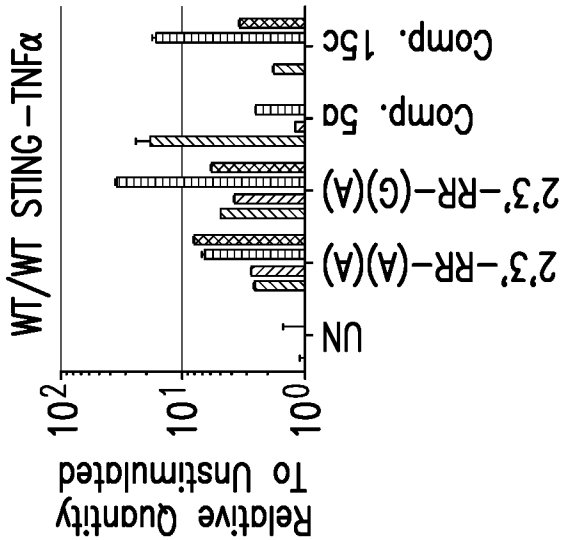


FIG. 7A

2H 100μM
2H 10μM
6H 100μM
6H 10μM

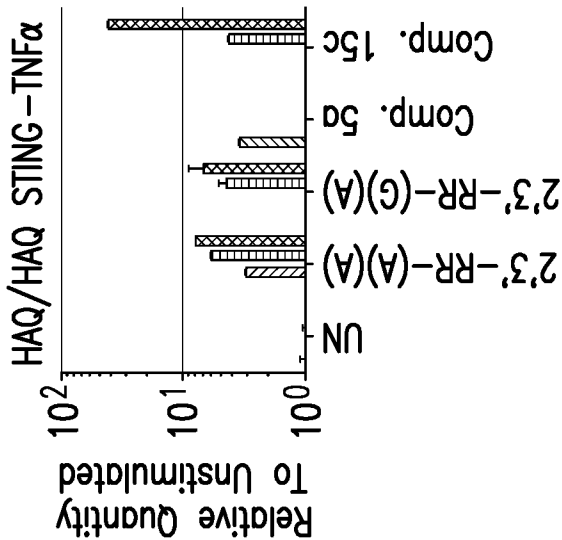


FIG. 7B

2H 100μM
2H 10μM
6H 100μM
6H 10μM

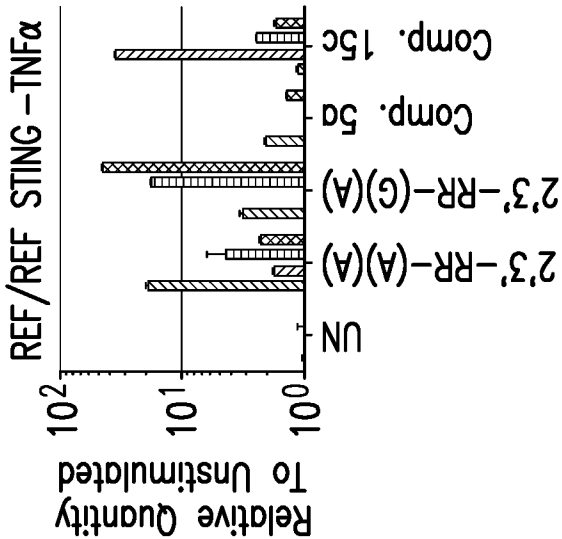


FIG. 7C

2H 100μM
2H 10μM
6H 100μM
6H 10μM

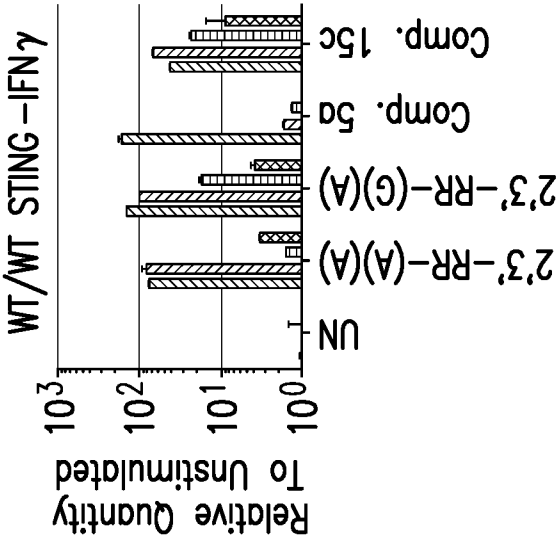


FIG. 8A

2H 100μM
2H 10μM
6H 100μM
6H 10μM

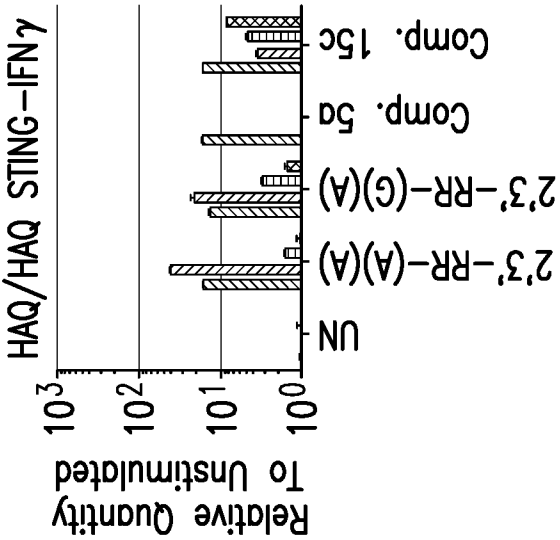


FIG. 8B

2H 100μM
2H 10μM
6H 100μM
6H 10μM

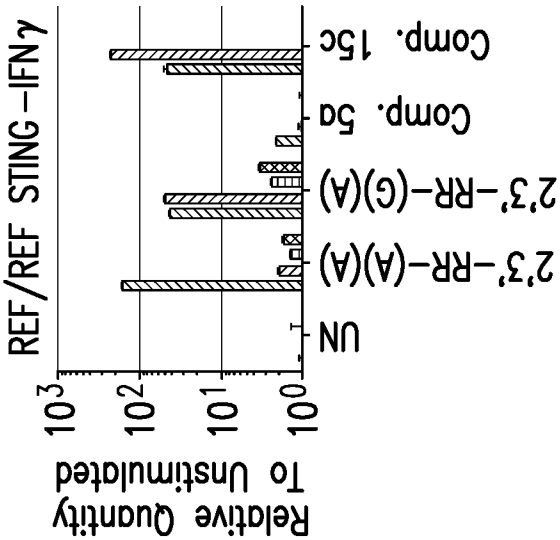


FIG. 8C

2H 100μM
2H 10μM
6H 100μM
6H 10μM

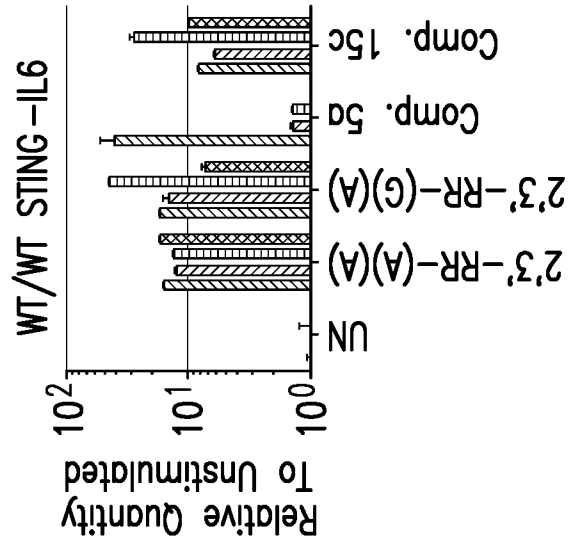


FIG. 9A

2H 100μM
2H 10μM
6H 100μM
6H 10μM

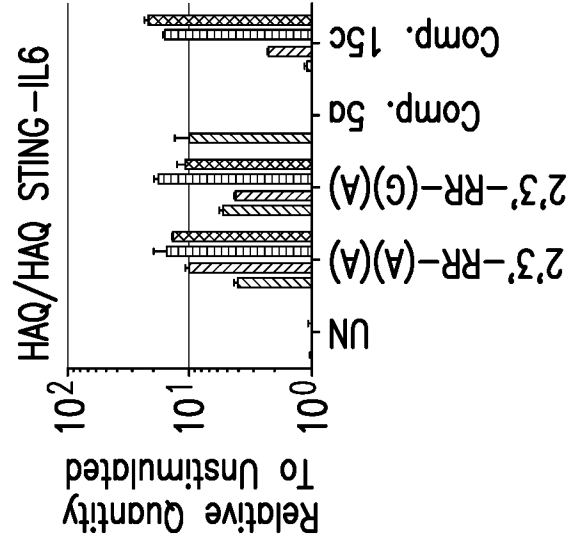


FIG. 9B

2H 100μM
2H 10μM
6H 100μM
6H 10μM

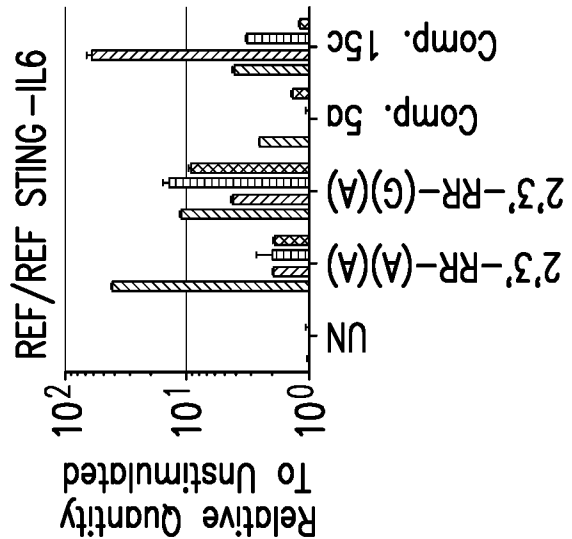


FIG. 9C

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2H 100μM
2H 10μM
6H 100μM
6H 10μM

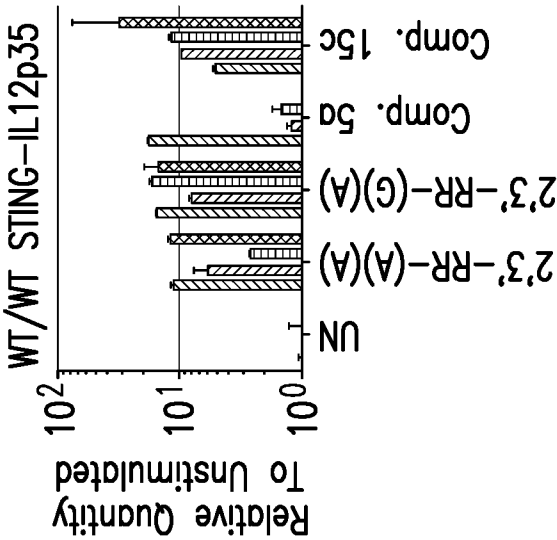


FIG. 10A

2H 100μM
2H 10μM
6H 100μM
6H 10μM

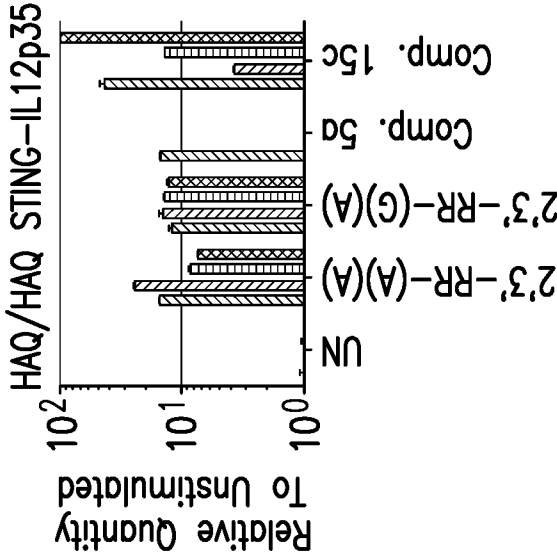


FIG. 10B

2H 100μM
2H 10μM
6H 100μM
6H 10μM

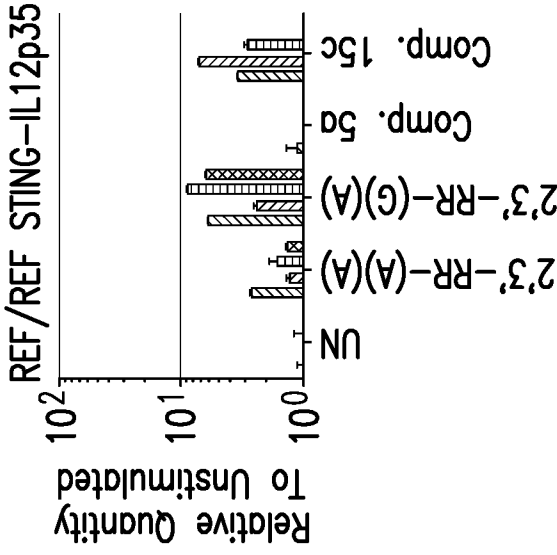


FIG. 10C

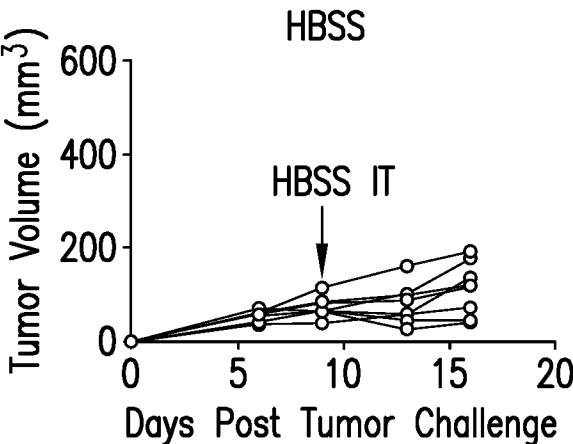


FIG.11A

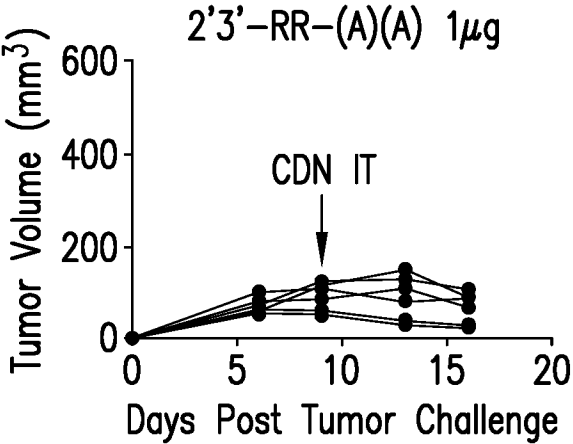


FIG.11B

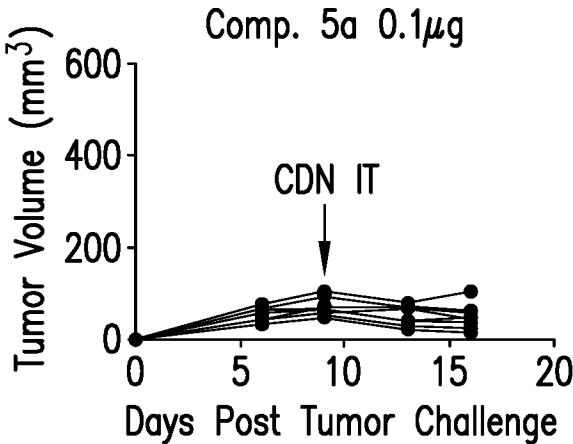


FIG.11C

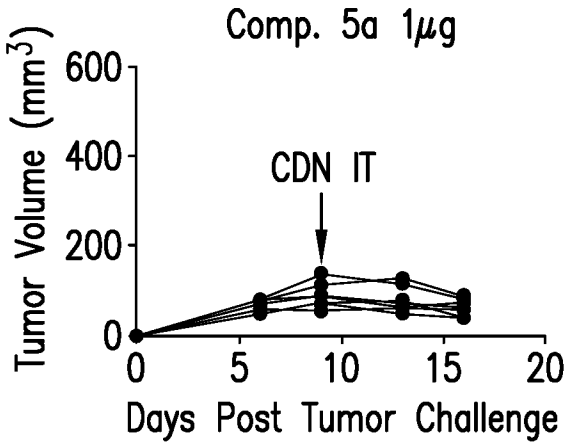


FIG.11D

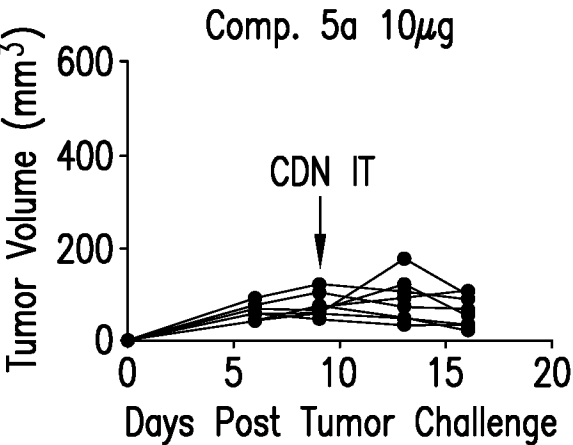


FIG.11E

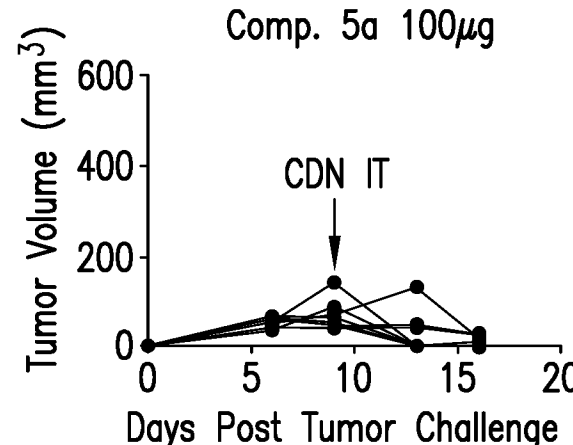


FIG.11F

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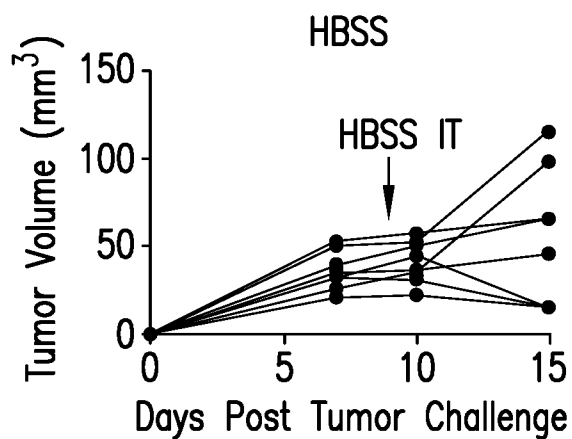


FIG.12A

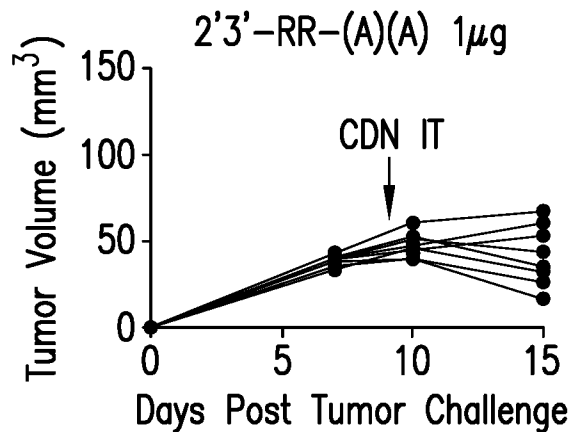


FIG.12B

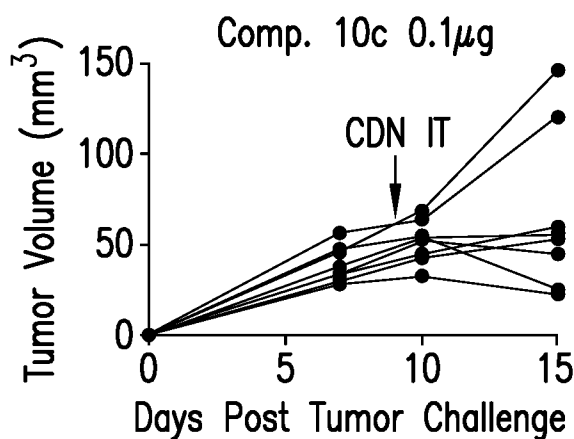


FIG.12C

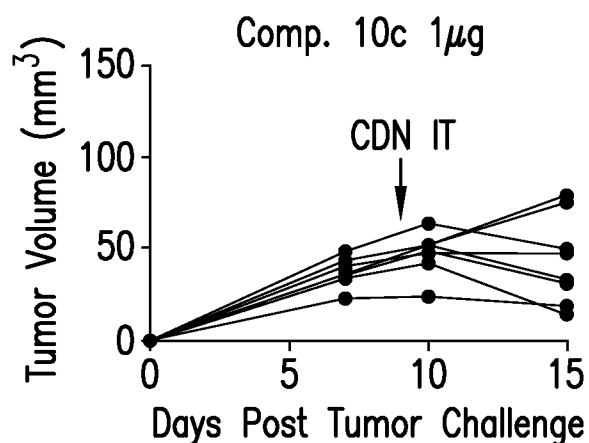


FIG.12D

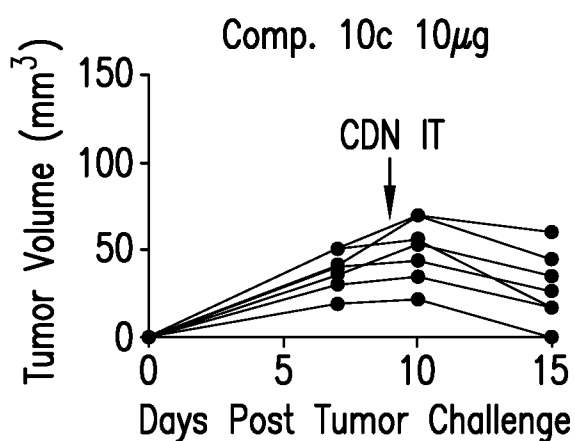


FIG.12E

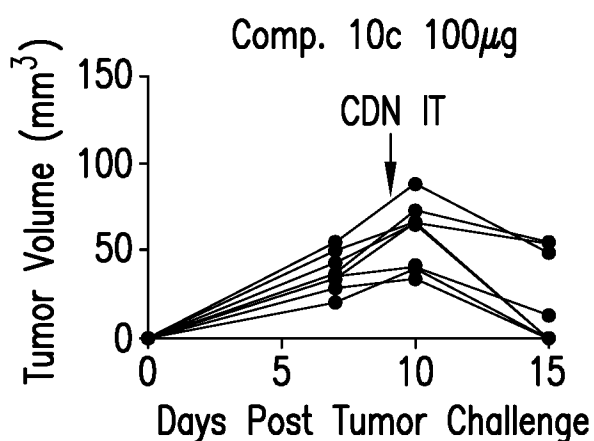


FIG.12F

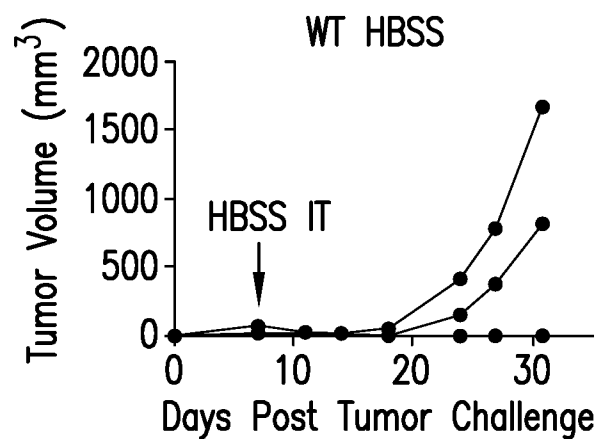


FIG.13A

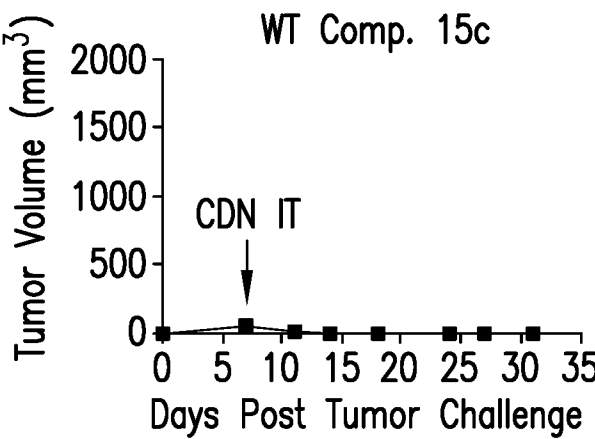


FIG.13B

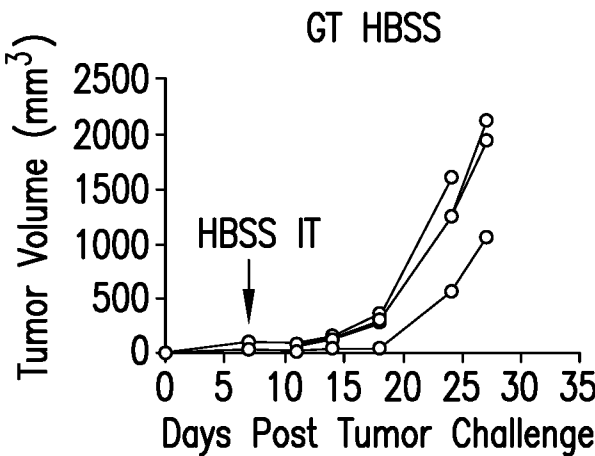


FIG.13C

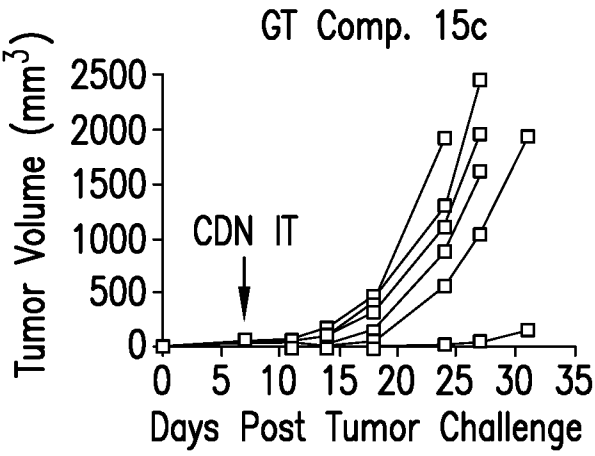


FIG.13D

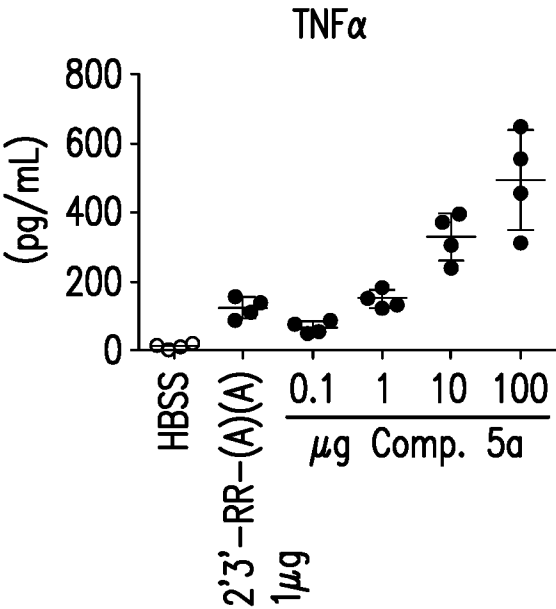


FIG.14A

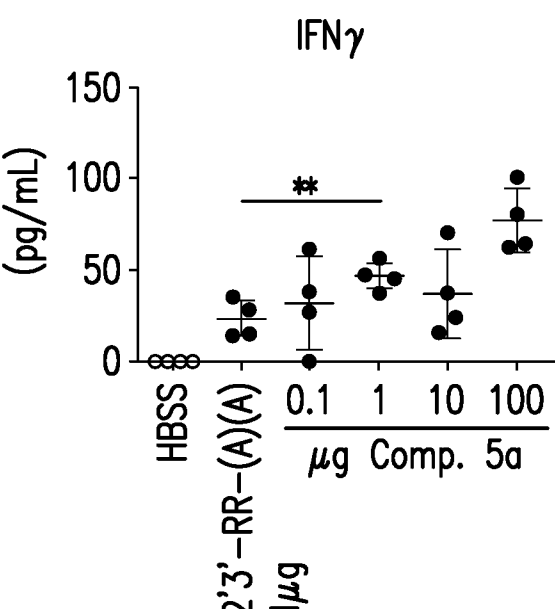


FIG.14B

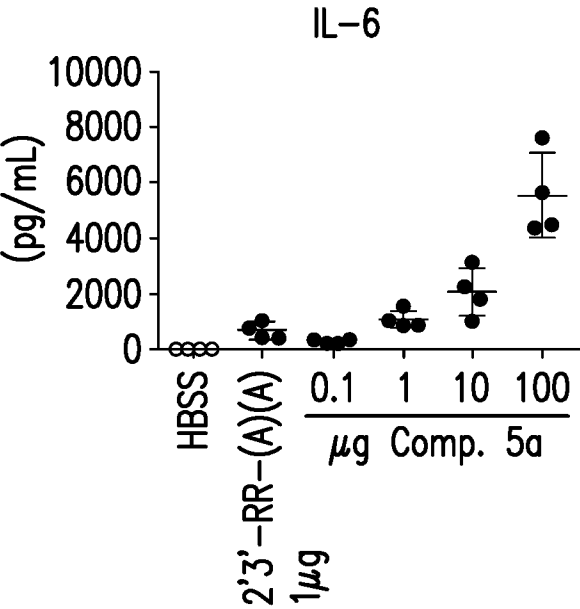


FIG.14C

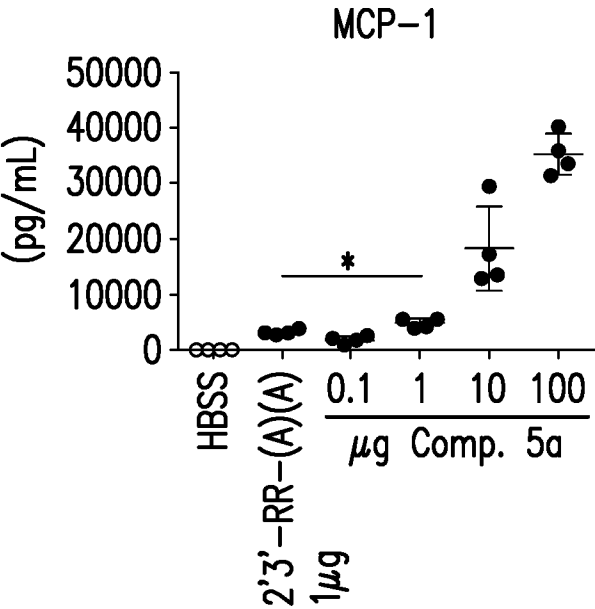


FIG.14D

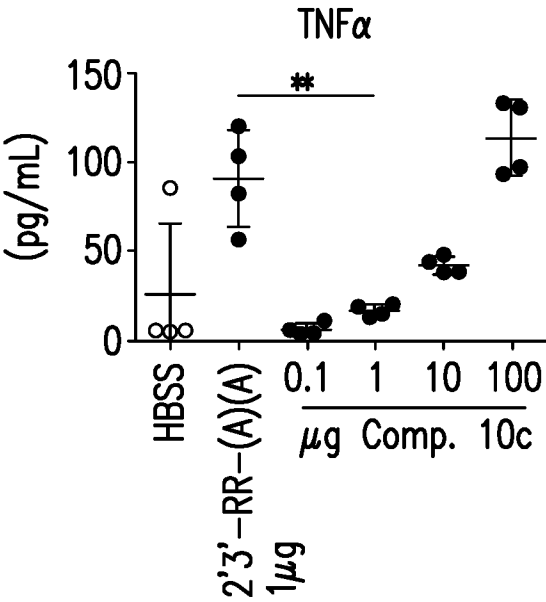


FIG.15A

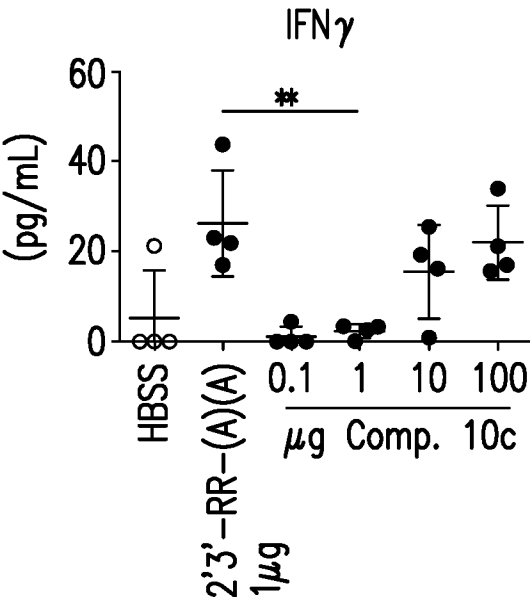


FIG.15B

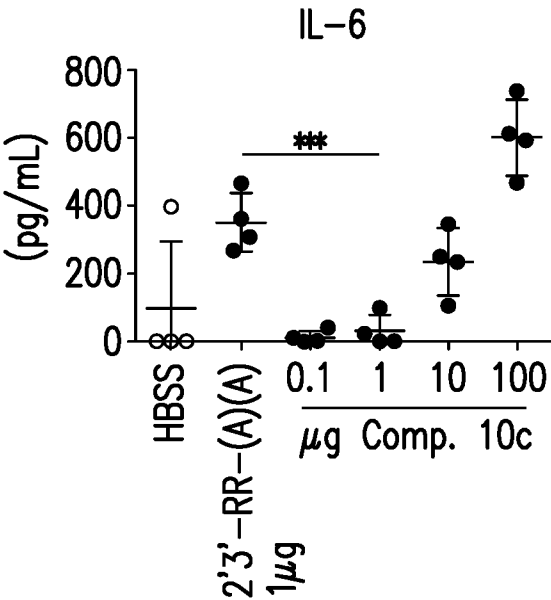


FIG.15C

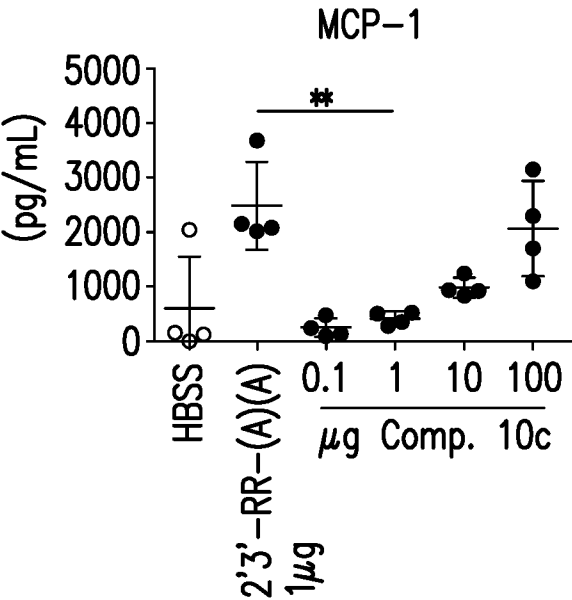


FIG.15D

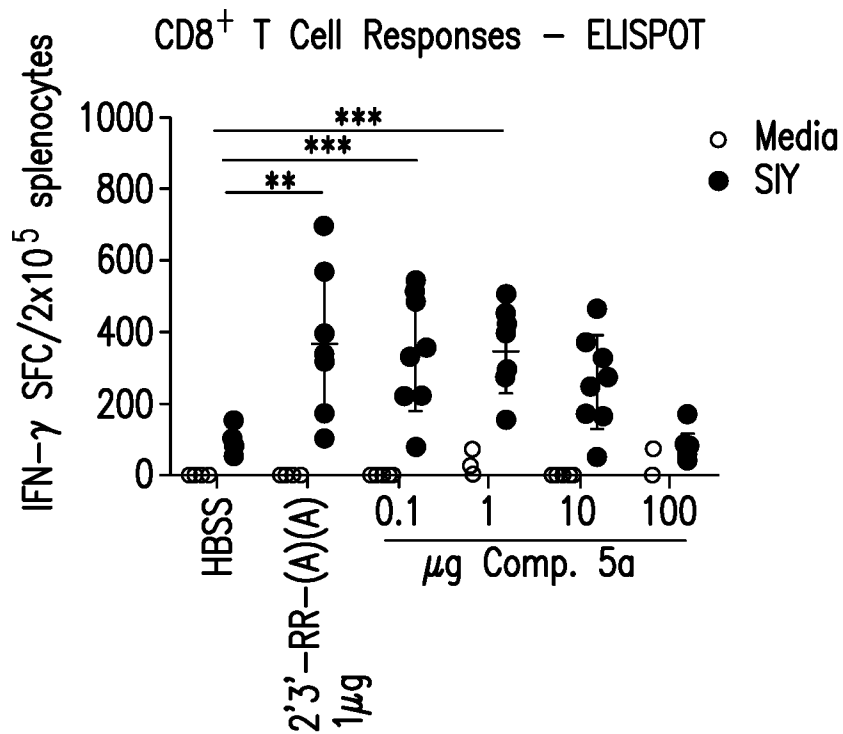


FIG.16A

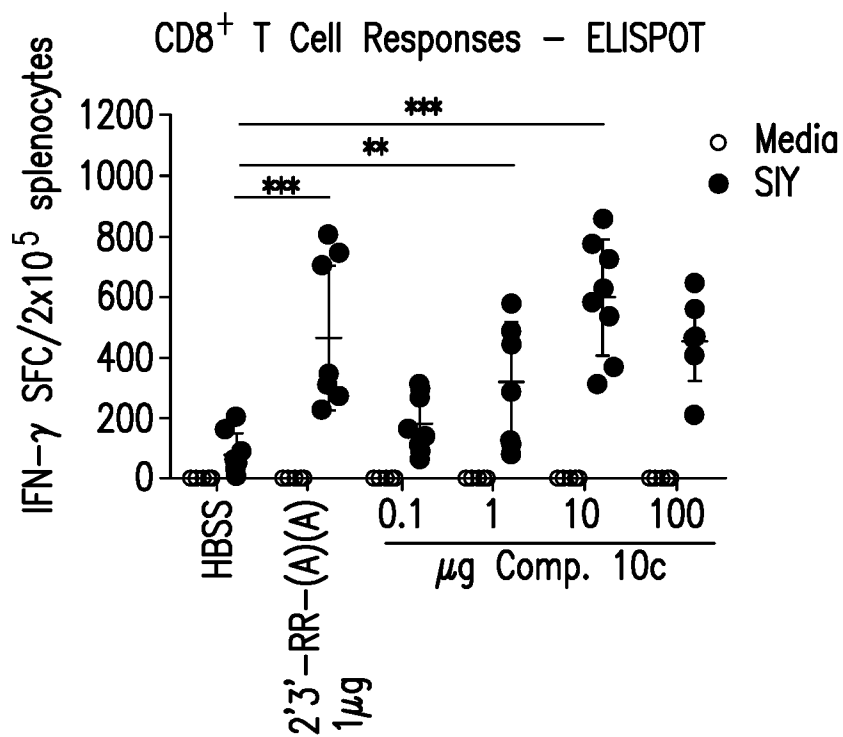


FIG.16B

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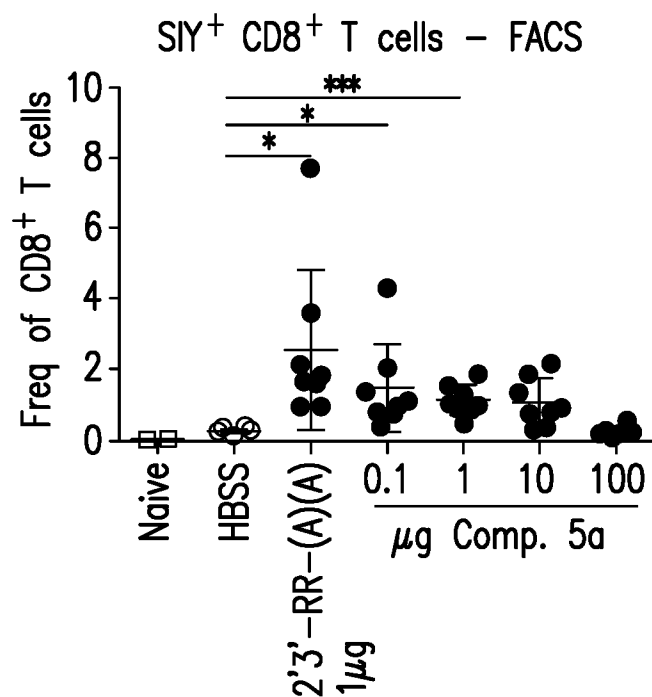


FIG.17A

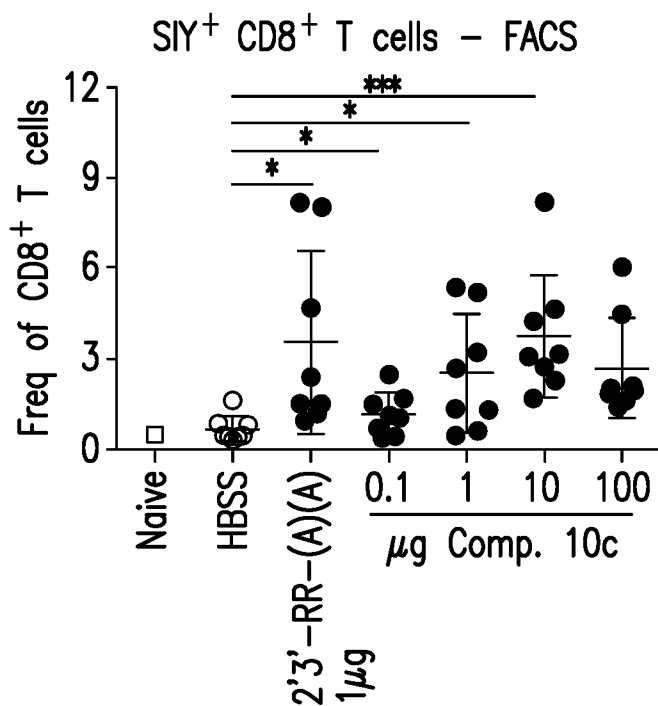


FIG.17B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/40535

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - C12N 15/113 (2017.01)
 CPC - C12N 2310/533, C12N 2310/14, C12N 15/1137

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Tang et al. "Agonist-Mediated Activation of STING Induces Apoptosis in Malignant B Cells" Cancer Research. 07 March 2016 (07.03.2016) vol 76, pg. 2137-2152 (supplementary pages 1-13 as separate document) < http://cancerres.aacrjournals.org/content/76/8/2137 >; pg. 2143, right col, para 2; supplementary figure 2	1-5
A	US 2012/0053226 A1 (Iyer et al.) 01 March 2012 (01.03.2012); para [0080]	1-5
A	US 8,461,124 B2 (Chattopadhyaya) 11 June 2013 (11.06.2013); Fig. 1; abstract	1-5
A	US 2015/297645 A1 (The Regents of the University of California) 22 October 2015 (22.10.2015); entire document	1-5

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 November 2017

Date of mailing of the international search report

07 DEC 2017

Name and mailing address of the ISA/US

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 P.O. Box 1450, Alexandria, Virginia 22313-1450
 Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/40535

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

- a. ☐ forming part of the international application as filed:
☐ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
☒ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/40535

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

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

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

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

3. ☒ Claims Nos.: 6-12, 25-28, 59-95
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/40535

--continued from Box No. III--

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: Claims 1-5, 13-24 and 29-58, directed to a compound of claim 1, that have the structure of formula I. The compound of claim 1 will be searched to the extent that it encompasses the first species of claim 1, represented by the first formula of claim 1, formula (I), wherein R1 and R2 are pyrimidine; R3 and R4 are OH; Y1 is O and Y2 is absent; Y3 is O and Y4 is absent; X1, X2 and X3 are present, X1 and X2 are -C(RbRc)- and X3 is -(CRbRc)-, selected to provide -X1-X2-X3- as -C(RbRc)-C(RbRc)-C(RbRc)-; X4' is O; X4 is O; bonded to the 2' carbon atom of Ring A; R5 is absent; R6 is H; R7 is H; Rb and Rc are H. It is believed that claims 1-5, read on this first named invention, and thus these claims will be searched without fee. Applicant is invited to elect additional compounds of claim 1, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be composition of claim 1, represented by the formula of claim 1, wherein R1 and R2 are pyrimidine; R3 and R4 are OH; Y1 is O and Y2 is absent; Y3 is O and Y4 is absent; X1, X2 and X3 are present, X1 and X2 are -C(RbRc)- and X3 is -(CRbRc)-, selected to provide -X1-X2-X3- as -C(RbRc)-C(RbRc)-C(RbRc)-; X4' is O; X4 is O; bonded to the 2' carbon atom of Ring A; R5 is absent; R6 is ORe; R7 is H; Rb and Rc are H; Re is alkyl (i.e., claims 1-4).

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

Special Technical Features:

Group I+ includes the technical feature of a unique compound of claim 1, which is not required by any other invention of Group I+.

Common technical features:

The inventions of Groups I+ share the technical feature of a compound of claim 1.

These shared technical features, however, do not provide a contribution over the prior art, as being obvious over US 2015/297645 A1 to The Regents of the University of California (hereinafter California) in view of US 2012/0053226 A1 to Iyer et al. (hereinafter Iyer). California discloses a compound similar to the compound of formula I wherein: R1 and R2 are purine; R3 and R4 are OH; Y1 is O and Y2 is absent; Y3 is O and Y4 is absent; X4' is O; X4 is O bonded to the 3' carbon of ring A; R6 is absent; R5 is OH; R7 is H (Fig. 3C: compound listed), but does not disclose X2 is absent, and X1 and X3 are both present. However, Iyer discloses a dinucleotide wherein X2 is absent, X1 and X3 are both present; X1 is O and X3 is -C(RbRc)-; selected to provide -X1-X3- as -O-C(RbRc)-; Rb and Rc are H (para [0080]: LNA dinucleotide). It would have been obvious to one with skill in the art to prepare derivatives of the compound disclosed by California, a cyclic dinucleotide; comprising a locked nucleic acid dinucleotide, as disclosed by Iyer, as said compounds are utilized to treat RNA/DNA viruses such as HCV (California, para [0119]; Iyer, para [0009]) in order to develop improved compounds for treatment.

As said compound and compositions were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the inventions of Groups I+. The inventions of Group I+ thus lack unity under PCT Rule 13.

Note: Claims 6-12, 25-28, 59-95 have been found to be unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).